Simvastatin Promotes Adult Hippocampal Neurogenesis by Enhancing Wnt/β-Catenin Signaling

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SUMMARY

Statins improve recovery from traumatic brain injury and show promise in preventing Alzheimer disease. However, the mechanisms by which statins may be therapeutic for neurological conditions are not fully understood. In this study, we present the initial evidence that oral administration of simvastatin in mice enhances Wnt signaling in vivo. Concomitantly, simvastatin enhances neurogenesis in cultured adult neural progenitor cells as well as in the dentate gyrus of adult mice. Finally, we find that statins enhance Wnt signaling through regulation of isoprenoid synthesis and not through cholesterol. These findings provide direct evidence that Wnt signaling is enhanced in vivo by simvastatin and that this elevation of Wnt signaling is required for the neurogenic effects of simvastatin. Collectively, these data add to the growing body of evidence that statins may have therapeutic value for treating certain neurological disorders.

INTRODUCTION

A large body of evidence indicates that statins, a class of drugs typically used to treat hyperlipidemia, are therapeutically beneficial for neurological disorders. Statins have been shown to improve outcome following traumatic brain injury and stroke (Chen et al., 2003; Karki et al., 2009; Lu et al., 2007; Mahmood et al., 2009; Wu et al., 2008). Simvastatin rescues cerebrovascular and memory-related deficits in mouse models of Alzheimer disease (AD) (Li et al., 2006; Tong et al., 2009, 2012), and recent meta-analysis of clinical studies concluded that statins provide a slight benefit in the prevention of AD and all-type dementia (Wong et al., 2013). While these effects have been attributed to reduction of inflammation, reduced oxidative stress, upregulated PI3K/AKT signaling, and enhanced neurogenesis, the mechanisms by which statins are beneficial in neurological disorders are not fully understood.

Previously, we reported a chemical genetic screen that revealed that several statins activate a β-catenin-responsive luciferase reporter (BAR) in a cell-based assay (Biechele et al., 2010). This result supports prior in vitro studies that have shown that statins modulate Wnt/β-catenin signaling (henceforth referred to as Wnt signaling) in human neuronal cells (Salins et al., 2007), in rat mesangial cells (Lin et al., 2008), and in mouse embryonic stem cells (Qiao et al., 2011). Given that Wnt signaling is a key regulator of adul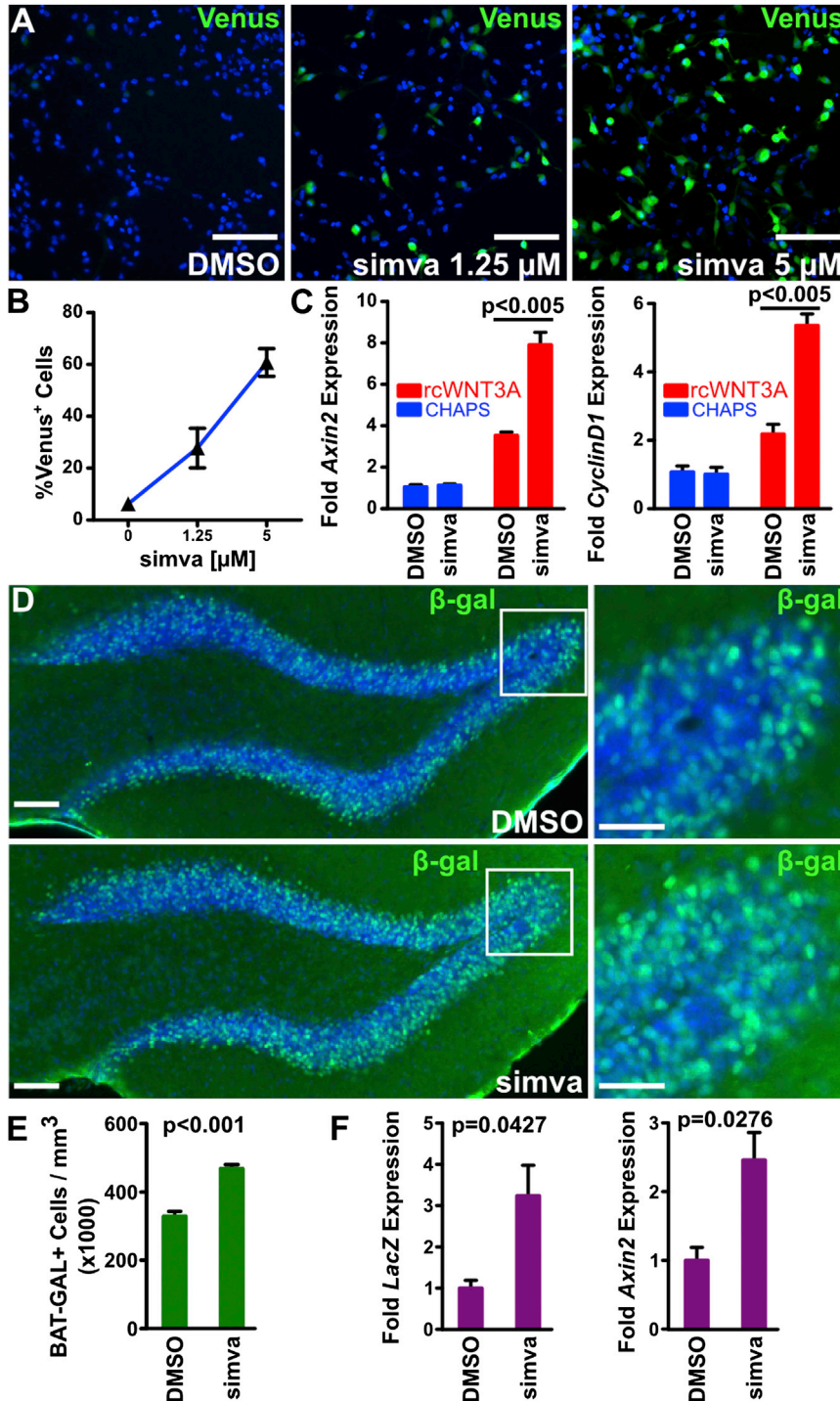t hippocampal neurogenesis (Jang et al., 2013; Kuwabara et al., 2009; Lie et al., 2005; Luo et al., 2010; Mao et al., 2009; Seib et al., 2013), we sought to determine whether statin-mediated enhancement of the Wnt pathway can occur in this region of the brain and to characterize any downstream effects on neurogenesis. We chose to focus on simvastatin (simva), as it is a lipophilic statin capable of crossing the blood-brain barrier (Tamai and Tsuji, 2000) and is commonly studied in neural contexts.

We find that simva enhances Wnt signaling in the adult hippocampus and that Wnt signaling is required for statins to enhance neuronal specification in differentiating adult neural progenitor cells (aNPCs). Through examination of various stage-specific markers in vivo, we determine that simva treatment increases the number of newborn neurons in the dentate gyrus (DG) by enhancing proliferation of intermediate precursor cells (IPCs) in the subgranular zone (SGZ). Finally, we determine that the effect of simva on the Wnt pathway is independent of cholesterol and is mediated by inhibition of isoprenoid biosynthesis.

RESULTS

Simva Enhances Wnt Signaling In Vitro and In Vivo

We recently reported that lovastatin and fluvastatin enhance Wnt signaling (Biechele et al., 2010). In the present study, we extended our analysis to simva, a statin of clinical relevance to neurological disease. To monitor

Figure 1. Simva Enhances Wnt Signaling In Vitro and In Vivo

(A) Venus reporter expression in aNPCs treated with various concentrations of simva in combination with rcWNT3A (20 ng/ml), counterstained with Hoechst (scale bars, 100 μm).

(B) Quantification of Venus+ cells (n = 5 independent wells).

(C) Expression of Wnt target genes in aNPCs treated with 5 μM simva or DMSO in combination with rcWNT3A or CHAPS (n = 3 independent wells).

(D) β-gal staining of DG from BAT-GAL mice treated with simva or DMSO, counterstained with DAPI (left scale bars, 100 μm; right-inset scale bars, 50 μm).

(E) Quantification of β-gal+ cell density (n = 7–8 mice per group).

(F) Real-time PCR analysis of Wnt target genes in hippocampi of BAT-GAL mice (n = 3 mice per group).

Results are presented as mean ± SEM (B, C, E, and F). Statistical analysis was performed with the Student's unpaired t test.

Wnt activity, we transduced cultured aNPCs with BAR driving expression of Venus fluorophore. Consistent with prior studies (Wexler et al., 2009), we saw no reporter activity under basal culture conditions, and addition of simva by itself did not induce reporter expression (not shown). However, in combination with a low dose (20 ng/ml) of recombinant WNT3A ligand (rcWNT3A), simva promoted a dose-dependent increase in the percentage of Venus+ cells following 4 days of treatment (Figures 1A and 1B).

If, as suggested above, simva were able to synergize with Wnt signaling, then one would predict that such treatment should increase the expression of endogenous Wnt target

genes. To test this, we treated aNPCs with simva or DMSO, in combination with either rcWNT3A or vehicle control (CHAPS), and used quantitative real-time PCR to monitor the levels of two genes that are known to be directly regulated by Wnt signaling in adult neural cells, *Axin2* and *CyclinD1* (Mao et al., 2009). As expected, rcWNT3A promoted increases in *Axin2* and *CyclinD1* expression compared to CHAPS, while simva alone had negligible effects. However, the expression of these genes was greatly enhanced by the combination of simva and rcWNT3A, similar to the synergy seen with the BAR reporter. We observed a 7.7-fold increase in *Axin2* and a 5.2-fold increase in *CyclinD1* expression (Figure 1C). Thus, both BAR reporter and expression levels of β-catenin target genes show that simva enhances Wnt signaling in cells where the pathway is activated at a low level.

We then directly investigated whether dosing mice with simva via oral gavage enhances Wnt signaling in the brain. Based on our in vitro data, we focused on the DG of the hippocampus, one of the germinal brain regions where aNPCs reside and where WNT3A is secreted throughout adulthood (Garbe and Ring, 2012; Lie et al., 2005). We treated adult C57BL/6J mice harboring a reporter transgene (BAT-GAL) that drives expression of nuclear-targeted β-galactosidase (β-gal) in response to Wnt signaling (Maretto et al., 2003), and which has been used to measure Wnt signaling in the DG (Garbe and Ring, 2012; Mazumdar et al., 2010). Based on previous studies, we treated BAT-GAL mice with either 10 mg/kg simva or DMSO by oral gavage daily for 7 days (Chen et al., 2003; Karki et al., 2009). Mice were sacrificed 4 hr after the final drug treatment. We observed a ~1.4-fold increase in the density of nuclei containing β-gal in the DG of mice treated with simva (Figures 1D and 1E), while the volume of the DG was unchanged between treatment groups (not shown). To test whether these results reflect changes in expression levels of Wnt targets, we collected total RNA from the hippocampus and performed real-time PCR analysis. We measured a significant increase in the average expression of both reporter gene *LacZ* and endogenous target gene *Axin2* in mice treated with simva compared with vehicle-treated mice (Figure 1F). Together with the BAT-GAL immunostaining, these results demonstrate that systemic administration of simva enhances Wnt signaling in the DG of adult mice.

Simva Enhances Neuronal Specification via Wnt Signaling

We then investigated the effect of simva on aNPCs during differentiation. We induced differentiation in aNPCs as described previously (Luo et al., 2010; Palmer et al., 1999; Smrt et al., 2007), in the presence of drug treatment for 4 days and stained for lineage-specific markers (Figure 2A). While simva slightly reduced the total number of cells detected following differentiation (Figure 2B), simva treatment lead to a 3.6-fold increase in the number of TUJ1+ neurons and a significant decrease in the number of GFAP+ astroglial cells (Figures 2C and 2D). To further assess the effect of simva on aNPC differentiation, we measured expression of linage-specific genes using real-time PCR. mRNA levels of the neuron-specific genes *Tuj1* and *NeuroD1* were significantly increased following differentiation in aNPCs treated with simva (Figure 2E). Levels of the astroglial genes *Gfap* and *Aqp4* were lower in simva-treated aNPCs, but the difference was not statistically significant (Figure 2F). These results, consistent with previous reports of Wnt enhancement during aNPC differentiation (Luo et al., 2010), indicate that simva influences lineage specification in aNPCs toward increased production of neurons and decreased production of astroglia.

In order to test whether the effect of simva on aNPC differentiation is due to enhanced Wnt signaling, we also tested simva in combination with a Wnt pathway antagonist. For this, we employed the small molecule XAV939. Importantly, XAV939 blocks simva-mediated enhancement of Wnt signaling in aNPCs (Figure S1 available online). We reasoned that if simva enhances neuronal differentiation via enhanced Wnt signaling, then a combination of XAV939 and simva should not elicit this effect.

As expected, XAV939 treatment significantly decreased the number of neurons formed following aNPC differentiation. We found that in aNPCs treated concurrently with XAV939 and simva, the percentage of TUJ1+ cells was equivalent to DMSO-treated cells and significantly reduced as compared to cells treated with simva alone (Figures 2A and 2C). Additionally, levels of the neuronal genes *Tuj1* and *NeuroD1* were decreased with combined simva and XAV939 treatment compared with DMSO (Figure 2D), while levels of the astrocytic genes *Gfap* and *Aqp4* where not significantly changed (Figure 2E). These data demonstrate that blocking Wnt signaling abolishes the ability of simva to enhance neuronal differentiation and suggest that this effect of simva is Wnt signaling-dependent.

Simva Enhances Adult Hippocampal Neurogenesis

Having observed that simva enhances Wnt signaling in the DG of adult mice and increases neuronal specification in cultured aNPCs, we next assessed the effects of the simva treatment described above on in vivo hippocampal neurogenesis. To assess overall cell proliferation in the SGZ, we first examined the DNA replication marker MCM2 (Figure 3A). Quantification of cell numbers using confocal z stack images revealed that mice treated with simva had ~1.7-fold more MCM2+ cells per DG compared to
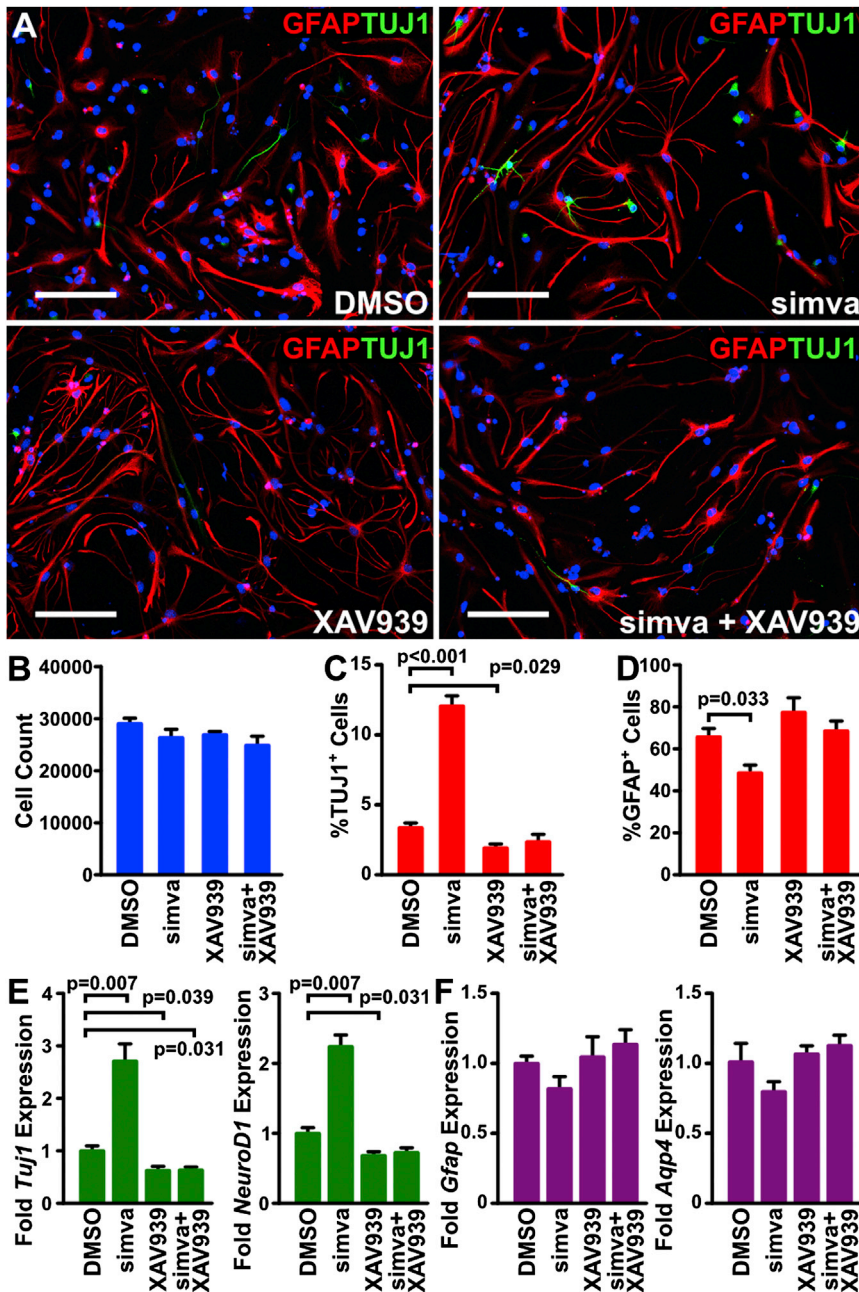
Figure 2. Simva Enhances Neuronal Specification via Wnt Signaling

