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Olig2 Targets Chromatin Remodelers To Enhancers To Initiate Oligodendrocyte Differentiation

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Summary

Establishment of oligodendrocyte identity is crucial for subsequent events of myelination in the central nervous system (CNS). Here, we demonstrate that activation of ATP-dependent SWI/SNF chromatin-remodeling enzyme Smarca4/Brg1 at the differentiation onset is necessary and sufficient to initiate and promote oligodendrocyte lineage progression and maturation. Genome-wide multistage studies by ChIP-seq reveal that oligodendrocyte-lineage determination factor Olig2 functions as a pre-patterning factor to direct Smarca4/Brg1 to oligodendrocyte-specific enhancers. Recruitment of Smarca4/Brg1 to distinct subsets of myelination regulatory genes is developmentally regulated. Functional analyses of Smarca4/Brg1 and Olig2 co-occupancy relative to chromatin epigenetic marking uncover novel stage-specific cis-regulatory elements that predict sets of transcriptional regulators controlling oligodendrocyte differentiation. Together, our results demonstrate that regulation of the functional specificity and activity of a Smarca4/Brg1-dependent chromatin-remodeling complex by Olig2, coupled with transcriptionally-linked chromatin modifications, is critical to precisely initiate and establish the transcriptional program that promotes oligodendrocyte differentiation and subsequent myelination of the CNS.

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Introduction

Oligodendrocyte, the specialized cell type for myelination in the central nervous system (CNS), plays an important role in brain development and neuronal function. Its development comprises a stepwise process including commitment of oligodendrocyte precursor cells (OPCs) from multipotent neural progenitors, OPC differentiation into immature and mature myelinating oligodendrocytes (Pfeiffer et al., 1993). Failure of remyelination by oligodendrocytes due to injury or disease impairs rapid propagation of action potentials and leads to nerve degeneration, and is associated with demyelinating disorders such as multiple sclerosis (MS) and leukodystrophies (Berger et al., 2001; Trapp et al., 1998). The observation that OPCs are present within demyelinating MS lesions, but fail to differentiate into myelinating cells, suggests that induction of OPC differentiation is a critical event for successful remyelination (Chang et al., 2002; Franklin and Ffrench-Constant, 2008).

How then is the transcriptional program responsible for precisely initiating oligodendrocyte differentiation in a temporally specific manner? Oligodendrocyte development depends on stage-specific transcription factors, including Olig2, Sox10, YY1, Olig1, MRF and Zfp191, as well as small non-coding RNAs such as miR-219 (Emery, 2010; Li et al., 2009). For instance, the basic helix-loop-helix (bHLH) transcription factor Olig2 functions as an oligodendrocyte lineage determination factor by controlling OPC specification, differentiation and myelination (Lu et al., 2002; Yue et al., 2006; Zhou and Anderson, 2002). However, whether this list is complete, and how these factors work remains unclear since very few oligodendrocyte-specific cis-regulatory elements have been identified and functionally dissected to fully elucidate the control of their target gene expression. Identification of lineage-specific cis-regulatory elements, in conjunction with the cognate transcription factors that also change at crucial transitions of differentiation, would help to narrow the search for the key regulators of oligodendrocyte development.

Differentiation from OPCs to mature oligodendrocytes was shown to undergo rapid and substantial nuclear and chromatin reorganization (Nielsen et al., 2002), suggesting that chromatin reorganization is crucial for the onset of differentiation. There are two major classes of enzymes that remodel chromatin: enzymes that covalently modify histones and enzymes of ATP-dependent remodeling complexes (Becker and Horz, 2002; Haberland et al., 2009; Jenuwein and Allis, 2001). For instance, mammalian SWI/SNF like Brg1/Brm associated factors (BAF) chromatin-remodeling complexes are able to use energy derived from ATP hydrolysis to alter chromatin structures and regulate nuclear processes such as transcription (Wu et al., 2009; Yoo and Crabtree, 2009). At present, however, the role and functional specificity of chromatin-remodeling enzymes during CNS myelination remains elusive.

To investigate gene activation events during the onset and progression of OPC differentiation, we performed whole-genome chromatin immunoprecipitation-sequencing (ChIP-Seq) to map the global gene transcripts that are targeted and activated by RNA polymerase II (RNAPII) at different stages. We identified that the Smarca4/Brg1 gene, encoding the central catalytic ATPase subunit of the SWI/SNF chromatin-remodeling complex, is most significantly targeted by RNAPII at the onset of OL differentiation. Remarkably, we found that the Brg1 chromatin remodeler is transcriptionally pre-patterned by Olig2 to achieve the target binding specificity and is both necessary and sufficient to activate expression of myelination-associated genes and oligodendrocyte lineage progression. In coupling with epigenetic histone mark signatures, Brg1 and Olig2 co-occupancy further predicts and reveals novel lineage specific enhancer elements and critical regulators for oligodendrocyte differentiation at a genome-wide level. Our studies uncover a dynamic transformation in transcription and epigenetic marking that occur during

oligodendrocyte lineage progression, provide a genome-wide view of oligodendrocyte development in unusually fine resolution, and unmask an unanticipated mechanism wherein activation of ATP-dependent chromatin remodeling complexes and regulation of Brg1 functional specificity by Olig2 coupled with transcriptionally-linked chromatin modifications are critical steps to precisely initiate and establish the transcriptional program for promoting oligodendrocyte differentiation.

Results

Genome-wide mapping of RNA polymerase II targeting reveals activation of Smarca4/Brg1 at the onset of oligodendrocyte differentiation

To identify the genes that are activated during the transition of OPC differentiation, we performed ChIP-seq to determine genome-wide RNAPII binding targets at different phases of differentiation. RNAPII occupancy throughout the entire gene body provides a direct readout of transcriptional activity, particularly for genes rapidly responding to developmental and extrinsic cues, and thus yields insights beyond what is typically achieved by mRNA expression profiling (Adelman and Lis, 2012; Hah et al., 2011). OPCs isolated from neonatal rats were cultured in differentiation medium (Chen et al., 2007) for one day to initiate the OPC differentiation process (Figure 1A). Expression of the early differentiation marker *Cnp* increased significantly with modest expression of mature myelin genes such as *Mag, Mbp* and *Plp*. The cells at this differentiation initiation stage were designated as immature oligodendrocytes (iOL). In contrast, four days after treatment, the cells robustly expressed myelin genes including *Mbp, Plp, Mag* and *Cnp* and became mature oligodendrocytes (designated as mOL) (Figure 1A, B).

By analyzing RNAPII targeting in OPC and iOL, we observed a significant enrichment of target genes linked to chromatin remodeling activities during the transition from OPC to iOL, including nucleosome assembly, positioning and chromatin reorganization (Figure 1C; Table S1 and Dataset S1). These data suggest that the initiation of oligodendroglial differentiation involves extensive chromatin remodeling and reorganization of the transcriptional machinery.

Strikingly, the most prominent and significant RNAPII binding target in iOL cells determined by *p*-value and FDR (false discovery rate <0.05) were mapped to the *Smarca4*/*Brg1* locus including intragenic exons and 3′ UTR regions (Figure 1D). An extensive and significant enrichment of RNAPII occupancy or pausing on the *Brg1* gene body across mainly the exons was detected in iOLs over OPCs (Figure 1D). *Brg1* was actively transcribed and upregulated in iOL cells in response to differentiation cues (Figure 1D, E), consistent with the notion that RNAPII enrichment and accumulation throughout the gene body facilitate rapid or synchronous activation of gene expression in response to signaling cues (Adelman and Lis, 2012). Further analysis of the binding targets found that RNAPII targeting on the locus of other SWI/SNF complex subunits including *BAF45b*, *BAF45d, BAF250b* and *BAF60a* (Wu et al., 2009) also increased from OPC to iOL (Figure 1D). Since RNAPII ChIP-seq could capture both actively transcribed and paused genes, we then performed qRT-PCR and Western blot analysis to confirm upregulation of RNAPII targeted Brg1 SWI/SNF complex components during OPC to iOL transition (Figure 1E, G). These findings indicate that expression of the SWI/SNF remodeling complex is activated at the onset of oligodendrocyte differentiation. In contrast, RNAPII occupancy on the locus of Brm1, the only homolog of Brg1 ATPase, was comparable between iOLs and OPCs (Figure 1F). Such a phenomenon of Brg1 activation was not observed in other cell types, including the change from mouse embryonic stem cells to embryonic fibroblasts (Min et al., 2011), or neurons in response to neuronal activity (Kim et al., 2010), or human breast cancer cells (MCF) in response to estrogen (Hah et al., 2011)(Figure S1). These observations suggest

that activation of the Brg1 chromatin-remodeling complex is a unique event to initiate the critical transition of OPC differentiation.

