Galectin-3 diminishes Wnt signaling in the postnatal subventricular zone

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Abstract
Postnatal subventricular zone (pSVZ) stem and progenitor cell proliferation is regulated by several developmental signaling pathways such as Wnt/β-catenin. However, the molecular regulation of Wnt function in the pSVZ is poorly understood. We previously showed that Wnt signaling is upregulated in an SVZ gliomagenesis in vivo model. As well, the pro-inflammatory molecule Galectin-3 (Gal-3) increases Wnt signaling in cancer cells and is expressed in the SVZ. Therefore, we asked if Gal-3 has a similar function on Wnt signaling in the pSVZ. We interrogated Wnt signaling using a signaling reporter as well as immunohistochemistry and showed that Wnt signaling predominates upstream in the pSVZ lineage but is downregulated in migrating neuroblasts. Biochemical analysis of SVZ cells, in vivo and in neurosphere stem/progenitor cells, showed that Gal-3 physically interacts with multiple forms of β-catenin, which is a major downstream regulator of Wnt signaling. Functional analyses demonstrated, in vitro and in vivo, that Gal-3 knockdown increases Wnt signaling and conversely that Gal-3 OE inhibits Wnt/β-catenin signaling in the pSVZ. This latter result suggested that Gal-3, which is consistently increased in brain injury, may decrease pSVZ proliferation. We showed that Gal-3 OE decreased proliferation without altering cell cycle re-entry and that it increased p27Kip1, a molecule which induces cell cycle exit. Our data uncover a novel regulator of Wnt signaling in the SVZ, Gal-3, which does so in a manner opposite to cancer.

KEYWORDS
β-catenin, Galectin-3, stem cell niche, subventricular zone, Wnt

1 INTRODUCTION

The postnatal subventricular zone (pSVZ) stem cell niche comprises quiescent NSCs that, when activated, give rise to transit-amplifying progenitors (TAPs).1,2 TAPs stay local, go through several rounds of division, and generate both neuronal and glial progeny. These migrate actively to the olfactory bulbs (OBs) and to the forebrain, respectively, and then differentiate.1,2 Whereas many classes of molecules are known to regulate SVZ proliferation and migration, much remains to be discovered.3 We have shown that in the adult SVZ, Galectin-3 (Gal-3) is necessary for neuroblast migration,3 and in the postnatal SVZ its upregulation increases gliogenesis via bone morphogenetic protein (BMP) signaling.4 In addition to these homeostatic roles, Gal-3 is increased in and around the lateral ventricle (LV) in perinatal hypoxia and in multiple sclerosis and it
regulates SVZ proliferation in two models of the latter disease. Here, we sought to further determine the postnatal function and signaling partners of Gal-3 in the SVZ.

Wnt/β-catenin signaling promotes SVZ NSC proliferation, self-renewal, and cell cycle re-entry. Other work has shown that Wnt signaling is sufficient to promote oligodendrogenesis. Wnt signaling also drives neurogenesis in the other major postnatal stem cell niche, the subgranular zone. Previous reports have concentrated on the role of Wnt signaling in the dorsal (subcallosal) SVZ, a distinct portion of the SVZ that gives rise to dopaminergic neurons and a small number of excitatory neurons, postnatally. Here, we observed β-catenin expression throughout the lateral wall of the SVZ. Thus, we sought to determine if active Wnt signaling occurs in this major pSVZ domain.

Gal-3 can induce Wnt signaling in several types of cancer cells, but if it regulates Wnt in the SVZ, was unknown. Here, we provide novel evidence that Gal-3, in contrast to what is known in colorectal and pancreatic cancer cell lines, negatively regulates Wnt signaling in the SVZ. We specifically focused on the largest portion of the pSVZ, the lateral aspect flanking the striatum. We controlled Gal-3 expression via in vivo electroporation and in vitro nucleofection and interrogated how this influenced Wnt/β-catenin signaling. Overall, our work demonstrates novel constitutive roles for Gal-3 in the pSVZ, it binds to β-catenin, reduces Wnt signaling and decreases proliferation.

