Hypoxia Epigenetically Confers Astrocytic Differentiation Potential on Human Pluripotent Cell-Derived Neural Precursor Cells

Tetsuro Yasui,1,2 Naohiro Uezono,1 Hideyuki Nakashima,1 Hirofumi Noguchi,1 Taito Matsuda,1 Tomoko Noda-Andoh,3 Hideyuki Okano,3 and Kinichi Nakashima1,4,*
1Department of Stem Cell Biology and Medicine
2Department of Otorhinolaryngology
Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Fukuoka 812-8582, Japan
3Department of Physiology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
4Laboratory of Molecular Neuroscience, Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara 630-0192, Japan
*Correspondence: kin1@scb.med.kyushu-u.ac.jp
http://dx.doi.org/10.1016/j.stemcr.2017.05.001

SUMMARY

Human neural precursor cells (hNPCs) derived from pluripotent stem cells display a high propensity for neuronal differentiation, but they require long-term culturing to differentiate efficiently into astrocytes. The mechanisms underlying this biased fate specification of hNPCs remain elusive. Here, we show that hypoxia confers astrocytic differentiation potential on hNPCs through epigenetic gene regulation, and that this was achieved by cooperation between hypoxia-inducible factor 1α and Notch signaling, accompanied by a reduction of DNA methylation level in the promoter region of a typical astrocyte-specific gene, Glial fibrillary acidic protein. Furthermore, we found that this hypoxia culture condition could be applied to rapid generation of astrocytes from Rett syndrome patient-derived hNPCs, and that these astrocytes impaired neuronal development. Thus, our findings shed further light on the molecular mechanisms regulating hNPC differentiation and provide attractive tools for the development of therapeutic strategies for treating astrocyte-mediated neurological disorders.

INTRODUCTION

The mammalian CNS is composed mainly of three neural cell types, neurons, astrocytes, and oligodendrocytes, all of which are generated from common multipotent neural precursor cells (NPCs) (Namihira and Nakashima, 2013; Svendsen et al., 1998). With recent advances in stem cell culture techniques, NPCs derived from human pluripotent stem cells (hPSCs), and embryonic and induced pluripotent stem cells (hESCs and hiPSCs), have been shown to recapitulate neural development to some extent in vitro (Takahashi et al., 2007; Thomson et al., 1998; Yu et al., 2007) and to serve as a model for various neurological disorders (Israel et al., 2012; Marchetto et al., 2011; Park et al., 2008; Sanchez-Danes et al., 2012). However, although human NPCs (hNPCs) derived from hPSCs differentiate efficiently into neurons, an extremely low fraction of them generate astrocytes over a period of 4 weeks after the induction of differentiation (Hu et al., 2010). Recent studies have shown that hNPCs require prolonged culture (typically around 100–200 days) under sphere-forming conditions to efficiently differentiate into astrocytes (Edri et al., 2015; Kondo et al., 2013; Krencik et al., 2011; Williams et al., 2014), thus retarding human astrocyte functional research that is relevant to neurological diseases.

Constituting about 40% of all cells in the brain, astrocytes have long been classified as mere passive supporting cells that, for example, promote survival and functional synaptic formation of neurons; however, astrocytes are also essential for the phagocytic elimination of synapses, which refines neuronal circuit development (Allen and Barres, 2009). Because these roles of astrocytes are very important for brain function, astrocytes are indispensable components in CNS integrity (Allen et al., 2012; Christopherson et al., 2005; Hamilton and Attwell, 2010; Haydon and Nedergaard, 2015; Kucukdereli et al., 2011; Molofsky et al., 2012; Ullian et al., 2001; Zhang et al., 2016). Therefore, astrocyte dysfunction is thought to be implicated in various neurological disorders including Rett syndrome (RTT), which is caused by methyl-CpG binding protein 2 (MECP2) mutations (Amir et al., 1999; Bienvenu and Chelly, 2006; Tsujimura et al., 2015). Mutant (MT) MECP2-expressing astrocytes derived from RTT-hiPSCs have recently been reported to have adverse effects on neuronal maturation compared with their isogenic wild-type (WT) MECP2-expressing astrocytes (Williams et al., 2014). However, little progress in human astrocyte functional analysis has been made because, as noted above, differentiation of hPSC-derived hNPCs into astrocytes is a time-consuming process.