Brg1 is required and sufficient for the initiation of OPC differentiation and maturation

To determine the developmental state of Brg1+ cells in the oligodendrocyte lineage, we co-labeled Brg1 with the stage-specific markers for differentiated oligodendrocytes (CC1+) or their precursors (PDGFRα+) in the spinal cord. Brg1 was strongly expressed in CC1+ differentiated oligodendrocytes but weakly in PDGFRα+ OPCs (Figures 2A, B and S2). The proportion of CC1+ among Brg1+ cells in the spinal white matter at P7 and P21 are 74±3.5% and 83.0±4.0%, respectively (> 500 cell count; n=3), suggesting that Brg1 is largely confined to differentiating cells in the oligodendrocyte lineage.

To assess the functional role of Brg1 in oligodendrocyte development *in vivo*, we ablated Brg1 expression in the oligodendrocyte lineage directed by Olig1-Cre (Xin et al., 2005; Ye et al., 2009) in *Brg1* conditional knockout mice (Sumi-Ichinose et al., 1997) (Figure S2B). We observed that all resulting mutant $Brg1^{flox/flox}$;$Olig1^{Cre+/-}$ mice (referred to as Brg1cKO), but not their control littermates, developed myelin-deficient phenotypes including generalized tremors, hindlimb paralysis and seizures beginning at postnatal week 2. The mice died at postnatal week 3–4 in contrast to the normal lifespan of wild-type and heterozygotes ($Brg1^{flox/+}$;$Olig1^{Cre+/-}$). The myelinating optic nerve tract from Brg1cKO mice at P14 was translucent (Figure 2C). In addition, expression of myelin genes such as *Mbp* (myelin basic protein) and *Plp1* (proteolipid protein) was diminished in the spinal cord and forebrain of mutant mice at P14 (Figure 2D and Figure S2C). In light of these findings, we further examined myelin sheath assembly by electron microscopy. In contrast to the large number of myelinated axons that are observed in control mice at P14, myelinated axons were hardly detectable in the optic nerve and spinal cord of Brg1cKO mutants (Figure 2E), indicating a severe myelination deficit and therefore, a requirement for Brg1 function.

Despite the deficiency in myelin gene expression, the OPC marker PDGFRα was detected in the spinal cord and brain of Brg1 mutants at P7, P14 and P21, a stage before animals die (Figures 2F and S3). The number of OPCs and their proliferation rate (percentage of BrdU+ proliferating OPCs) in *Brg1* mutants were comparable with those of control mice (Figure 2G and S3), suggesting that most OPCs in *Brg1* mutants are maintained. We did not detect any significant cell death in the CNS of Brg1cKO mice at these stages based on cleaved Caspase-3 immunoreactivity (data not shown), although there was an increase of cell death when OPCs isolated from Brg1cKO animals underwent differentiation *in vitro* (Figure S4). It is possible that we miss a narrow window for cell death detection *in vivo* by activated Caspase 3 due to activation of microglia, which could rapidly remove dying cells. It is worthy noting that *Brg1* mutants did not exhibit significant alterations of astrocytes, neurons or microglia identified by GFAP, NeuN and Iba1, respectively, in the brain (Figure S2).

To determine the Brg1 downstream targets that regulate oligodendrocyte differentiation, we carried out RNA-seq profiling analysis using the oligodendrocyte-enriched optic nerves of control and Brg1cKO mice at P14 (Table S2). In *Brg1* mutants, gene ontology analysis displayed an enrichment of downregulated genes related to myelination and lipid-protein synthesis (Figure 3A, B), consistent with deficient myelin formation. In contrast, upregulation of differentiation inhibitors including ID2/4, Nfia/b, Sox5 and β-catenin (Ctnnb1) was observed (Figure 3A). Similarly, qRT-PCR analysis indicated that myelination-associated genes including critical differentiation regulators such as Gm98/MRF and Sox10 were markedly downregulated (Figure 3C). Together, these observations suggest that Brg1 is required for OPC differentiation and myelination in the CNS but not their proliferation and survival.

To determine whether Brg1 is sufficient to promote OPC differentiation, we transfected control and Brg1-expressing vectors into OPCs isolated from neonatal rat cortices. We found that overexpression of Brg1 in cultured OPCs significantly promoted the expression of myelin genes *Mbp, Plp* and *Cnp* (Figure 3D). However, the Brg1 ATPase-dead (K798R) mutant, which is capable of forming a complex with other BAF subunits (Peterson et al., 1994), displayed a dominant negative function to inhibit myelin gene expression while activating differentiation inhibitors such as Hes1 and Id2 (Figure 3E), suggesting that ATPase activity is critical for Brg1 function to promote myelination programs. Furthermore, we observed that Brg1 overexpression led to a significant increase of mature MBP+ oligodendrocytes that harbored complex processes as compared with the control (Figure 3F, H).

To investigate the differentiation capacity of OPCs in the absence of Brg1 in vitro, we purified OPCs from control and Brg1cKO neocortices using immunopanning. Control OPCs readily differentiated into MBP+ mature oligodendrocytes. In contrast, no MBP+ oligodendrocytes were detected in *Brg1* deficient OPCs (Figure 3G). In addition, we found that re-introduction of *Brg1* to the *Brg1*-knockdown OPCs could rescue, at least partially, for the defects in myelin gene expression (Figure 3I), suggesting that Brg1 is responsible for promoting the myelination program. Together, these observations demonstrate that activation of the Brg1 chromatin remodeler is both essential and sufficient for initiating and promoting OPC differentiation and maturation.

Brg1 selectively targets myelination-related genes during oligodendrocyte differentiation

To identify the direct target genes through which Brg1 regulates the onset of oligodendrocyte differentiation, we performed the ChIP-seq analysis for Brg1 genome-wide occupancy in OPCs and iOLs respectively (Figure 4A). We identified 5465 binding sites (fold enrichment >=5, p value $\leq 10^{-9}$) within iOL datasets (Figure 4B and Dataset S2), the majority of which were present in evolutionarily conserved intergenic regions or introns of the gene loci (Figure 4C, D). In contrast, Brg1 occupancies in OPCs were much fewer and only 451 significant regions were identified (Figure 4B). Essentially, all Brg1 peaks in OPCs co-localized with those identified in iOLs but at a much-reduced intensity (Figure 4A, B). The weak Brg1-chromatin occupancy in OPCs is likely due to spontaneous differentiation of a small population of OPCs in culture. Although Brg1-binding around transcription start sites (TSS) displays a higher binding density, ~80% of Brg1-binding peaks were localized distantly from the TSS (Figure 4D), suggesting that Brg1 mainly targets to the intergenic enhancer regions.

Gene ontology analysis indicated that genes overrepresented in Brg1-binding targets were mainly related to myelination, oligodendrocyte differentiation and cell cycle arrest (Figure 4E), such as *Cnp, Cldn11, Klf9* and *Zfp191* (Figure 4F). The frequency and intensity of Brg1 binding substantially increased in iOLs over OPCs, indicating a recruitment of the Brg1 SWI/SNF complex to key differentiation gene loci during the initiation of OPC differentiation.

To further determine whether Brg1 targeting regions in iOLs are unique to oligodendrocytes, we compared Brg1 iOL binding sites with Brg1 targets in embryonic stem cells (ESCs) (Ho et al., 2009) and neural precursors in E11.5 forebrain (Rada-Iglesias et al., 2011), respectively. We observed that very few (< 3%) of Brg1 occupied sites in iOLs overlapped with those in ESCs and embryonic forebrain (Figure 4G), suggesting that the chromatin remodeler Brg1 is recruited by a lineage-specific factor(s) to occupy a specific subset of genes that are unique for oligodendrocyte differentiation.

Brg1 is recruited to oligodendrocyte lineage-specific cis-regulatory elements pre-occupied by the determination factor Olig2

De novo analysis of Brg1 occupancy revealed that Brg1 targeted to evolutionarily conserved elements (Figure 5A). Approximately 71% Brg1 binding regions contained an enhancer box (E-box, CANNTG) motif, which is typically recognized by bHLH transcription factors (Figure 5A).

Strikingly, the most overrepresented consensus motif of Brg1 binding sites matches the motif targeted by the bHLH factor Olig2 in embryonic stem cells (Figure 5A) (Mazzoni et al., 2011). Given the critical role of Olig2 in oligodendrocyte lineage progression, we hypothesized that Brg1 cooperates with Olig2 to target differentiation-promoting genes, and hence carried out ChIP-seq analysis to identify Olig2 binding sites in OPCs and iOLs. Olig2 bound to a large variety of genomic regions in OPCs including 21,901 significant binding sites (fold enrichment >=5, p value $\leq 10^{-9}$). The binding sites of Olig2 in OPCs essentially persisted in differentiating iOLs with 25,787 significant binding sites (Dataset S3). We further detected that the majority of Brg1 binding regions in iOLs overlapped with Olig2 signals in OPCs and iOLs, suggesting that Brg1 binds to chromatin regions pre-occupied by Olig2 in OPCs (Figure 5B, C).

Motif analysis of binding sites reveals that the majority of Olig2 and Brg1 targeted sites share essentially identical consensus sequences carrying the E-box motif (Figure 5D). Recent studies illustrate that there are at least five evolutionarily conserved enhancer regions (Kuspert et al., 2011) that regulate expression of Sox10, an essential factor for oligodendrocyte differentiation. Olig2 was shown to bind to the U2 enhancer in the distal 5′-flank of the Sox10 (Kuspert et al., 2011). Our ChIP-seq data from iOLs demonstrated that Brg1 and Olig2 co-occupied these conserved Sox10 enhancers containing E-box motifs in addition to the U2 region (Figure 5E). All these enhancer regions contain the consensus Brg1/Olig2 binding motif including the E-box (Figure 5E). In addition, the binding patterns of Olig2 and Brg1 co-occupancy were also detected in enhancers of other key myelination related genes including *Mbp* and *Ugt8a/Cgt* (Figure 5F, G).

To further determine whether Olig2 and Brg1 could physically associate with each other during oligodendrocyte differentiation, we performed co-immunoprecipitation (co-IP) assays. Brg1 weakly interacted with Olig2 in OPCs (Figure 5H), however, this interaction was dramatically enhanced when OPCs were induced to differentiate after 24 hr treatment in differentiation medium (Figure 5H), suggesting that Brg1 is strongly associated with Olig2 when OPCs undergo the differentiation process. To verify the Brg1-Olig2 interaction at the endogenous protein level, we carried out co-IP assays using mouse brain tissues at P10 and demonstrated that Brg1 interacted with Olig2 *in vivo* (Figure 5I). Together, these observations suggest that Brg1 is an integral component of the transcriptional control of myelination via interaction with Olig2 at the onset of OPC differentiation.

Distinct genomic distribution of Brg1 and Olig2 targeting at different stages of oligodendrocyte differentiation

Oligodendrocyte development involves sequential phases of maturation including initiation and progression of differentiation. To investigate whether Brg1 and Olig2 exhibit temporally distinct regulatory functions, we further performed ChIP-seq of Brg1 in mature oligodendrocytes (mOLs).

When comparing Brg1 genomic occupancy among OPCs, iOLs and mOLs, we observed dynamic changes of Brg1 targeting sites at each stage. These Brg1 targets can be clustered into two categories: targets that elicit intense Brg1 signal beginning at either iOL or mOL stage (Figure 6A). When compared to an oligodendrocyte transcriptome database (Dugas et

al., 2006), genes targeted by Brg1 in iOLs belong to a cohort of early expressing genes referred to as “early differentiation genes” (Dugas et al., 2006), including *Cnp, Mbp, Klf9* and *Sirt2* (Figure 6B), while those targeted by Brg1 in mOLs mainly act as “late differentiation genes” including *Fnbp1, Ank3, Eml1*, *Tmem123,* and *Mylk* (Dugas et al., 2006) (Figure 6C). Strikingly, the majority of the Brg1 target genes in mOLs were involved in cytoskeletal remodeling including microtubule/cytoskeleton-based transport and microtubule polymerization and depolymerization (Figure 6D). Among them, Fnbp1 and Ank3/AnkG have been shown to be critical for membrane curvature-dependent actin polymerization and clustering of voltage-gated Na channels at axon initial segments, respectively (Takano et al., 2008; Zhou et al., 1998).

Similar dynamic changes of Olig2 target occupancy were also observed during oligodendrocyte maturation. Despite comparable target occupancy between iOLs and OPCs, Olig2 binding decreased in mOLs (Figure 6E). Olig2 targets in mOLs were mainly involved in the regulation of cytoskeletal processes and organization, particularly the Rho family of GTPases such as Cdc42 and Rac1 (Figures 6F and S5), which are required for oligodendrocyte maturation and the correct formation of myelin sheaths in the CNS (Thurnherr et al., 2006). These observations suggest that the late differentiation genes targeted by Olig2 and Brg1 are required for maintenance of the differentiated state and process elaboration. Collectively, these results suggest that Brg1 and Olig2 target distinct subsets of target genes during early and late phases of differentiation to control oligodendrocyte lineage progression.

Brg1 and Olig2 co-occupancy coupled with distinct histone modifications controls expression of critical regulators for oligodendrocyte differentiation

Brg1 functions primarily as a transcriptional activator containing multiple functional domains including a helicase ATP-binding domain and a bromodomain (Wu et al., 2009; Yoo and Crabtree, 2009). The latter recognizes and interacts with acetylated lysine residues in histones to promote chromatin remodeling and gene transcription. We hypothesized that Brg1 is recruited by Olig2 and interacts with an activating histone mark, K27 acetylation at histone 3 (H3K27Ac), which distinguishes active enhancers from inactive/poised enhancer elements (Creyghton et al., 2010), to direct the transcription of critical genes for differentiation initiation.

To address the hypothesis, we performed ChIP-seq in OPCs and iOLs with activating histone marks, H3K27Ac and H3K4me3, which signify gene activation at enhancers and transcriptional start sites (TSS), respectively (Martin and Zhang, 2005; Strahl and Allis, 2000), and compared them with Brg1 and Olig2 genomic occupancy. Among the targets that are co-occupied by Brg1/Olig2 and the respective active histone marks, we identified a cohort of transcriptional regulators including previously identified regulators of oligodendrocyte differentiation such as MRF, Sox10, Olig1/2, Zfp191 and Sip1/Zfhx1b (Emery, 2010; Li et al., 2009; Weng et al., 2012) (Figure 7A, B). *MRF,* which was downregulated in *Brg1* mutants (Figure 7B), has a similar mutant phenotype to Brg1 with defects in differentiation but not OPC formation (Emery et al., 2009). We observed that Brg1 and Olig2 were recruited to enhancer regions of *MRF* designated by flanking H3K27Ac peaks (Figure 7B; Dataset S4). Of particular interest, Brg1 recruitment coincided with a dramatic increase in the activating H3K27Ac mark at the onset of OPC differentiation (Figure 7B). In addition, a surge in another activating histone mark H3K4me3 (Dataset S5) at the promoter region proximal to the TSS site of the MRF gene locus was observed in differentiating OLs as compared to OPCs. This is consistent with the activation of MRF expression upon OPC differentiation.

Based on Olig2 and Brg1 co-occupancy together with the increase in active histone modification signatures, we identified a cohort of potential transcriptional regulators previously uncharacterized in oligodendrocyte development (Figure 7A), including new oligodendrocyte-enriched transcription factors (e.g. Sall1 and ETV6), transcription initiation factors (e.g. Med27 and TAF2) and a cohort of histone modifiers such as histone demethylase Kdm6b, and Cdyl, a chromodomain protein with histone acetyltransferase activity (Figures 7A and S6). Brg1 knockdown by RNAi resulted in significant down-regulation of these factors including Cdyl, Kdm6b and Sall1 (Figure 7D). Importantly, like Brg1 knockdown, siRNA knockdown of *Cdyl, Kdm6b* or *Sall1* significantly inhibited myelin gene expression during OPC differentiation (Figure 7E, F) while upregulating differentiation inhibitors such as Hes5 (Figure 7F). The siRNA knockdown studies also indicate that expression of these differentiation regulators is, at least in part, mutually dependent on one another (Figures 7D and S6).

In the promoter region of oligodendrocyte differentiation inhibitors such as Hes1 and PDGFRα, however, levels of enrichment of the H3K27Ac histone mark were substantially reduced in oligodendrocytes as compared with OPCs, concurrent with reduced Olig2 binding (Figure 7G, H). In contrast, the level of the repressive histone mark H3K9me3 (Dataset S6), which is associated with gene repression (Martin and Zhang, 2005; Strahl and Allis, 2000), was increased during differentiation (Figure 7G, H). As a control, the level of the H3K27Ac histone mark on the promoter of the housekeeping gene β-actin was comparable between OPCs and differentiating oligodendrocytes (Figure 7I). These data suggest that Olig2 and Brg1 targeting coupled with the transcriptionally-linked epigenetic landscape establishes the gene transcription program to promote oligodendrocyte differentiation.

Discussion

Our genome-wide, base-resolution timecourse of chromatin and transcriptome changes sheds light on the molecular basis of oligodendrocyte development. In this study, we uncover that activation of a Brg1-based ATP-dependent SWI/SNF chromatin-remodeler is sufficient for initiating transcriptional programs to establish oligodendrocyte identity. Importantly, we find that the oligodendrocyte-lineage determination factor Olig2 transcriptionally pre-patterns the chromatin-remodeling factor Smarca4/Brg1 to the enhancer elements to control oligodendrocyte differentiation. The identification of stage-specific enhancers at the genome-wide level unveils a series of new regulatory factors that control oligodendrocyte development. Our genome-wide multistage study further reveals a mechanism of oligodendrocyte differentiation by linking the transcription factor control with stage- and target-specific epigenetic regulation and chromatin remodeling, such mechanistic insight has not been previously defined for CNS myelination. Thus, our results provide a genome-wide map of transcriptional and epigenetic changes that define stepwise oligodendrocyte lineage progression, an instructive reference to gene regulatory networks and epigenomics, and important elements and principles for defining the complex process of myelination in the vertebrate CNS.