2 | METHODS

2.1 | Animals

C57BL/6 mice were used for in vivo electroporation, and CD1 or C57BL/6 mice for in vitro neurosphere culture, nucleofection, Western blotting, and protein coimmunoprecipitation. Both CD1 and C57BL/6 mice were purchased from Harlan UK (Oxon, United Kingdom). Animals were kept in rooms with a lighting schedule of 12 hours light/darkness and with standard diet and water ad libitum. The experiments were performed in accordance with the UK Animals (Scientific Procedures) 1986 Act, UK Home Office. All animal work was approved by the UK Home Office, License #30/2496, and the University of Oxford Department of Physiology, Anatomy and Genetics Departmental Ethical Review Committee.

2.2 | In vivo brain electroporation

Electroporation was performed as in Reference 18. Briefly, P2 pups were anesthetized by hypothermia. One to two microliters of plasmid(s) solution (2 μg/μL per plasmid with 0.1% Fast Green in Endotoxin-free TE [Qiagen]) was injected into the right LV of C57BL6 mice. Electroporation: five 50 ms 100 V pulses with 850 ms intervals, using CUY650-P5 tweezers (Sonidel) connected to an ECM830 square wave electroporator (BTX). Pups recovered in a 36°C heating chamber for 15 to 20 minutes and then returned to the dam. Mice were perfused 3, 7, or 17 days postelectroporation (DPE).

2.3 | Thymidine analog injection

BrdU (Sigma Aldrich) and EdU (Life Technologies) were reconstituted in sterile normal saline at 10 mg/mL. A single i.p. injection of BrdU or EdU (50 mg/kg) was given.

2.4 | Nucleofection

NSC/neural progenitor cell cells (1 × 10⁶ to 4 × 10⁶) from tertiary neurospheres were nucleofected with 5 μg DNA/plasmid/condition using an Amaxa Biosystems Nucleofector I as per the Amaxa Mouse Neural Stem Cell Nucleofector Kit instructions.

2.5 | Image acquisition and quantification

Confocal images were acquired on a Zeiss LSM 710 microscope. All images represent single optical planes, unless noted. All quantifications were done by an observer blinded to experimental condition. For colocalization studies, ×40 z-stacks and ×20 z-stacks (12 optical sections, each 1.3 μm apart) were quantified on Volocity 6.3 (Improvision) and ImageJ. At least three images from three sections were quantified per animal and considered technical replicates. The sections spanned the LV at the level of, and anterior to, the crossing of the anterior commissure. Quantifications were done in the lateral SVZ only, and the dorsolateral horn was excluded. Only DAPI+ cells were included, and cells the nuclei of which were not completely within the z-stack were excluded. p27Kip1 was ubiquitously expressed in the SVZ; therefore, the p27Kip1 signal histogram was thresholded in FIJI (NIH) software to allow counting p27Kip1 high cells only, which corresponded to the top 2.5% of the histogram. For quantification of neurosphere numbers, 10 entire wells/condition from 96-well plates were imaged on a Life technologies EVOS FL Auto Fluorescent microscope and TIFF files quantified in Volocity 6.3.

Significance statement

Galectin-3 (Gal-3) is frequently increased in cancer and injury. It regulates inflammation and subventricular zone (SVZ) neurogenesis, yet the signaling pathways whereby it does so are poorly understood. Gal-3 increases Wnt signaling in cancer cells but here we show that it binds to β-catenin in SVZ cells and negatively regulates Wnt signaling. Gal-3 also decreased proliferation and increased cell cycle exit. Gal-3 decreased Wnt signaling in the same cells with increased bone morphogenetic protein signaling. Since Gal-3 is druggable, these studies suggest Gal-3 modulators could be used to manipulate Wnt signaling in the clinical setting.
Galectin-3 expression and β-catenin signaling are downregulated in neuroblasts. A, Wnt reporter plasmid and in vivo brain electroporation scheme. All labeled cells expressed mCherry, but only cells with active Wnt signaling expressed dGFP. B, Approximate anatomical locations of panels (C), (D), and (E). C–E, Single optical planes from confocal microscopy showing Wnt signaling is active in labeled cells in the subventricular zone (C). Galectin-3 (Gal-3) is expressed in a subset of those cells. The proximal RMS, D, and distal RMS, E, expressed less Gal-3 and downregulated Wnt signaling. Scale bars = 100 μm in main panels and 50 μm in insets. RMS, rostral migratory stream.
2.6 | Neurosphere generation and culture

