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Region specific oligodendrocyte transcription factor expression in a model of neonatal hypoxic injury

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ABSTRACT

White matter injury (WMI) of prematurity is associated with a spectrum of neurological disorders ranging from mild cognitive and behavioral deficits to cerebral palsy. Translational studies have implicated impaired oligodendrocyte development after hypoxia as the primary cause of WMI, but the underlying mechanisms remain poorly understood. The goal of this study was to identify alterations in the expression of oligodendrocyte precursor cell transcription factors in a mouse model of transient mild global hypoxia.

Postnatal day (P) 7 mouse pups were exposed to hypoxia (7.5% O_2) for 60 minutes. We compared oligodendrocyte differentiation and subsequent myelin formation between hypoxia and sham animals at P9, P14 and P28 by examining the expression of key transcription factor regulators of oligodendrocyte differentiation (Ascl1, Olig1, Olig2, and Nkx2.2), as well as APC, a mature oligodendrocyte marker, in the major white matter regions including the corpus callosum, external capsule and anterior commissure. We also examined the effect on myelin formation by examining two myelin specific protein constituents, myelin associated glycoprotein (MAG) and myelin basic protein (MBP), in white matter tracts and whole brain lysate respectively.

We found that transient hypoxia at P7 altered the expression of Ascl1, Olig1 and Nkx2.2, resulting in delayed myelination in the external capsule. In addition, our study showed that oligodendrocyte progenitor cells specified several days prior to a hypoxic event are more susceptible to maturation arrest than those specified shortly prior to hypoxia. Our results suggest that alterations of Ascl1, Olig1 and Nkx2.2 underlie impaired oligodendrocyte differentiation and deficient myelination in WMI. These transcription factors are potential therapeutic targets for the treatment of WMI in preterm infants.

1. Introduction

Survival rates for very low birth weight and extremely low birth weight infants have risen significantly in the past decade (Hamilton et al., 2015). Along with this increase in survival, the incidence of various chronic neurologic diseases in premature infants, ranging from cognitive deficits and learning disabilities to epilepsy and cerebral palsy, have also increased dramatically (Allen, 2008; Allin et al., 2011; Northam et al., 2011; Stephens and Vohr, 2009). Over time, these chronic neurologic deficits represent a major public health burden as the costs related to preterm birth-related disabilities increase with higher survival rates (Clements et al., 2007; Petrou et al., 2003; Russell et al., 2007). Recent studies have identified white matter injury (WMI) as a predominant pathological feature associated with chronic neurologic deficits due to premature birth. Such WMI encompasses a broad spectrum of lesions ranging from cystic periventricular leukomalacia to non-cystic (diffuse or focal) hypomyelination. In recent years, non-cystic periventricular leukomalacia has become the most predominant form of WMI in preterm newborns (Khwaja and Volpe, 2008; Volpe, 2001, 2009).

Animal studies have demonstrated that developing oligodendrocytes are particularly susceptible to various brain insults, suggesting that oligodendrocyte differentiation arrest is the main mechanism of WMI (Back et al., 2002; Back et al., 2007; Buser et al., 2012; Segovia et al., 2008). Several hypotheses about the mechanisms of oligodendrocyte differentiation arrest and/or attenuation have been reported, including increased oxidative damage and activation of toxic inflammatory processes (Akundi and Rivkees, 2009; Back et al., 2002; Back

Abbreviations: AC, anterior commissure; CC, corpus callosum; EC, external capsule; MAG, myelin associated glycoprotein; MBP, myelin basic protein; OPC, oligodendrocyte progenitor cells; WMI, white matter injury

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et al., 2007; Brazel et al., 2004; French et al., 2009; Rice et al., 1981); however the specific molecular mechanisms that impede oligodendrocyte differentiation programs after hypoxic injury have not been fully uncovered.

There is a correlation between the development of oligodendrocyte lineage in mice and the development of oligodendrocytes in humans, making rodents useful models to study WMI in preterm infants. In terms of oligodendrocyte development and myelination, postnatal day (P) 7 in mice roughly corresponds to a 30–36 week gestational age infant (Craig et al., 2003). Classical animal studies have primarily used a model of injury that combines hypoxia and ischemia. This approach results in massive neuronal loss due to necrosis and apoptosis, in addition to inflammation and subsequent axonal disintegration that obscures distinctions of primary and secondary damage in the white matter (Brazel et al., 2004; Kaindl et al., 2009; Rice et al., 1981; van der Kooij et al., 2009; Vannucci, 1990; Vannucci and Vannucci, 2005). By contrast, the use of global hypoxia as a model of injury seeks to reduce many of the complex pathological changes caused by ischemia in order to clarify the molecular pathways involved in the arrest or attenuation of oligodendrocyte development in a less biased environment. Specifically, models of acute systemic hypoxia (Strasser et al., 2016; Trollmann et al., 2014) and chronic hypoxia have been able to elicit WMI similar to that observed in preterm infants, interestingly, changes in these models are characterized by differentiation abnormalities rather than decreased cell viability (Buser et al., 2012; Jablonska et al., 2012; Scafidi et al., 2014; Segovia et al., 2008).