The interleukin-6 family of cytokines, including leukemia inhibitory factor (LIF), are well known to efficiently induce astrocytic differentiation of late-gestational (lg) NPCs by activating the janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling
pathway (Bonni et al., 1997; Nakshima et al., 1999; Weible and Chan-Ling, 2007). However, these cytokines are incapable of inducing astrocytic differentiation of mid-gestational (mg)NPCs because astrocytic genes, such as Glial fibrillary acidic protein (GFAP), are silenced by DNA methylation (Fan et al., 2005; Takizawa et al., 2001). Thus, mgNPCs have a strong tendency to differentiate into neurons rather than astrocytes. mgNPCs prepared from embryonic day 11 (E11) mouse telencephalon can be induced with moderate efficiency to differentiate into astrocytes after culturing for 4 days (nominally corresponding to E15), while astrocytic differentiation is effectively induced in IgNPCs prepared directly from E15 mouse telencephalon. We have previously shown that this weaker acquisition of astrocytic differentiation potential by mgNPCs cultured in dishes is due to the high oxygen level compared with that in vivo (Mutoh et al., 2012). The atmosphere contains 21% O2 (160 mm Hg), whereas interstitial oxygen concentration ranges from 1% to 5% (7–40 mm Hg) in mammalian tissues including the embryonic brain (Mohyeldin et al., 2010; Simon and Keith, 2008). Thus, 21% O2 (atmospheric) is actually physiologically abnormal in vivo; however, because cell cultures are generally conducted in 21% O2, and it is common to define atmospheric O2 concentration as normoxia, we refer to 21% O2 as normoxia in this study. Notably, when we cultured E11 mgNPCs for 4 days under hypoxia (2% O2), the cells differentiated efficiently into astrocytes, to a level comparable with that of E15 IgNPCs. We also revealed that demethylation of Gfap in mgNPCs is enhanced in hypoxic culture compared with that in normoxia (21%) (Mutoh et al., 2012).

Given these findings, we hypothesized that the inefficient astrocytic differentiation of hPSC-derived hNPCs is due to a retarded or suspended transition from mid- to late-gestational stages of NPC development, so that hypoxia should confer astrocytic differentiation potential on hNPCs as we observed in mouse mgNPCs. We therefore cultured hPSC-derived hNPCs under hypoxic conditions and found that this is indeed the case. The hNPCs differentiated rapidly (within 4 weeks) into astrocytes, and this was inversely correlated with the methylation status of the GFAP promoter. We also show that conferral of astrocytic differentiation potential on the hNPCs is achieved by a collaboration between hypoxia-inducible factor 1α (HIF1α) and Notch signaling. Furthermore, we show that astrocytes derived from RTT-hiPSCs using our method impair aspects of neuronal development such as neurite outgrowth and synaptic formation, indicating that our protocol will accelerate investigations of the functions of neurological disorder-relevant astrocytes in vitro.

RESULTS

Astrocytic Differentiation Potential of hNPCs Is Inversely Correlated with DNA Methylation Status in the GFAP Promoter

We first re-examined the differentiation tendencies of four hNPC lines established from hiPSCs (AF22 and AF24), hESCs (AF23) (Falk et al., 2012), and human fetal brain (CB660) (Sun et al., 2008) by immunocytochemistry with antibodies against the neuron and astrocyte markers tubulin β 3 class III (TUBB3) and GFAP, respectively. Whereas fetal brain-derived CB660 could efficiently differentiate into both TUBB3-positive neurons and GFAP-positive astrocytes after a 4-week differentiation period, the astrocyte population was extremely low in AF22 and AF23 (Figures 1A and 1B). Moreover, only a small fraction of AF22 and AF23 differentiated into astrocytes even when stimulated with LIF, which activated STAT3 in these cells (Figures S1A and S1B). Interestingly, AF24 (hNPCs established from CB660-derived hiPSCs) also barely differentiated into astrocytes even in the presence of LIF (Figures 1A, 1B, S1A, and S1B). These results suggest that the capacity to differentiate into astrocytes is restricted in hNPCs if they are derived from hPSCs, regardless of the properties of the original cells. Since it has been shown that mouse mgNPCs have a limited astrocytic differentiation potential due to the hyper-methylation status in astrocytic gene promoters (Namihira et al., 2009; Takizawa et al., 2001), we next examined the methylation status of the GFAP promoter as a representative gene promoter in these cells (Figure 1C). Bisulfite sequence analysis revealed a high-methylation status for the GFAP promoter in AF22, 23, and 24 but not in CB660 (Figures 1D and 1E). These methylation statuses were inversely correlated with the astrocytic differentiation ability of each cell line (Figures 1B and 1E).

Hypoxia Increases Astrocytic Differentiation of hNPCs in Association with Demethylation of the GFAP Promoter

hNPCs with low astrocytic differentiation potential (AF22, 23, and 24) were all established from hPSCs, and had never been exposed to hypoxia during or after their establishment (Falk et al., 2012). In contrast, CB660 hNPCs were prepared directly from a human fetal brain around gestational week 8 (Sun et al., 2008), indicating that they had been under hypoxia until at least this time because embryonic tissues including brain are in hypoxic conditions (Mohyeldin et al., 2010; Simon and Keith, 2008). Since we have previously shown that hypoxia confers astrocytic differentiation potential on mouse mgNPCs (Mutoh et al., 2012), we speculated that the difference in astrocytic differentiation between these hNPCs is attributable to their exposure...
دریافت فوری متن کامل مقاله

امکان دانلود نسخه تمام متن مقالات انگلیسی
امکان دانلود نسخه ترجمه شده مقالات
پذیرش سفارش ترجمه تخصصی
امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
امکان دانلود رایگان ۲ صفحه اول هر مقاله
امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
دانلود فوری مقاله پس از پرداخت آنلاین
پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات