PERSPECTIVE

Bone morphogenetic protein signaling: a promising target for white matter protection in perinatal brain injury

Prematurely born newborns, as well as those born at term, may suffer from several types of brain injury including hypoxic-ischemic injury, intracranial hemorrhage, both intraventricular and parenchymal, and injury that is the consequence of intrauterine growth restriction (IUGR). Injury of all types can impact the motor and cognitive abilities of survivors. The mechanisms leading to disability are not completely understood. Here we discuss the role of the bone morphogenetic protein (BMP) signaling pathway in newborn brain injury. We review the evidence that the BMP signaling pathway is activated in various injury types and discuss the downstream effects of its activation and possible interventions to curtail the effects of this pathway's activation. In addition, we identify interactions with other signaling pathways important in neurodevelopment.

Perinatal brain injury: The newborn period is a time of risk for neurologic injury. Newborns born at the limits of viability are at particularly high risk. In a recent study, survivors born at 23 weeks gestation had only a 17.9% rate of survival without severe neurodevelopmental impairment (NDI). Severe NDI was defined as a cognitive or motor score on the Bayley Scales of Infant and Toddler Development (Bayley III) greater than 2 standard deviations below the mean, a Gross Motor Function Classification System (GMFCS) level of 4 or 5, or bilateral blindness or deafness not corrected by bilateral amplification (Rysavy et al., 2015). For preterm newborns, NDI is often associated with intraventricular hemorrhage (IVH) and/or periventricular leukomalacia, infection, patent ductus arteriosus, chronic lung disease and necrotizing enterocolitis. In contrast, for newborns born at term, NDI is often associated with birth asphyxia or hypoxic-ischemic encephalopathy (HIE). At term, the incidence of HIE in the developed world is 1–3 per 1000 live births. Risk factors for HIE include abnormal cardiotocography, prolonged membrane rupture, thick meconium, shoulder dystocia, tight nuchal cord, sentinel events and failed vacuum delivery. The rate of death or moderate to severe brain injury following HIE ranges from 44% to 62%, depending on whether the infant received therapeutic hypothermia, with severe NDI defined similarly as for preterms except for the use of the Bayley II, a GMFCS level of 3 to 5, and deafness that can be corrected by amplification (Natarajan et al., 2014). Another clinical entity seen at term and near-term gestation is arterial ischemic stroke, which has an incidence of between 1 in 2300 to 5000 live births. Long term sequelae of arterial ischemic stroke includes cerebral palsy, attention problems, behavioral problems, speech and language delay and epilepsy (Lehman and Rivkin, 2014). There is emerging evidence that perinatal risk factors such as IUGR also result in long-term impairment in cognitive, language, and motor development in both preterm and term infants compared to their age matched counterparts. IUGR is defined as a significant reduction in fetal growth rate resulting in birth weight < 10^{th} percentile for gestational age and is estimated to occur in 5% to 7% of all pregnancies. The most common identifiable cause of IUGR is uteroplacental insufficiency. Population-based cohort studies have shown significant motor delays and as much as a 5–7 fold increased risk of developing cerebral palsy in growth restricted term infants (Levine et al., 2015). Thus newborns, both preterm and term, are a patient population particularly burdened by brain injury. For this reason, there is a high interest in developing therapeutic interventions for this patient population that will preserve as much cognitive and/or motor function as possible.

BMPs in neurodevelopment: Injury during the newborn period is unique in that it occurs during critical stages of brain development. The brain continues to develop postnatally and the normal newborn period presents a time of intense myelination of neurons. In preterms, the brain is in even earlier stages of development. It is important to note here that the newborn period represents a time when myelination normally occurs and this represents a particularly vulnerable time for the developing brain. One important pathway that, in part, regulates oligodendroglial development is the BMP pathway. BMP signaling is employed in many aspects of embryonic development, with a common theme in regulating the differentiation of progenitors. During neurodevelopment, chick, Xenopus and mouse studies have shown BMPs, members of the TGF beta protein family, to be important morphogens, especially in fate changes of progenitors to become neurons, astrocytes and oligodendrocytes. While essential during embryonic brain development, here we focus on BMP's role during later stages of fetal and neonatal life, when white matter development is occurring in earnest.