Oligodendrocyte differentiation and myelination are complex processes. Several transcription factors have proven to be crucial for proliferation, migration and differentiation in oligodendrocyte lineage cells. Ascl1 is a pivotal basic helix-loop-helix (bHLH) transcription factor for neuronal and oligodendrocyte generation and differentiation (Guillemot and Joyner, 1993). In fact, Ascl1 is one of the first transcription factors that is expressed in specified oligodendrocyte progenitor cells (OPCs). Several studies have placed Ascl1 as a key regulatory transcription factor involved in myelination and remyelination in the embryonic and adult brain (Kim et al., 2008; Kim et al., 2007; Nakatani et al., 2013; Parras et al., 2004; Parras et al., 2007). Ascl1 exhibits bimodal expression patterns with its initial expression during oligodendrocyte specification and its later reappearance that coincides with the start of myelin gene expression. In a knockout model, Ascl1 absence in the telencephalon decreases the number of OPCs in the white matter, emphasizing the importance of this transcription factor in myelination. Furthermore, Ascl1 is also required for OPC proliferation in remyelination (Nakatani et al., 2013; Sugimori et al., 2007; Vue et al., 2014).

Other transcription factors are active during oligodendrocyte differentiation and may be regulated by the expression of Ascl1 (Ligon et al., 2006; Sugimori et al., 2008; Wang et al., 2006). The bHLH factors Olig1 and Olig2 are crucial for oligodendrocyte differentiation and maturation, and their expression and subcellular localization are indicators of specific developmental stages of OPC development. Olig2 is crucial for oligodendrocyte lineage specification and is highly preserved during development (Zhu et al., 2012). Olig1 is essential for OPC maturation in the cerebrum, and its expression correlates with the onset of myelination. In addition, the homeodomain transcription factor Nkx2.2 plays an important role during differentiation of oligodendrocytes, whereas its ablation delays oligodendrocyte maturation; its interaction with Olig2 and Olig1 has been reported in previous studies (Cai et al., 2010; Okahara et al., 2014; Othman et al., 2011; Sugimori et al., 2007; Zhu et al., 2014). Subsets of studies have demonstrated that Ascl1 plays a crucial role in specification and differentiation of oligodendrocytes through the regulation of Nkx2.2 and Olig1 (Nakatani et al., 2013; Parras et al., 2004; Parras et al., 2007; Sugimori et al., 2008). It has been proposed that there are changes to the expression of these transcription factors following ischemia and stroke models (Iwai et al., 2010; McIver et al., 2010; Zhang et al., 2011), but there has not been a study of how these transcription factors on oligodendrocyte differentiation are altered following mild global hypoxia.

In this study, we sought to investigate the effect of mild transient global hypoxia utilizing the expression of a select group of transcription factors Ascl1, Olig2, Olig1 and Nkx2.2 as possible mechanisms for oligodendrocyte differentiation arrest. We also sought to identify the periods of increased susceptibility to hypoxia in the oligodendrocyte precursor subpopulations that may lead to a delay in differentiation through the use of a fate-mapping transgenic animal model. Elucidating specific molecular targets and susceptible periods involved in the pathogenesis of oligodendrocyte developmental arrest following hypoxia may reveal possible therapeutic targets for the prevention and treatment of WMI in preterm infants.

2. Materials and methods

2.1. Ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All procedures used were approved by Loma Linda University Institutional Animal Care and Use Committee (IACUC # 8120036).

2.2. Animals

Animals were initially housed at Cincinnati Children's Hospital Medical Center where naïve animals were characterized; subsequently breeding pairs were then moved and housed under the same conditions at the Loma Linda University Animal Care facility with water and food *ad libitum* on a 12 h light/dark cycle. Pups were housed with their dams until sexual maturity (P21); they were then separated by sex in an appropriately sized cage.

Ascl1-GFP transgenic (Tg) mice (AU176Gsat/Mmnc, identification number 000295-UNC) were obtained from the Mutant Mouse Regional Resource Center, a NIH funded strain repository, and were donated to the MMRRC by Nathaniel Heintz, Ph.D. The Rockefeller University, GENSAT (Schmidt et al., 2013). These animals were backcrossed to a CD1 background. In addition, transgenic animals were identified by PCR analysis using tail/ear DNA with the following primers: CACATGAAGCAGCACGACTT; AGTTCACCTTGATGCCGTTC.

Ascl1-CreER BAC (CAG-GAT-EGFP) mice (Battiste et al., 2007) were generously provided by Dr. Jane Johnson (University of Texas Southwestern); Transgenic animals were identified by PCR analysis using tail/ear punch DNA with primers to *Cre*: ggacatgttcagggatcgccaggcg; gcataaccagtgaaacagcattgctg. Tamoxifen (Sigma T5648) dissolved in corn oil was used for recombination at P5 by an intraperitoneal injection at a dose of 3 mg/20 g of body weight.

2.3. Hypoxia

To achieve hypoxia, we placed P7 pups for both transgenic lines (Ascl1-GFP and Ascl1-CreER BAC) in a glass chamber (submerged in a 37 °C water bath to maintain normothermia) through which a humidified mixture of oxygen (7.5% during 60 min) and balanced nitrogen flowed at 3 L/min. Sham littermates were also taken from the dam and placed in the opened glass chamber for the same amount of time. Both hypoxia and sham littermates were returned to their dams until the designated harvest points. All comparisons of transcription factors were made between shams and animals receiving hypoxia. Four to six animals were analyzed for each time point and condition, unless there were poor-quality sections or inadequate staining where the region of interest was not sufficiently visualized, for which the slide was excluded from analysis.