(A) Staining for astroglial marker GFAP and neuronal marker TUJ1 in differentiated aNPCs treated with DMSO, 5 μM simva, 2.5 μM XAV939, or a combination of simva and XAV939, counterstained with Hoechst (scale bars, 100 μm).

(B) Cell count for each condition following differentiation.

(C) Quantification of TUJ1+ cells.

(D) Quantification of GFAP+ cells.

(E) Real-time PCR analysis of neuronal-specific genes.

(F) Real-time PCR analysis of astroglial-specific genes.

Results are presented as mean ± SEM, n = 3 independent wells (B–F). Statistical analysis as performed with the Student's unpaired t test. See also Figure S1.

control (Figure 3B). To test whether the simva-mediated increase in DG cell proliferation affects the formation of new neurons, we examined the immature neuron marker DCX. We did not find a significant difference in the number of DCX+ cells per DG for simva versus control (not shown). However, when we injected mice with a single dose of EdU (50 mg/kg) 24 hr prior to perfusion to specifically label cells in S phase toward the end of treatment, we saw that the number of DCX+/EdU+ cells per DG was increased 1.4-fold with simva treatment (Figures

3C and 3D). To test whether simva treatment affects cell survival, we counted the apoptotic nuclei within the same area of the DG. Cleaved caspase-3 (cCASP3) staining revealed no significant difference in the number of cCASP3+ cells per DG in simva-treated mice (Figure S2).

The increase in the number of proliferating cells within the SGZ that we observed as a result of simva treatment could be due to increased IPC proliferation or increased production of IPCs from radial-glia like early precursors (RGLs) (Bonaguidi et al., 2011). We looked to see what
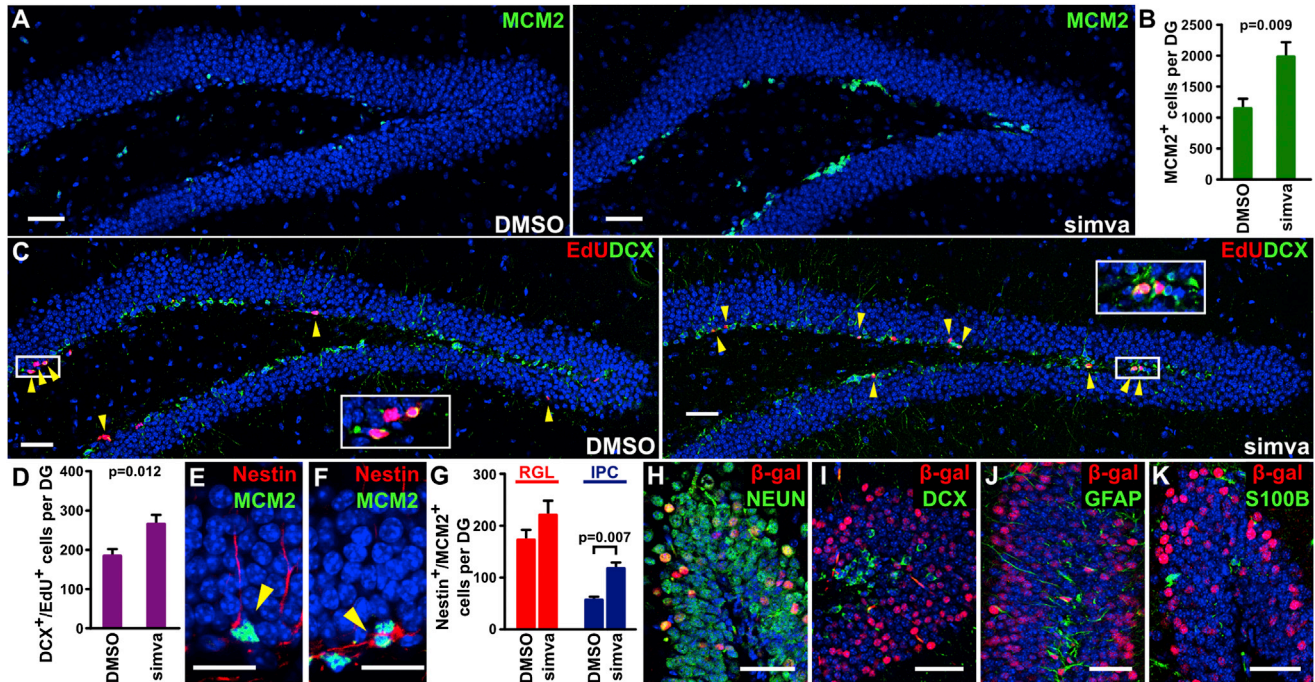
Figure 3. Simva Enhances Adult Hippocampal Neurogenesis

(A) Staining for proliferation marker MCM2 in the DG of mice treated with simva or DMSO, counterstained with DAPI (scale bars, 50 μm).

