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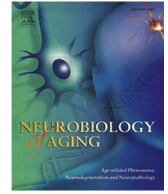

Increased bone morphogenetic protein signaling contributes to age-related declines in neurogenesis and cognition

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ABSTRACT

Aging is associated with decreased neurogenesis in the hippocampus and diminished hippocampus-dependent cognitive functions. Expression of bone morphogenetic protein 4 (BMP4) increases with age by more than 10-fold in the mouse dentate gyrus while levels of the BMP inhibitor, noggin, decrease. This results in a profound 30-fold increase in phosphorylated-SMAD1/5/8, the effector of canonical BMP signaling. Just as observed in mice, a profound increase in expression of BMP4 is observed in the dentate gyrus of humans with no known cognitive abnormalities. Inhibition of BMP signaling either by over-expression of noggin or transgenic manipulation not only increases neurogenesis in aging mice, but remarkably, is associated with a rescue of cognitive deficits to levels comparable to young mice. Additive benefits are observed when combining inhibition of BMP signaling and environmental enrichment. These findings indicate that increased BMP signaling contributes significantly to impairments in neurogenesis and to cognitive decline associated with aging, and identify this pathway as a potential druggable target for reversing age-related changes in cognition.

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1. Introduction

Many cognitive functions are well preserved into old age, but impairments in memory and the rapid use of novel information are common (Bishop et al., 2010). The dentate gyrus (DG) of the hippocampus is crucial in the integrative learning and memory functions that typically decline during aging. Increasing evidence suggests that newly generated neurons in the subgranular zone (SGZ) of the DG are vital for this cognitive functioning (Clelland et al., 2009; Deng et al., 2010; Marin-Burgin and Schinder, 2012; Ming and Song, 2011; Yassa and Stark, 2011; Zhang et al., 2008), and neurogenesis declines with age in parallel with cognitive impairments (Kuhn et al., 1996). Age-related deficits in neurogenesis and cognition can be improved with long-term exposure to running or an enriched environment (Kempermann et al., 2002; Kronenberg et al., 2006; van Praag et al., 2005). Although most enhancements in neurogenesis by environmental enrichment (EE) are linked to exercise (Kobilo et al., 2011; Mustroph et al., 2012), EE without running has been shown to increase neurotrophic factor release and enhance synaptogenesis (Birch et al., 2013; Zhao et al., 2014). Furthermore, a combination of running and EE has additive effects on neurogenesis (Fabel et al., 2009). These studies suggest that signaling pathways that are activated by exposure to exercise or to EE can reverse the age-associated decline in neural stem/progenitor cell (NSC/NPC) proliferation in the SGZ.

Bone morphogenetic protein (BMP) signaling is one of the many pathways that are regulated by exposure to running or EE (Gobeske et al., 2009). BMP signaling regulates NSC fate in both the developing and adult brain (Bond et al., 2012). In the hippocampus, BMP signaling is regulated by changes in both BMP ligands and inhibitors (Bond et al., 2014; Gobeske et al., 2009; Mira et al., 2010). BMP4 is the primary BMP family member in the adult hippocampus and noggin is its main inhibitor (Lim et al., 2000; Mikawa and Sato, 2011; Mikawa et al., 2006). BMP signaling promotes quiescence of hippocampal NSC/NPC, and inhibition of BMP signaling recruits both NSC and NPC back into cell cycle with accelerated generation of new neurons (Bond et al., 2014). The proneurogenic effect of running correlates with increased expression of noggin, decreased expression of BMP4, and an overall decrease of BMP signaling in the DG (Gobeske et al., 2009). These observations suggest a possible role for BMPs in the neurogenic deficits present in the aging brain.

Here, we identify the BMP signaling pathway as a key regulator of age-related cognitive decline and demonstrate that inhibiting

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this pathway improves neurogenesis and hippocampus-dependent cognitive function in mice. We show that there are significant age-related increases in BMP4 expression in human and mouse hippocampus suggesting that increased BMP signaling may underlie age-related changes in neurogenesis and cognition in humans as well as mice.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice were housed in groups of 3–5 per cage, except during enrichment exposures, with food and water ad libitum in a facility with constant temperature, humidity, and a 14/10-hour light/dark cycle. Experimental protocols were approved by Northwestern University's Center for Comparative Medicine and by the Institutional Animal Care and Use Committee.

2.2. Generation of transgenic mice and tamoxifen administration

Generation of BMP receptor type 2 (BMPRII) floxed mutant mice ($BMPRII^{fx/fx}$) and breeding to Ascl1-$CreER^{TM}$; $BMPRII^{flx}$; $Rosa^{zsGreen/+}$ was described previously (Beppu et al., 2005; Bond et al., 2014; Kim et al., 2011). The transgenic mice were maintained with heterozygous floxed or heterozygous wild-type BMPRII and recombined cells expressed zsGreen (ZG) fluorescent reporter. Male and female mice were used and no sex-related differences in neurogenesis were observed. 4-hydroxy-tamoxifen (Sigma Aldrich #T5648, 30 mg/mL solution in 10% ethanol and 90% corn oil) was administered at 10–12 months of age via intraperitoneal injections using an insulin syringe according to animal weight (180 mg/kg). Five consecutive daily injections were given per animal to ensure efficient recombination, and the animals were killed on the specified day after the day of initial injection. All animal procedures followed NIH guidelines and were approved by the Animal Care and Use Committee of Northwestern University.