During spinal cord development, BMPs are expressed most highly along the dorsal midline, with a decreasing concentration gradient moving distally, defining specific domains. In other words, domains are defined by high, intermediate and low BMPs levels. Similarly, sonic hedgehog (Shh) is expressed most highly in the ventral midline and defines its own concentration gradient moving distally from the ventral midline. Together, these 2 gradients create a pattern from which specific cell types arise. The same blueprint operates in the development of the forebrain. As in the spinal cord, BMPs are expressed dorsally in the roof plate and Shh is expressed ventrally in the forebrain. Dorsal structures are dependent on BMP signaling whereas ventral structures are dependent on inhibition of BMP signaling by endogenous inhibitors such as noggin and chordin. In contrast to the spinal cord, BMPs decrease proliferation and increase apoptosis in embryonic forebrain explant culture (Furuta et al., 1997). This is not completely unexpected as unique combinatorial signals are observed at different locations and different time points. An example is BMP regulation of cell fate. In the fetal subventricular zone, BMP inhibits oligodendrogliogenesis and promotes astrogliogenesis synergistically with the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway (Fukuda et al., 2007). During fetal life, BMP instructs differentiation of both cortical and cerebellar neural progenitors, neuronal precursors and neurons. Later, BMP regulates neurite outgrowth, axonal guidance, dendrite morphology and synapse stabilization. When cells were cultured from rat brains at varying times of cortical development, exogenous BMP2 had different effects. Lower concentrations (1–10 ng/mL) induced neuronal and astroglial proliferation but inhibited oligodendroglial differentiation. Higher doses of BMP (100 ng/mL) reduced cell viability and oligodendroglial differentiation, while noggin promoted it. When cells were cultured from P2 brains, BMP2 and BMP7 reduced oligodendroglial differentiation and stimulated astrocyte formation. *In vivo*, enforced expression of BMP4 in mouse neurons led to marked increases in astrocytes and reduced density of oligodendrocytes (Gomes et al., 2003).

Thus, BMPs are involved in proliferation of stem cells, specification of neural stem cell lineage, then proliferation and differentiation of progenitors from all 3 lineages sequentially, first neurons, then astrocytes and oligodendrocytes.

Evidence for changes in BMP signaling in perinatal brain injury: The preponderance of evidence for changes in BMP signaling in brain injury derives from the adult stroke literature. Yet BMP signaling in perinatal brain injury has been minimally studied. Given especially the adult stroke findings, it is logical to examine BMP signaling in perinatal brain injury.

Perinatal hypoxia-ischemia: Although focal stroke is observed in the neonate, global hypoxia-ischemia (HI) is more frequently observed, and this type of injury has been the focus of our lab. Because loss of white matter and mature oligodendrocytes (OLs) following HI and blocked differentiation of oligodendroglial progenitor cells (OPCs) following recurrent HI have been observed, we have specifically investigated the role of BMP in oligodendroglial differentiation. We have shown that BMP4 expression and the downstream effector of canonical BMP signaling, SMAD 1/5/8, is increased following global perinatal HI using the Vannucci injury model in which postnatal day 7 mice are subjected to common carotid artery ligation then 8% hypoxia for 60 minutes. Given that BMPs negatively regulate the differentiation of neural stem cells into oligodendroglia, we hypothesized that downregulation of BMP signaling would result in rescue of oligodendroglia. We first tested this hypothesis using a noggin-overexpresser transgenic mouse engineered to express noggin after completion of neuronal differentiation by driving expression from the NSE promotor (NSE-noggin) and found an increase in OPCs and OLs 7 days post injury in injured NSE-noggin mice compared to injured wild type (WT) mice. In addition, we found an improvement in ambulation 14 days post injury in NSE-noggin mice compared to injured WT mice (Dizon et al., 2011). Using this strategy, it was not possible to attribute outcomes to changes in BMP signaling within oligodendroglia exclusively; outcomes could potentially be caused by changes in signaling within neurons or astrocytes alone or in combination with oligodendroglia. In addition, noggin overexpres-

sion was present prior to injury. Thus, to test the hypothesis that downregulation of BMP signaling within oligodendroglia and after injury effects rescue, we used the same injury model in mutant mice in which the BMP receptor 2 subtype is conditionally and inducibly expressed within OPCs ($NG2Cre^{ERT2}$;$BMPR2^{fl/fl}$). We found that this strategy did not increase OPCs and OLs yet still prevented loss of myelin, rescued motor function and protected the brain generally as evidenced by decreased ventriculomegaly (Dettman et al., 2018). Preliminary data from the $NG2Cre^{ERT2}$;$BMPR1a^{fl/fl}$ mouse suggests that downregulation of BMP signaling specifically through the BMPR1a receptor subtype may result in more robust rescue of oligodendroglia. Together our data suggests that increased BMP signaling associated with perinatal HI negatively impacts the survival of OPCs and OLs and suppresses the production of proteins important in myelination.