Activation of ATP-dependent Brg1 chromatin-remodeling complex drives oligodendrocyte lineage progression

The Brg1 chromatin remodeler was originally thought to be a ubiquitously expressed factor, however, unexpectedly we find that Brg1, but not Brm1, is strongly transcribed and upregulated at the onset of differentiation during oligodendrocyte lineage progression. Brg1 upregulation and activation have not been reported in other cellular processes such as during the transition from embryonic stem cells to embryonic fibroblasts, activation of neuronal cells or estrogen response in human breast cancer cells (Figure S1), suggesting that

activation of Brg1 may be specific for promoting the temporal transition of oligodendrocyte precursor differentiation. What dictates the upregulation of Brg1 from OPC to iOL transition remains unknown at present. Our ChIP-seq data indicate that genomic occupancy by Olig2 precedes that of Brg1 during oligodendrocyte lineage progression. Olig2 targets the locus of Brg1 in OPCs, but not vice versa (Figure S1). In addition, Brg1 is down-regulated in the oligodendrocyte-enriched corpus callosum of Olig2-ablated mutants (Figure S1), suggesting that Olig2 might have a role in regulating Brg1 expression. Alternatively, it is possible that regulatory miRNAs such as miR-219 and miR-338 (Dugas et al., 2010; Zhao et al., 2010) might control Brg1 expression during the OPC transition.

Our data suggest that Brg1 regulates the OPC-to-oligodendrocyte transition in a stage-specific manner. Deletion of Brg1 by Olig1-Cre does not appear to affect the developmental transition from Olig+ pMN progenitors to form OPCs at embryonic stages (Figure S3C), despite blocking the transition from OPCs into oligodendrocytes. In addition, inhibition of Brg1 does not cause global downregulation in gene expression programs since downregulation of Brg1 *in vivo* and *in vitro* inhibits differentiation activators while activating differentiation inhibitors (Figure 3A, E). Furthermore, Brg1 targets are significantly overrepresented by factors that are required for oligodendrocyte differentiation. These observations suggest a specific function of Brg1 during the transition from OPC to oligodendrocytes.

Olig2 directs the functional specificity of the Brg1-dependent chromatin-remodeling complex to establish oligodendrocyte identity

Our studies uncover Olig2 as a pre-patterning factor that directs the recruitment of Brg1 to oligodendrocyte lineage-specific cis-regulatory elements during the critical transition from OPC to iOL. The regulation of the functional specificity and activity of the chromatin remodeler Brg1 by Olig2, coupled with distinct epigenetic changes during developmental transition, likely plays a key role in the activation of the stage-specific transcriptional program for oligodendrocyte development. These studies provide important insights into the molecular mechanism by which Olig2, a factor long known to be involved in oligodendrocyte lineage, acts to regulate the development of this lineage.

Once considered to be general mediators of transcription, the Brg1-based SWI/SNF chromatin-remodeling complexes are increasingly recognized for their cell type or tissue-specific functions during development (Euskirchen et al., 2011; Yoo and Crabtree, 2009). Much of this specificity is thought to stem from post-translational modification of SWI-SNF subunits and/or variations in subunit composition such as neuronal specific BAF complex (nBAF) (Lessard et al., 2007; Wu et al., 2007). We observe that Brg1 binding targets in oligodendrocyte lineage cells are specifically associated with the differentiation program for oligodendrocytes but not embryonic stem cells or neural progenitors. However, whether there exists an oligodendrocyte-specific BAF complex with unique subunit composition, and what controls the functional specificity of the Brg1 chromatin-remodeling complex for targeting oligodendrocyte-specific genes remain unresolved at present.

Upregulation of Brg1 complex components, together with a surge of physical association of Olig2 with the Brg1 complex at the onset of OPC differentiation, suggests that Olig2 can functionally pre-pattern Brg1 complex targeting on oligodendrocyte-specific enhancers and therefore directs Brg1 chromatin remodeling activity specifically to activate the oligodendrocyte differentiation program. This is in contrast to neuronal cells wherein a change in Brg1 complex composition acts as a developmental switch in the nervous system (Wu et al., 2009; Yoo and Crabtree, 2009). Given the essential role of Olig2 in neural subtype switch (Li et al., 2011; Zhu et al., 2012), regulation of the functional specificity and activity of a Brg1 chromatin-remodeling complex by Olig2 should have important

implications on the molecular mechanisms of neural subtype specification and lineage development.