(B) Quantification of MCM2 cell number.

(C) Costaining for immature neuron marker DCX in cells labeled with a single dose of EdU 24 hr prior to sacrifice. (Insets show colocalization, yellow arrows indicate DCX$^+$/EdU$^+$ cells; scale bars, 50 μm.)

(D) Quantification of DCX$^+$/EdU$^+$ cell number.

(E) Yellow arrow: activated early precursor cell (RGL), Nestin$^+$/MCM2$^+$ with radial morphology (scale bar, 25 μm).

(F) Yellow arrow: proliferating intermediate precursor cell (IPC), Nestin$^+$/MCM2$^+$ with horizontal morphology (scale bar, 25 μm).

(G) Quantification of activated RGL and proliferating IPC cell number.

Results are presented as mean ± SEM, n = 8 mice per group, statistical analysis performed with Student's unpaired t test (B, D, and G).

(H–K) β-gal costaining with (H) mature neuron marker NEUN, (I) DCX, (J) astroglial and immature precursor marker GFAP, and (K) astrocyte marker S100B. Scale bars, 50 μm.

Counterstaining with DAPI (A, C, E, F, and H–K). See also Figure S2.

effect simva has on these populations by labeling for progenitor marker Nestin alongside MCM2. Activated RGLs, which asymmetrically divide to form new IPCs, were identified as Nestin$^+$/MCM2$^+$ with radial morphology (Figure 3E). Proliferating IPCs were identified as Nestin$^+$/MCM2$^+$ cells with horizontal morphology (Figure 3F). We observed a modest, but not statistically significant, increase the number of activated RGLs per DG in simva-treated mice. Meanwhile, the number of proliferating IPCs per DG was increased 2-fold in simva-treated mice (Figure 3G).

BAT-GAL staining revealed that Wnt signaling is active in some, but not all, of the cells in the DG of both simva- and control-treated mice (Figure 1D). To identify the cell types that exhibit active Wnt signaling following simva treatment, we costained tissue for β-gal and a panel of cell-type-specific markers. We observed that a majority of nuclear β-gal expression occurred in cells labeled with the mature neuron-specific protein NEUN in the granule cell layer (GCL) (Figure 3H), with very little β-gal in immature neurons (DCX$^+$) (Figure 3I). We saw significant β-gal expression in GFAP$^+$ cells in both the GCL and SGZ (Figure 3J). While GFAP can label both astrocytes and immature precursors, cells with nuclear β-gal expression were rarely labeled with the astrocyte marker S100B (Figure 3K).

We found that simva increases overall cell proliferation in the SGZ, leading to an increase in the number of newly formed neurons. When we looked to delineate between different progenitor pools, we found that IPC proliferation was significantly increased. Additionally, we observed Wnt reporter expression in both GFAP$^+$ and NEUN$^+$ cells following simva treatment. Taken together, these data suggest that oral simva treatment enhances adult neurogenesis in the mammalian hippocampus.
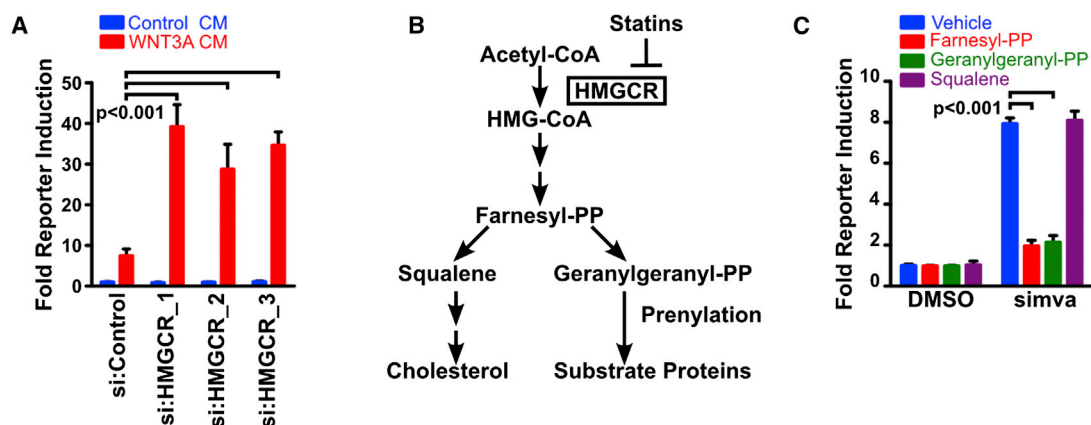
Figure 4. Simva Enhances Wnt Signaling via Depletion of Isoprenoids
(A) BAR luciferase assay in SH-SY5Y cells transfected with control (nontargeting) siRNA or one of three independent siRNAs targeting *HMGCR* and treated with WNT3A CM or control CM.
(B) The sterol biosynthetic pathway is blocked by statins, leading to depletion of isoprenoids and cholesterol.
(C) BAR luciferase assay in SH-SY5Y cells treated with simva in combination with products of the sterol biosynthetic pathway: 10 µM GGPP and 10 µM FPP, and 10 µM squalene.
Results are presented as mean ± SEM; n = 3 independent wells (A and C). Statistical analysis was performed with the Student's unpaired t test. See also Figures S3 and S4.