***Intraventricular hemorrhage*:** BMPs were shown to be upregulated in a model of intraventricular hemorrhage induced by intraperitoneal glycerol in rabbits. Inhibition of BMP signaling resulted in functional recovery following IVH. In this model, IVH was associated with hypomyelination and gliosis. Moreover, inhibition of BMP signaling following IVH using recombinant human noggin resulted in normalization of SMAD 1/5/8, rescue of OLs and myelin and recovery of motor function recovery (Dummula et al., 2011).

There are currently no studies examining BMP signaling in perinatal non-IVH intracranial hemorrhage or stroke, however BMP signaling has been studied extensively in adult stroke.

***Intrauterine growth restriction*:** Recent studies have shown that a significant factor in the pathogenesis of the NDI seen with IUGR is white matter injury characterized by damage to OLs, impaired myelination and astrogliosis. Major consequences of IUGR due to uteroplacental insufficiency include chronic hypoxia and induction of oxidative stress, which has been shown to inhibit oligodendroglial differentiation. Elevated BMP4 has been demonstrated in a rat model of IUGR created by bilateral ligation of the uterine artery. Moreover, it has been demonstrated that OPCs cultured postnatally from this model retained increased BMP4 expression and impaired differentiation that was reversed with the BMP inhibitor noggin (Reid et al., 2012). Our laboratory utilizes a novel *in vivo* model of IUGR that mimics preeclampsia, the most common cause of uteroplacental insufficiency and IUGR in developed countries, using a thromboxane A2 analog (Fung et al., 2011). We are currently testing if oligodendroglial and white matter loss and motor deficits occur in this model and if BMP signaling is involved.

Blood-brain barrier (BBB) permeability and BMP signaling: Intriguingly, increased signaling through BMP receptors in OPCs after brain injury may be stimulated by non-BMP ligands and by blood-derived signals. Peterson et al. (2017) found that the blood-derived coagulation factor fibrinogen deposits in the brain following BBB disruption, activating the BMP signaling pathway in OPCs and suppressing remyelination. Fibrinogen activated the phosphorylation of Smad 1/5/8, altered the expression of BMP targets such as Id1, Id2, Hes1, Hey1 and Lef1 and promoted the differentiation of OPCs into astrocytes. The authors found that fibrinogen activation of BMP signaling required the activin A type receptor 1 (ACVR1) and could be blocked by 4-[6-(4-isopropoxyphenyl) pyrazolo [1,5-a]pyrimidin-3-yl]quinoline (DMH1) (an ACVR1 inhibitor) but not noggin. While the mechanism of activation of BMP signaling appears to differ from the one that directly involves BMP4 and noggin, it has similar effects on OPCs and white matter development, mainly inhibiting OL maturation and myelination. This finding may be particularly applicable in perinatal brain injury as BBB permeability is increased in both prematurity as well as with the injury and inflammation following perinatal brain injury.

In conclusion, data from our lab and others strongly suggest that downregulation of BMP signaling demonstrates promise as a strategy to ameliorate NDI from several types of perinatal brain injury. The strength of this strategy lies in its ability to address protection at multiple points in the pathway and at multiple stages of oligodendroglial differentiation (**Figure 1**).

Jill Chang, Robert W. Dettman, Maria L.V. Dizon*
Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA (Chang J, Dizon MLV)
Stanley Manne Research Institute, Chicago, IL, USA (Dettman RW)
***Correspondence to:** *Maria L.V. Dizon, M.D., m-dizon@northwestern.edu.*
***orcid:** 0000-0002-0102-4157 (Maria L.V. Dizon)*
***Accepted:** 2018-05-22*

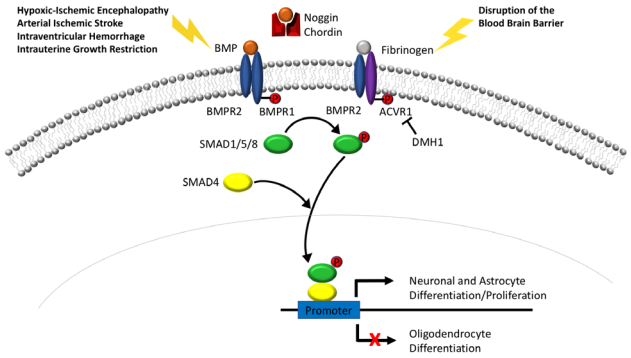

Figure 1 Activation of bone morphogenetic protein (BMP) signaling in perinatal brain injury.
Generalized scheme for activation of BMP signaling in neural cells. Brain injury can result in increased expression of BMP ligands (left) and/or disruption of the blood brain barrier (right). This activates signaling through either BMP receptors or activin A type receptor 1 (ACVR1) to increase phosphorylation of SMAD 1/5/8. Noggin/chordin can inhibit signaling through BMP receptors but not ACVR1. Signaling through ACVR1 can be inhibited by 4-[6-(4-isopropoxyphenyl)pyrazolo [1,5-a] pyrimidin-3-yl]quinoline (DMH1). Increased phospho-SMAD 1/5/8 results in increased activation of BMP response genes and alters oligodendroglial differentiation.