Hypothermia was induced in P4 mice, followed by decapitation, brain extraction, and SVZ dissection. Brains were washed in Hank’s Balanced Salt solution before coronal sectioning into 500 μm slices on a McIlwan tissue chopper. The lateral wall of the LV was micro-dissected under a Leica MZ12 dissecting microscope. SVZ tissue was dissociated by incubation in Accutase (Sigma) for 10 minutes at 37°C, followed by trituration using a p1000 pipette and declumped by passing through a 40 μm cell strainer (BD Falcon). Next, we washed cells 2x in Neurobasal A+ (NB-A+) (Neurobasal-A medium +1% B-27 + 1 x GlutaMax + Penicillin/Streptomycin 1 x; Gibco). The cell pellet was then suspended in NB-A+ and cells counted using a hemocytometer and/or Scepter 2.0 (Millipore), and viability was quantified using Trypan blue (Flukar) exclusion. After the final wash, SVZ cells were diluted to 100 cells/μL in growth medium (NB-A+ GF) (NB-A+ supplemented with 20 ng/mL EGF (Sigma E9644) and 20 ng/mL FGF-2 (R&D Systems 233-FB) and seeded into nontissue culture-treated six wells plates (BD Falcon) cultured in a 37°C-5% CO2 incubator. For culture at clonal density, cells were diluted to 10 cells/μL and cultured in Costar 96-well plates for 7 days before passaging or analysis.

2.7 | Statistical analyses

All quantifications were done blind to experimental condition using a random coding system. Differences between two groups were assessed using unpaired t test when normality could be checked using

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**FIGURE 2** Galectin-3 and β-catenin coimmunoprecipitation. A,B, Coimmunoprecipitation (IP) shows that Gal-3 complexes with β-catenin in total, A, and membrane, B, SVZ protein. C, Reverse coimmunoprecipitation showing β-catenin pull-down from SVZ membrane protein and Gal-3 immunoblot. D, Gal-3 binds to various phosphorylated β-catenin (pβ-catenin) species in the cell membrane. E, Gal-3 complexes with β-catenin in tertiary neurosphere total protein. IB, Immunoblot; SVZ, subventricular zone
Shapiro-Wilk test (n = 5 or more) or the Mann-Whitney U nonparametric test (n = 3 or 4). For in vitro experiments, including quantitative polymerase chain reaction (qPCR) analysis of delta-delta Ct values from biological replicates, one sample t test was used after data normalization to control. Comparisons between three or more groups were conducted using analysis of variance with Tukey or Sidak post hoc tests as appropriate in experiments with n ≥ 5; otherwise, the Kruskal-Wallis test was used. Significance was set at P < .05. Analyses and graphical representations were performed in Microsoft Excel 2011 and GraphPad Prism 6 (GraphPad) software packages. Values were presented as mean ± SEM.

3 RESULTS

3.1 Wnt signaling and Gal-3 expression is downregulated with SVZ lineage progression

We first studied Gal-3 expression in Wnt/β-catenin signaling cells, and found downregulation of both as cells progressed through the SVZ lineage. We electroporated a bicistronic Wnt/β-catenin signaling reporter plasmid into P2 SVZs, sacrificed mice 3 DPE (Figure 1A). Cells electroporated with this plasmid express mCherry but only cells with active Wnt signaling express destabilised green fluorescent protein (dGFP). The latter feature ensures that only cells with active or recent Wnt signaling will be labeled. We determined Gal-3/GFP coexpression along the anteroposterior axis (Figure 1B). In the anterior SVZ, a subset of electroporated cells coexpressed Gal-3 and GFP, confirming Gal-3 expression and active Wnt/β-catenin signaling (Figure 1C). Conversely, in both the posterior and anterior rostral migratory stream (RMS), Gal-3 immunofluorescence and GFP were minimal and not detected in mCherry+ cells, which had migrated there from the SVZ (Figure 1D,E). Together, these studies confirmed downregulation of Gal-3 and Wnt/β-catenin signaling with SVZ lineage progression.