Simva Enhances Wnt Signaling via Depletion of Isoprenoids

Finally, we investigated the mechanism by which simva can enhance Wnt signaling. Statins antagonize HMG-CoA-reductase (HMGCR), the rate-limiting enzyme in the sterol biosynthetic pathway. This pathway is responsible for de novo synthesis of cholesterol as well as isoprenoids (Endo, 1992). To test whether inhibition of sterol biosynthesis enhances Wnt signaling, we used small interfering RNAs (siRNAs) to knock down *HMGCR* in human neuroblastoma (SH-SY5Y) cells harboring BAR driving luciferase. Similar to our aNPC data, we found that simva enhances Wnt signaling in these cells with a low dose of WNT3A conditioned media (CM), but not with control CM (Figure S3). We tested three unique siRNAs to knock down *HMGCR* (Figure S4) and a control nontargeting siRNA. Cells transfected with *HMGCR* siRNAs and treated with WNT3A CM showed significantly increased reporter induction (Figure 4A).

Downstream of HMGCR, sterol biosynthesis bifurcates to produce either the cholesterol-precursor squalene or the isoprenoids farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) (Fears, 1981) (Figure 4B). Statins, via inhibition of the sterol biosynthetic pathway, deplete cellular pools of cholesterol, FPP, and GGPP (Hughes, 1996). To test whether any of these products are involved in simva enhancement of Wnt signaling, we treated SH-SY5Y BAR cells with 5 µM simva, WNT3A CM, and exogenous FPP, GGPP, or squalene. We reasoned that if statin-mediated depletion of any of these metabolites caused the enhancement of the Wnt pathway, then adding the responsible metabolite back to the cells would inhibit the ability of simva to enhance BAR activity. Notably, addition of 10 µM GGPP or 10 µM FPP to simva-treated cells significantly reduced simva-mediated Wnt enhancement, while 10 µM squalene did not have an effect (Figure 4C). Since FPP is used to synthesize GGPP, the effect may be due to depletion of GGPP alone or a combination of FPP and GGPP depletion. Therefore, we conclude that simva enhances Wnt signaling via depletion of isoprenoids and not cholesterol.

DISCUSSION

Simva is under investigation for its potential therapeutic effects outside of hyperlipidemia treatment. While statins have been reported to enhance Wnt signaling in vitro, it was heretofore not known whether statins can enhance this pathway in vivo and in the context of neurogenesis. Here we provide evidence that oral simva treatment enhances Wnt signaling in the mammalian adult hippocampus. This is significant in that aside from lithium, no other clinically approved compound has been demonstrated to enhance Wnt signaling in the brain (Zimmerman et al., 2012).

The observations in this study are consistent with reports of increased hippocampal neurogenesis due to both simva treatment (Chen et al., 2003; Lu et al., 2007; Wu et al., 2008) and increased Wnt signaling (as cited in

introduction). Importantly, we demonstrate a link between these phenomena by probing Wnt's role in simva enhancement of neurogenesis in vitro, and subsequently investigating the effect of enhanced Wnt signaling during multiple stages of in vivo adult hippocampal neurogenesis.

While we showed a requirement for Wnt signaling in increased neuronal differentiation among simva-treated aNPCs, it remains possible that additional signaling pathways play a role in simva's effect on overall neurogenesis. Further, while others have demonstrated beneficial neurological effects of simva in disease models (as cited in introduction), the present study was performed using healthy animals and did not monitor later stages of neuronal development or behavioral outcomes.

To help map the biological connection between enhanced Wnt signaling and enhanced neurogenesis, we examined costaining of BAT-GAL with various cell-type-specific markers following simva treatment. However, a comparison showing differences in temporal expression patterns between different in vivo Wnt reporters presents a potential caveat to this approach (Garbe and Ring, 2012).

The mechanism underlying statin enhancement of Wnt signaling had not been previously reported. Providing initial insight, we show that HMGCR loss of function is sufficient to enhance the Wnt pathway. Furthermore, we demonstrate that simva acts on Wnt signaling by depleting isoprenoids, rather than through a cholesterol-dependent mechanism. Prenylation guides membrane localization of small GTPases such as RAS and RHO-associated kinases and other signaling proteins (Zhang and Casey, 1996), and serves as a regulatory mechanism for these enzymes that can be targeted therapeutically (Gelb et al., 2006). To this point, recent studies have measured an age-dependent increase of isoprenoid levels in brains of mice (Hooff et al., 2012) and have identified an overabundance of isoprenoids in the brains of AD patients (Eckert et al., 2009). The identity of the specific prenylated protein or proteins responsible for the effect of simva on the Wnt pathway remains elusive. However, there are a number of prenylated proteins known to regulate Wnt signaling (e.g., RAC1 and RHOA) that may serve as candidates for future studies (Schlessinger et al., 2009).

EXPERIMENTAL PROCEDURES

BAR Venus Experiment with aNPCs

aNPCs were plated on polyornithine- (Sigma-Aldrich) and laminin-coated (Life Technologies) optical imaging plates (Corning) in proliferation media containing FGF (Life Technologies) and epidermal growth factor (EGF; PeproTech) (see Supplemental Experimental Procedures). Drugs were added as indicated 24 hr after plating. Following 4-day drug treatment, Hoechst 33342 dye (Sigma-Aldrich) was added at 1 μg/ml.

Mouse Experiments

Eight- to 10-week-old C57BL/6J WT and BAT-GAL mice were used in this study. All animal-related procedures were approved by the Institutional Animal Care and Use Committee of the University of Washington and were conducted in accordance with the guidelines of the National Institutes of Heath. For oral gavage, simva was dissolved in DMSO and diluted in 1% carboxymethylcellulose (Sigma-Aldrich) in water. For EdU experiments, mice were injected intraperitoneally with 50 mg/kg EdU dissolved in DMSO. For details on tissue collection, see the Supplemental Experimental Procedures.

aNPC Differentiation

To induce differentiation, aNPCs were plated on polyornithine- and laminin-coated optical imaging plates with differentiation media containing 5 μM forskolin (Sigma-Aldrich) and 1 μM retinoic acid (Sigma-Aldrich) and lacking EGF and fibroblast growth factor (see Supplemental Experimental Procedures).