***doi:** 10.4103/1673-5374.235025*
***Copyright transfer agreement:** The Copyright License Agreement has been signed by all authors before publication.*
***Plagiarism check:** Checked twice by iThenticate.*
***Peer review:** Externally peer reviewed.*

References

Dettman RW, Birch D, Fernando A, Kessler JA, Dizon MLV (2018) Targeted knockdown of bone morphogenetic protein signaling within neural progenitors protects the brain and improves motor function following postnatal hypoxia-ischemia. Dev Neurosci 40:23-38.
Dizon ML, Maa T, Kessler JA (2011) The bone morphogenetic protein antagonist noggin protects white matter after perinatal hypoxia-ischemia. Neurobiol Dis 42:318-326.
Dummula K, Vinukonda G, Chu P, Xing Y, Hu F, Mailk S, Csiszar A, Chua C, Mouton P, Kayton RJ, Brumberg JC, Bansal R, Ballabh P (2011) Bone morphogenetic protein inhibition promotes neurological recovery after intraventricular hemorrhage. J Neurosci 31:12068-12082.
Fukuda S, Abematsu M, Mori H, Yanagisawa M, Kagawa T, Nakashima K, Yoshimura A, Taga T (2007) Potentiation of astrogliogenesis by STAT3-mediated activation of bone morphogenetic protein-smad signaling in neural stem cells. Mol Cell Biol 27:4931-4937.
Fung C, Brown A, Cox J, Callaway C, McKnight R, Lane R (2011) Novel thromboxane A2 analog-induced IUGR mouse model. J Dev Orig Health Dis 2:291-301.
Furuta Y, Piston DW, Hogan BL (1997) Bone morphogenetic proteins (BMPs) as regulators of dorsal forebrain development. Development 124:2203-2212.
Gomes WA, Mehler MF, Kessler JA (2003) Transgenic overexpression of BMP4 increases astroglial and decreases oligodendroglial lineage commitment. Dev Biol 255:164-177.
Lehman LL, Rivkin MJ (2014) Perinatal arterial ischemic stroke: presentation, risk factors, evaluation, and outcome. Pediatr Neurol 51:760-768.
Levine TA, Grunau RE, McAuliffe FM, Pinnamaneni R, Foran A, Alderdice FA (2015) Early childhood neurodevelopment after intrauterine growth restriction: a systematic review. Pediatrics 135:126-141.
Natarajan G, Shankaran S, Pappas A, Bann C, Tyson JE, McDonald S, Das A, Hintz S, Vohr B, Higgins R; Extended Hypothermia Subcommittee of the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network (2014) Functional status at 18 months of age as a predictor of childhood disability after neonatal hypoxic-ischemic encephalopathy. Dev Med Child Neurol 56:1052-1058.
Petersen MA, Ryu JK, Chang KJ, Etxeberria A, Bardehle S, Mendiola AS, Kamau-Devers W, Fancy SPJ, Thor A, Bushong EA, Baeza-Raja B, Syme CA, Wu MD, Rios Coronado PE, Meyer-Franke A, Yahn S, Pous L, Lee JK, Schachtrup C, Lassmann H, et al. (2017) Fibrinogen activates BMP signaling in oligodendrocyte progenitor cells and inhibits remyelination after vascular damage. Neuron 96:1003-1012.e7.
Reid MV, Murray KA, Marsh ED, Golden JA, Simmons RA, Grinspan JB (2012) Delayed myelination in an intrauterine growth retardation model is mediated by oxidative stress upregulating bone morphogenetic protein 4. J Neuropathol Exp Neurol 71:640-653.
Rysavy MA, Li L, Bell EF, Das A, Hintz SR, Stoll BJ, Vohr BR, Carlo WA, Shankaran S, Walsh MC, Tyson JE, Cotten CM, Smith PB, Murray JC, Colaizy TT, Brumbaugh JE, Higgins RD; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network (2015) Between-hospital variation in treatment and outcomes in extremely preterm infants. N Engl J Med 372:1801-1811.