3.2 Gal-3 physically interacts with β-catenin

It was unknown if Gal-3 binds β-catenin in SVZ cells. We tested this using immunoprecipitation and Western blots for β-catenin. This showed Gal-3 is complexed to β-catenin in both whole SVZ protein and membrane protein preparations (Figure 2A,B). We next confirmed binding between Gal-3 and β-catenin with reverse coimmunoprecipitation; pulling down β-catenin in the SVZ membrane protein fraction and immunoblotting for Gal-3 (Figure 2C). An 68 kDa band corresponding to the dimeric form of Gal-3 was detected. In contrast, Gal-3 did not bind to the epidermal growth factor receptor in SVZ cells, as expected or to neural cell adhesion molecule (data not shown), supporting the specificity of the above results. We then asked if the Gal-3 β-catenin interaction was activation-state specific. Therefore, we blotted for several phosphorylated β-catenin (p-β-catenin) forms after Gal-3

FIGURE 3 Galectin-3 regulates Wnt signaling in the subventricular zone stem cell niche. A, Experimental scheme: Wnt reporter plus control (nontargeting shRNA) or Gal-3 knockdown (shGal-3) or Gal-3 overexpression (Gal-3 OE) plasmids, and timeline of in vivo electroporation. B, Confocal images of nuclei (DAPI), mCherry (all electroporated cells) and dGFP (active Wnt signaling) in controls, shGal-3, and Gal-3 OE groups. High magnification and orthogonal views of boxed areas shown next to panels. Scale bars = 20 μm in panels and 10 μm in insets. C, Quantification of (B). One-way ANOVA, Tukey’s test for multiple comparisons. n = 7 to 9. ANOVA, analysis of variance. DAPI, 4’,6-diamidino-2-phenylindole; dGFP, destabilized green fluorescent protein.
immunoprecipitation from SVZ membrane protein (Figure 2D). β-catenin phosphorylated at serine residues 33/37/45 and threonine 41 is targeted for proteasomal degradation and conversely, β-catenin phosphorylated at serines 552 or 675 is transcriptionally active. Gal-3 was complexed to all phosphorylated forms tested, suggesting it physically interacts with both active and inactive β-catenin states (Figure 2D). To verify binding in NSC/TAPs, we immunoprecipitated Gal-3, and then blotted for β-catenin from tertiary neurospheres which are enriched in NSCs/TAPs (Figure 2E). Gal-3 was complexed with β-catenin in neurosphere protein preparations suggesting that at least some of the interaction was in NSCs/TAPs. Taken together, the results showed that Gal-3 and β-catenin were complexed together in SVZ NSC/TAPs, and that the interaction takes place at least in part in the plasma membrane regardless of β-catenin phosphorylation status.

3.3  |  Gal-3 decreases Wnt/β-catenin signaling

To determine if Gal-3 levels alter Wnt/β-catenin signaling in vivo in the pSVZ, we electroporated plasmids into the SVZ of postnatal day 2 (P2) mice and then harvested brains 3 DPE (Figure 3A). We used nontargeting shRNA control plasmids, knockdown (shGal-3) plasmids, or Gal-3 overexpression (Gal-3 OE) plasmids, together with the Wnt/β-catenin signaling reporter plasmid. Compared to controls, the Wnt reporter showed higher GFP intensity in the shGal-3 group.