Antibody Staining, Imaging, and Quantification

BAR Venus aNPCs were imaged with a fluorescence microscope (Nikon), and antibody-stained aNPCs were imaged with a Nikon A1 confocal microscope. TUJ1$^+$ and GFAP$^+$ aNPCs were manually counted using ImageJ, and percentage was determined by dividing by total number of nuclei. BAT-GAL$^+$ cell density and DG volume was determined using stereology software and semiautomated counting with a fluorescence microscope. To quantify MCM2$^+$, DCX$^+$, EdU$^+$/DCX$^+$, and Nestin$^+$/MCM2$^+$ cells we collected z stacks and counted manually using ImageJ (see Supplemental Experimental Procedures).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.stemcr.2013.11.002.

REFERENCES

Biechele, T.L., Camp, N.D., Fass, D.M., Kulikauskas, R.M., Robin, N.C., White, B.D., Taraska, C.M., Moore, E.C., Muster, J., Karmacharya, R., et al. (2010). Chemical-genetic screen identifies riluzole as an enhancer of Wnt/β-catenin signaling in melanoma. Chem. Biol. 17, 1177–1182.

Bonaguidi, M.A., Wheeler, M.A., Shapiro, J.S., Stadel, R.P., Sun, G.J., Ming, G.L., and Song, H. (2011). In vivo clonal analysis

reveals self-renewing and multipotent adult neural stem cell characteristics. Cell *145*, 1142–1155.

Chen, J., Zhang, Z.G., Li, Y., Wang, Y., Wang, L., Jiang, H., Zhang, C., Lu, M., Katakowski, M., Feldkamp, C.S., and Chopp, M. (2003). Statins induce angiogenesis, neurogenesis, and synaptogenesis after stroke. Ann. Neurol. *53*, 743–751.

Eckert, G.P., Hooff, G.P., Strandjord, D.M., Igbavboa, U., Volmer, D.A., Müller, W.E., and Wood, W.G. (2009). Regulation of the brain isoprenoids farnesyl- and geranylgeranylpyrophosphate is altered in male Alzheimer patients. Neurobiol. Dis. *35*, 251–257.

Endo, A. (1992). The discovery and development of HMG-CoA reductase inhibitors. J. Lipid Res. *33*, 1569–1582.

Fears, R. (1981). The contribution of the cholesterol biosynthetic pathway to intermediary metabolism and cell function. Biochem. J. *199*, 1–7.

Garbe, D.S., and Ring, R.H. (2012). Investigating tonic Wnt signaling throughout the adult CNS and in the hippocampal neurogenic niche of BatGal and ins-TopGal mice. Cell. Mol. Neurobiol. *32*, 1159–1174.

Gelb, M.H., Brunsveld, L., Hrycyna, C.A., Michaelis, S., Tamanoi, F., Van Voorhis, W.C., and Waldmann, H. (2006). Therapeutic intervention based on protein prenylation and associated modifications. Nat. Chem. Biol. *2*, 518–528.

Hooff, G.P., Wood, W.G., Kim, J.-H., Igbavboa, U., Ong, W.-Y., Muller, W.E., and Eckert, G.P. (2012). Brain isoprenoids farnesyl pyrophosphate and geranylgeranyl pyrophosphate are increased in aged mice. Mol. Neurobiol. *46*, 179–185.

Hughes, A.D. (1996). The role of isoprenoids in vascular smooth muscle: potential benefits of statins unrelated to cholesterol lowering. J. Hum. Hypertens. *10*, 387–390.

Jang, M.-H., Bonaguidi, M.A., Kitabatake, Y., Sun, J., Song, J., Kang, E., Jun, H., Zhong, C., Su, Y., Guo, J.U., et al. (2013). Secreted frizzled-related protein 3 regulates activity-dependent adult hippocampal neurogenesis. Cell Stem Cell *12*, 215–223.

Karki, K., Knight, R.A., Han, Y., Yang, D., Zhang, J., Ledbetter, K.A., Chopp, M., and Seyfried, D.M. (2009). Simvastatin and atorvastatin improve neurological outcome after experimental intracerebral hemorrhage. Stroke *40*, 3384–3389.

Kuwabara, T., Hsieh, J., Muotri, A., Yeo, G., Warashina, M., Lie, D.C., Moore, L., Nakashima, K., Asashima, M., and Gage, F.H. (2009). Wnt-mediated activation of NeuroD1 and retro-elements during adult neurogenesis. Nat. Neurosci. *12*, 1097–1105.

Li, L., Cao, D., Kim, H., Lester, R., and Fukuchi, K. (2006). Simvastatin enhances learning and memory independent of amyloid load in mice. Ann. Neurol. *60*, 729–739.

Lie, D.-C., Colamarino, S.A., Song, H.-J., Désiré, L., Mira, H., Consiglio, A., Lein, E.S., Jessberger, S., Lansford, H., Dearie, A.R., and Gage, F.H. (2005). Wnt signalling regulates adult hippocampal neurogenesis. Nature *437*, 1370–1375.

Lin, C.-L., Cheng, H., Tung, C.-W., Huang, W.-J., Chang, P.-J., Yang, J.-T., and Wang, J.-Y. (2008). Simvastatin reverses high glucose-induced apoptosis of mesangial cells via modulation of Wnt signaling pathway. Am. J. Nephrol. *28*, 290–297.

Lu, D., Qu, C., Goussev, A., Jiang, H., Lu, C., Schallert, T., Mahmood, A., Chen, J., Li, Y., and Chopp, M. (2007). Statins increase neurogenesis in the dentate gyrus, reduce delayed neuronal death in the hippocampal CA3 region, and improve spatial learning in rat after traumatic brain injury. J. Neurotrauma *24*, 1132–1146.

Luo, Y., Shan, G., Guo, W., Smrt, R.D., Johnson, E.B., Li, X., Pfeiffer, R.L., Szulwach, K.E., Duan, R., Barkho, B.Z., et al. (2010). Fragile x mental retardation protein regulates proliferation and differentiation of adult neural stem/progenitor cells. PLoS Genet. *6*, e1000898.

Mahmood, A., Goussev, A., Kazmi, H., Qu, C., Lu, D., and Chopp, M. (2009). Long-term benefits after treatment of traumatic brain injury with simvastatin in rats. Neurosurgery *65*, 187–191, discussion 191–192.

Mao, Y., Ge, X., Frank, C.L., Madison, J.M., Koehler, A.N., Doud, M.K., Tassa, C., Berry, E.M., Soda, T., Singh, K.K., et al. (2009). Disrupted in schizophrenia 1 regulates neural progenitor proliferation via modulation of GSK3β/ß-catenin signaling. Cell *136*, 1017–1031.

Maretto, S., Cordenonsi, M., Dupont, S., Braghetta, P., Broccoli, V., Hassan, A.B., Volpin, D., Bressan, G.M., and Piccolo, S. (2003). Mapping Wnt/β-catenin signaling during mouse development and in colorectal tumors. Proc. Natl. Acad. Sci. USA *100*, 3299–3304.

Mazumdar, J., O'Brien, W.T., Johnson, R.S., LaManna, J.C., Chavez, J.C., Klein, P.S., and Simon, M.C. (2010). O2 regulates stem cells through Wnt/β-catenin signalling. Nat. Cell Biol. *12*, 1007–1013.

Palmer, T.D., Markakis, E.A., Willhoite, A.R., Safar, F., and Gage, F.H. (1999). Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. J. Neurosci. *19*, 8487–8497.

Qiao, L.J., Kang, K.L., and Heo, J.S. (2011). Simvastatin promotes osteogenic differentiation of mouse embryonic stem cells via canonical Wnt/β-catenin signaling. Mol. Cells *32*, 437–444.

Salins, P., Shawesh, S., He, Y., Dibrov, A., Kashour, T., Arthur, G., and Amara, F. (2007). Lovastatin protects human neurons against Abeta-induced toxicity and causes activation of beta-catenin-TCF/LEF signaling. Neurosci. Lett. *412*, 211–216.

Schlessinger, K., Hall, A., and Tolwinski, N. (2009). Wnt signaling pathways meet Rho GTPases. Genes Dev. *23*, 265–277.

Seib, D.R.M., Corsini, N.S., Ellwanger, K., Plaas, C., Mateos, A., Pitzer, C., Niehrs, C., Celikel, T., and Martin-Villalba, A. (2013). Loss of Dickkopf-1 restores neurogenesis in old age and counteracts cognitive decline. Cell Stem Cell *12*, 204–214.

Smrt, R.D., Eaves-Egenes, J., Barkho, B.Z., Santistevan, N.J., Zhao, C., Aimone, J.B., Gage, F.H., and Zhao, X. (2007). Mecp2 deficiency leads to delayed maturation and altered gene expression in hippocampal neurons. Neurobiol. Dis. *27*, 77–89.

Tamai, I., and Tsuji, A. (2000). Transporter-mediated permeation of drugs across the blood-brain barrier. J. Pharm. Sci. *89*, 1371–1388.

Tong, X.-K., Nicolakakis, N., Fernandes, P., Ongali, B., Brouillette, J., Quirion, R., and Hamel, E. (2009). Simvastatin improves cerebrovascular function and counters soluble amyloid-beta, inflammation and oxidative stress in aged APP mice. Neurobiol. Dis. *35*, 406–414.

Tong, X.-K., Lecrux, C., Rosa-Neto, P., and Hamel, E. (2012). Age-dependent rescue by simvastatin of Alzheimer's disease cerebrovascular and memory deficits. J. Neurosci. *32*, 4705–4715.

Wexler, E.M., Paucer, A., Kornblum, H.I., Palmer, T.D., and Geschwind, D.H. (2009). Endogenous Wnt signaling maintains neural progenitor cell potency. Stem Cells *27*, 1130–1141.

Wong, W.B., Lin, V.W., Boudreau, D., and Devine, E.B. (2013). Statins in the prevention of dementia and Alzheimer's disease: a meta-analysis of observational studies and an assessment of confounding. Pharmacoepidemiol. Drug Saf. *22*, 345–358.

Wu, H., Lu, D., Jiang, H., Xiong, Y., Qu, C., Li, B., Mahmood, A., Zhou, D., and Chopp, M. (2008). Simvastatin-mediated upregulation of VEGF and BDNF, activation of the PI3K/Akt pathway, and increase of neurogenesis are associated with therapeutic improvement after traumatic brain injury. J. Neurotrauma *25*, 130–139.

Zhang, F.L., and Casey, P.J. (1996). Protein prenylation: molecular mechanisms and functional consequences. Annu. Rev. Biochem. *65*, 241–269.

Zimmerman, Z.F., Moon, R.T., and Chien, A.J. (2012). Targeting Wnt pathways in disease. Cold Spring Harb. Perspect. Biol. *4*, 4.