2.3. Enrichment exposure

Up to 15 mice were housed together in special "enrichment" cages made of clear plastic (74 × 53 × 25 cm) with a wire cage top providing food and water ad libitum and allowing extensive exploratory climbing. In addition to social enhancement, enrichment objects provided mice with topological and kinesthetic diversity and were substituted piecewise with novel objects every 5–7 days. Running wheels were excluded from enrichment cages. Additional bedding material and igloo/shepherd-shack housing were also supplied (Gobeske et al., 2009).

2.4. Protein isolation and Western blotting

The DG was mechanically dissociated and lysed with T-PER Protein Extraction Reagent (Pierce) with 1× Halt Protease+Phosphotase inhibitor cocktail (Thermo Scientific). Protein samples were boiled for 10 minutes in strong denaturing conditions and 7–10 μg of sample was loaded into 4%–20% sodium dodecyl sulfate—polyacrylamide gels (Bio-Rad). Proteins were transferred onto polyvinylidene difluoride membranes at 4 °C for 1 hour, which were then blocked in tris-buffered saline with 0.1% Tween 20 with 5% nonfat dry milk or 5% bovine serum albumin for 1 hour at room temperature. Primary antibodies were diluted in blocking solution and incubated with membranes overnight at 4 °C. The membranes were incubated with horseradish peroxidase–conjugated secondary antibodies (1:2000, Santa Cruz Biotechnology) and developed using SuperSignal West Pico enhanced chemiluminescent reagent (Thermo Scientific). Primary antibodies include BMP4 (mouse IgG1, 1:1000, Origene), noggin (rabbit polyclonal, 1:4000, Chemicon), phospho-Smad1/5/8 (rabbit polyclonal, 1:1000, Chemicon), Smad1/5/8 (rabbit polyclonal, 1:1000, Cell Signaling). Specificity of the BMP4 antibody has been demonstrated previously (Masuhara et al., 1995), confirming no cross reactivity with TGFβ1 and BMP2, a BMP family member that shares over 90% identity with BMP4 (Feng et al., 1994). Densitometric analysis was performed using ImageJ software and intensity was measured relative to GAPDH unless noted.

2.5. Immunohistochemistry

Mice were killed with CO_2 inhalation, perfused with HBSS (Lonza) and fixed with 4% paraformaldehyde (PFA) in 1X PBS. Brains were fixed for 2 hours or overnight in 4% PFA and then cryoprotected in 30% sucrose solution overnight. Each brain was either frozen and sectioned into 40 μm sections on a microtome (HM 450, Microm) or embedded in Tissue-Tek OCT embedding compound (Sakura), frozen on dry ice and sliced into 20 μm sections on Leica CM3050S cryostat. Tissue samples were blocked in 1X PBS with 10% normal donkey serum or 10% normal goat serum and stained with primary antibody overnight in 1X PBS with 1% bovine serum albumin and 0.25% Triton X-100. Sections were washed with 1X PBS and treated with secondary Alexa Fluor-conjugated antibodies (Invitrogen, 1:200) and 4',6-diamidino-2-phenylindole nuclear stain (Invitrogen) for 1 hour at room temperature. Slides were washed with 1X PBS and mounted with Prolong Gold anti-fade reagent (Invitrogen). Primary antibodies used include glial fibrillary acidic protein (mouse IgG1, 1:1000, Sigma), doublecortin (DCX; goat polyclonal IgG, 1:500, Santa Cruz), bromodeoxyuridine (BrdU; mouse IgG1, 1:200, BD), chlorodeoxyuridine (CldU; rat, 1:250, Accurate), Ki67 (rabbit, 1:500, Novocastra), dsRed (rabbit, 1:1000, Invitrogen), NeuN (mouse IgG1, 1:500, Chemicon), Sox2 (polyclonal rabbit, 1:500, Chemicon), BMP4 (mouse IgG2b, 1:1000, Millipore), and BMP4 (mouse IgG1, 1:50, Origene).

2.6. Thymidine analog administration

For each experiment, either BrdU or CldU was dissolved in saline and administered via intraperitoneal injection using an insulin syringe according to animal weight. CldU was administered in a single injection 24 hours and 12 hours before killing at 42.5 mg/kg. BrdU was administered for 3 days 4 times a day and every 2 hours at 50 mg/kg. Owing to low levels of proliferation in the aged mouse brain, 3 days of BrdU was used to ensure enough cells were labeled for accurate cell counts. Before primary antibody incubation, sections stained for BrdU or CldU were incubated in 10-mM sodium citrate, 0.05% Tween 20, pH 6 for 20 minutes at 95 °C and then were allowed to cool for 30 minutes at room temperature. Sections were washed in PBS and then incubated in 2N HCl for 30 minutes at room temperature. Sections were washed in 0.1-M sodium tetraborate, pH 8.5 for 10 minutes, washed in PBS and continued normal immunohistochemistry (IHC) protocol.

2.7. Human tissue preparation

Tissue samples were graciously provided by the Northwestern Cognitive Neurology & Alzheimer's Disease Center (CNADC) Neuropathology Core. The samples all came from nondemented patients at the time of death. PFA-fixed wet human hippocampal tissue was sectioned on a microtome (HM 450, Microm) and stained as described previously. The samples were coded so that the subject was unknown during the analysis of the tissue.

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