![Figure 4](image-url)  
**Figure 4** Galectin-3 overexpression reduces SVZ proliferation. A, Electroporation as in Figure 3A. Confocal images of mCherry electroporated cells and PHI3+ cells. Merged images show orthogonal views. Arrows indicate mCherry+Phi3+ cells and arrowheads indicate mCherry−Phi3+ cells. Scale bar = 20 μm. B, Quantification of (A), Kruskal-Wallis test with Dunn’s test for multiple comparisons. n = 3 to 5 (experimental groups compared to control group). C, Electroporation as in Figure 3A. p27/Kip1+ cells. Arrows indicate p27 + Kip1high labeled cells, and arrowheads p27 + Kip1low/negative cells. Merged images show orthogonal views. Scale bar = 20 μm. D, Quantification of (C). Only p27 + Kip1high cells were counted, One-way ANOVA, Tukey’s test for multiple comparisons. n = 7 to 9. Mean ± SEM. *P < .05; ***P < .001; ****P < .0001. ANOVA, analysis of variance; PHI3, phospho-Histone-3; SVZ, subventricular zone.
and lower GFP fluorescence in the Gal-3 OE group (Figure 3B). Significantly more mCherry-labeled cells were GFP+ in the shGal-3 group (53.7% ± 1.2%), and fewer in the Gal-3 OE group (35.3% ± 2.4%), compared to controls (43.3% ± 1.9%) (Figure 3C). In previous work, we confirmed downregulated Gal-3 in the shGal-3 group compared to controls and upregulated Gal-3 expression in the Gal-3 OE group. Together, these data indicated Gal-3 negatively regulates Wnt/β-catenin signaling in the pSVZ in vivo. Driven by these in vivo results, we asked whether the functional relationship between Gal-3 and Wnt/β-catenin signaling is niche-dependent and could be detected in vitro. The data indicate that Gal-3 negatively regulates SVZ Wnt/β-catenin signaling in NSC/progenitors independent of niche factors (Figure S1 and Results section in Supporting Information).

We next queried if Gal-3 changes β-catenin expression levels. At 3 DPE, we compared controls with Gal-3 overexpression and Gal-3 knockdown electroporation. We immunostained each group for β-catenin and analyzed a total of 2195 electroprated (GFP+) cells. There were no statistically significant differences in the percent of GFP+ cells that expressed β-catenin upon Gal-3 modulation.

3.4 | Gal-3 overexpression reduces SVZ proliferation and increases cell cycle exit

Wnt/β-catenin signaling reportedly promotes NSC symmetric divisions, NSC maintenance, and TAP expansion. Given that Gal-3 negatively regulated Wnt/β-catenin signaling, we examined the role of Gal-3 in proliferation. We coelectroporated control, Gal-3 OE or knockdown constructs, and the Wnt reporter as in (Figure 3), and used the proportion of electroprated cells expressing phospho-Histone-3 (PHi3) to assess proliferation (Figure 4A). Gal-3 OE significantly reduced the percentage of PHi3+ electroprated cells (Figure 4B). This shows that Gal-3 gain-of-function not only reduced Wnt signaling but also diminished SVZ proliferation. However, increased Wnt signaling in Gal-3 knockdown was insufficient to increase proliferation (Figure 4B). β-catenin loss-of-function induces cell cycle exit in embryonic cortical precursors. Thus, we asked if Gal-3 modulates p27/Kip1, a cyclin-dependent kinase inhibitor that promotes cell cycle exit in the SVZ. Gal-3 OE significantly increased the proportion of p27/Kip1+ labeled SVZ cells, while Gal-3 knockdown had no effect (Figure 4A,B). These data suggest Gal-3 overexpression increased cell cycle exit possibly accelerating lineage progression. We found a reduction in proliferation in Gal-3 OE only, no significant differences were identified between Gal-3 KD and controls. We also found that only Gal-3 OE increased the percentage of p27/Kip1+ cells, indicating an increase in cell-cycle exit. Thus, we next determined whether the reduction in proliferation and increase in cell-cycle exit was due to reduced cell cycle re-entry. To explore the temporal dynamics of Gal-3 OE-induced cell cycle exit, wild type (WT) P2 mice were electroporated with control GFP or Gal-3 OE plasmids and pulsed with BrdU 2 DPE and then with EdU 2 hours before brains...
were harvested (3 DPE) (Figure 5A). In line with our PH13 data, Gal-3 OE significantly reduced the percentage of GFP+ cells that were BrdU+, that is, proliferating 2 DPE (Figure 5B,C) or EdU+ (2 hours before sacrifice, quantification not shown). The percentage of GFP+ electroporated cells that were BrdU+EdU+ was also significantly reduced (Figure 5D), supporting reduced proliferation. However, the proportion of EdU+BrdU+GFP+ cells among BrdU+GFP+ cells, which represents cell cycle re-entry, was not altered (Figure 5E). These data show that Gal-3 OE reduced proliferation 2 and 3 DPE and promoted cell cycle exit before 2 DPE, rapidly reducing the population growth fraction, without altering cell cycle re-entry.

We previously showed that Gal-3 positively regulates BMP signaling.4 Here, we asked whether Wnt signaling and BMP signaling occur in the same SVZ cell population. We thus immunostained for pSmad1/5/8+ (the hallmark of BMP signaling) in mice electroporated with the Wnt reporter and harvested brains 3 DPE. We found that the majority of pSmad1/5/8+ cells coexpressed GFP, indicating that both BMP and Wnt signaling were active in the same cells in which Gal-3 exerted its effects (Figure S2A). We next explored if Gal-3’s modulation of BMP signaling mediates its effect on Wnt signaling. We nucleofected NSCs in vitro with the Wnt reporter and treated them with recombinant BMP4 for 24 hours before flow cytometric analysis (Figure S2C-E). BMP4 treatment for 24 hours did not change the proportion of cells with total or high Wnt signaling (Figure S2C,D). Moreover, analysis of growing neurospheres treated with BMP4 for 48 hours showed no difference in expression of the transcript levels of the Wnt target Axin2 compared to controls (Figure S2E). These data show that Gal-3 OE in the lateral pSVZ inhibits Wnt/β-catenin signaling and thereby reducing lateral pSVZ proliferation. The reduction in SVZ proliferation upon Gal-3 OE could have resulted in reduced rates of OB neurogenesis. However, in previous work and here again, we showed that after postnatal Gal-3 OE, the total number of newborn neurons born in the SVZ that migrated to the OB did not change significantly.4 In contrast, Wnt signaling was activated by Gal-3 knockdown. However, this did not induce proliferation, perhaps due to incomplete Gal-3 knockdown, or because of other compensatory mechanisms that keep the cell cycle in check.

How Gal-3 regulates Wnt/β-catenin signaling is not clear. One potential mechanism is suggested from our data. Given the binding between Gal-3 and β-catenin in cell membranes, Gal-3 may sequester β-catenin and hinder its nuclear translocation. Alternatively, Gal-3 may change β-catenin phosphorylation and activation; however, we found that Gal-3 binds β-catenin irrespective of its phosphorylation state. Additional studies are needed to ascertain the exact molecular mechanism whereby Gal-3 regulates β-catenin function. Gal-3 can regulate several signaling pathways such as Wnt, Notch, and EGFr in different cell types.16,28,29 Interestingly, we have evidence that loss of Gal-3 increases phosphorylation of the EGFr3 and thus activates it; however, in two separate studies, we could not find binding of Gal-3 to the receptor.5,4 This was in contrast to cancer cells in which Gal-3 and EGFr were found to bind and thereby alter internalization.28 Thus, Gal-3 has different binding partners and different functions in various contexts.

Previous work has indicated that Wnt/β-catenin maintains SVZ NSCs.7,8 Wnt/β-catenin signaling drives NSC self-renewal and proliferation.7,8 We thus expected Gal-3 to decrease NSC self-renewal. However, in our previous work, Gal-3 OE did not alter lateral pSVZ NSC proportions, self-renewal, activation, or OB neurogenesis.4 Wnt/β-catenin’s effects on SVZ progenitor proliferation are inconsistent; with various reports supporting positive,9 negative,30 or no regulation.31 The effects of Gal-3 on Wnt signaling may be gliogenic since in our earlier study Gal-3 increased the proportion of proliferative putative glial progenitors and was necessary for SVZ-derived striatal gliogenesis.4 It appears that Gal-3’s effects are indeed context-dependent; shortly after overexpression, it causes cell cycle exit—an effect tied to a reduction of Wnt signaling and subsequently it is necessary for gliogenesis.

Gal-3 is upregulated in many cancers and frequently exacerbates malignancy. This further justified determination of Gal-3 effects
on Wnt signaling in the pSVZ since the SVZ can be a source of tumours. In contrast to our findings, Gal-3 increases Wnt/β-catenin signaling in cancer cell lines from breast, colon, and pancreas. It is unclear how Gal-3 regulates Wnt signaling oppositely in cancer vs the SVZ, but several Wnt/β-catenin pathway components are mutated in cancer, which may contribute to Gal-3’s opposite actions. In other work, we generated a mouse model of gliomagenesis by selectively expressing a common human driver mutation (IDH1R132H) in the SVZ and found that Wnt signaling was significantly altered. We have preliminary evidence that Gal-3 expression increases in the IDH1R132H mutant SVZ, and future work could help decipher how the switch in Gal-3 function on Wnt signaling between homeostasis and cancer contributes to malignancy.

Gal-3 is unique among the 15 galectins in that it contains a C-terminal carbohydrate recognition domain (CRD) and also an N-terminal nonlectin domain. In contrast, other galectins contain two end-terminal CRD’s. Gal-3’s CRD binds to a variety of glycosaminoglycans and proteoglycans while the nonlectin domain allows several Gal-3 molecules to multimerize. Thus, Gal-3 has myriad effects mediated by cross-linking molecules found in the extracellular matrix, cell surface, or cytoplasm. Gal-3 expression in the SVZ neurogenic niches overlaps with that of Gal-1 and Gal-9 (Szele lab, unpublished); however, to our knowledge, other galectins have not been documented in the SVZ.

Interestingly, Gal-3 has opposite constitutive effects on Wnt and BMP signaling. In our previous study on postnatal Gal-3 functions in the SVZ, we showed that BMP signaling is increased by Gal-3 overexpression. Also, blocking signaling with floxed BMP1a mice demonstrated that BMP is necessary for the Gal-3 OE fate shift from oligodendrocyte to astrocyte genesis. Here, we show that Wnt and BMP signaling can occur in the same SVZ cells which is noteworthy, since Wnt and BMP have several opposing effects in SVZ proliferation and fate choices. Thus, Gal-3 may serve to harmonize these two major signaling pathways. Finally, since there was evidence that BMP inhibits Wnt signaling in the dorsal pSVZ, we tested this in the lateral pSVZ and found that BMP4 stimulation did not alter Wnt signaling. It will be fascinating in future studies to discover how these signaling pathways may be differentially coordinated in different SVZ subdomains.

5 | CONCLUSION

A comprehensive map of how signaling pathways are regulated in the SVZ niche paves the way for discovery of drugs to modulate its output in response to pathological states. Gal-3 is druggable and is a pharmacological target in fibrosis, heart disease, and several other maladies. We show in this study that increasing Gal-3 negatively modulates Wnt/β-catenin function in the pSVZ and also that it reduces proliferation. The developmental effects and molecular mechanisms uncovered here contribute to our understanding of normal pSVZ homeostatic mechanisms and have important implications in disease.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

O.A.-D.: conception/design, collection and/or assembly of data, data analysis and interpretation, manuscript writing; J.N., S.D.: collection and/or assembly of data, data analysis and interpretation; L.C.S.: collection and/or assembly of data; F.G.S.: conception/design, financial support, data analysis and interpretation, manuscript writing, final approval of manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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10

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SUPPORTING INFORMATION
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