Temporal control of oligodendrocyte lineage progression by dynamic distribution of Brg1/Olig2 targeting

Our genome-wide targeting studies at multiple developmental stages uncover a temporally regulated mechanism of Brg1 and Olig2 targeting in controlling stage-specific transcriptional programs for oligodendrocyte lineage initiation and maturation. At the onset of oligodendrocyte differentiation, Brg1 and Olig2 bind preferentially to the enhancers of differentiation-promoting genes. Bromodomain-containing Brg1 recruited by Olig2 may recognize activated histone acetylation marks such as H3K27Ac to promote expression of key differentiation activators such as MRF and Sox10.

Interestingly, upon oligodendrocyte maturation, accessibility of Brg1 and Olig2 in genomic loci is reduced, and Brg1 and Olig2 appear to redistribute to distinct target sites, while expression of myelin genes continues to increase. This suggests that Olig2-engaged Brg1 chromatin remodeling complex would be required transiently during the initial period of OPC differentiation to reset histones for activating a gene expression program, and that it would be sustained by histone marks or epigenetic memory. Strikingly, in maturating oligodendrocytes, despite the reduction of target gene binding sites, Brg1 and Olig2 binding redistributes to a set of genes critical for cytoskeleton reorganization and curvature-dependent actin polymerization, which are required for complex morphogenesis during myelination. These data suggest a stage-specific switch of Brg1/Olig2 targeting in the regulation of distinct sets of genes from initiating the differentiation process to maintaining the differentiation state during lineage progression. The mechanisms underlying the temporal specificity of Olig2/Brg1 targeting are not known at present. It is possible that stage-specific regulators (e.g. MRF and Sox10) activated by Olig2/Brg1 targeting at the differentiation onset could form a positive feedback loop to interact with Olig2 and/or Brg1, as suggested in other contexts (Liu et al., 2007; Weider et al., 2012), to control their targeting specificity at later stages of differentiation and maturation. Therefore, the coordination of Brg1/Olig2 functional dynamics at different stages is an integral component in the spatial and temporal control of oligodendrocyte development.

Our Brg1/Olig2 targeting studies in conjunction of epigenetic histone modification analysis also shed light on stage-specific oligodendrocyte cis-regulatory elements at a genome-wide level. Temporally specific Olig2 and Brg1 co-occupancy identifies and predicts cis-regulatory enhancer elements that are critical for differentiation initiation and maintenance during the myelination process. The identification of cis-regulatory elements activated de novo during the transition of OPC differentiation uncovers a cohort of new oligodendrocyte-enriched transcription factors, chromatin modifiers and signaling regulators, which will yield important clues to the regulation of the myelination process and undoubtedly illuminate a new landscape of therapeutic targets for promoting myelin repair in the CNS. The roles of the candidate cis-elements and their rules in engaging gene regulation should be greatly clarified by future analysis, to detect specific chromatin looping events, enhancer activation states e.g. mapped by association with p300 and HDACs, and DNA methylation. It is noteworthy that Brg1 expression is substantially upregulated in oligodendrocyte lineage cells in lysolecithin-induced demyelinating lesion during remyelination (Figure S7), suggesting a potentially important function of Brg1 in oligodendrocyte remyelination. Given the critical role of Brg1 in promoting oligodendrocyte differentiation, augmenting the activity of the ATP-dependent chromatin-remodeling enzyme might have therapeutic benefits for promoting myelin repair in demyelinating diseases.

Experimental procedures

For full materials, see Extended Experimental Procedures. Briefly, *Brg1*$^{lox/lox}$ mice (Sumi-Ichinose et al., 1997) were crossed with *Olig1-Cre* mice to generate Brg1cKO (*Brg1*$^{lox/lox}$*;Olig1Cre*$^{+/-}$) and heterozygous control (*Olig1Cre*$^{+/-}$*; Brg1*$^{lox/+}$) mice. All animal use and studies were approved by ethical committees at Sichuan University, China and by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center at Dallas, USA. Rat and mouse OPCs were isolated and cultured according to the procedures as previously described (Chan et al., 2004; Chen et al., 2007). siRNAs for *Brg1, Cdyl, Kdm6b* and *Sall1* were purchased from Sigma, St Louis, MO. Co-immunoprecipitation, ChIP-seq and RNA-seq were carried out and analyzed as previously reported (Flavell et al., 2008; Weng et al., 2012). Details are described in Extended Experimental Procedures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Research highlights

1. Activation of the Brg1 chromatin-remodeling complex drives OPC differentiation
2. Olig2 pre-patterns and directs Brg1 targeting to oligodendrocyte-specific enhancers
3. Functional and stage-dependent Olig2/Brg1 targeting in oligodendrocyte development
4. Olig2/Brg1 targeting coupled with epigenetic marking defines oligodendrocyte identity

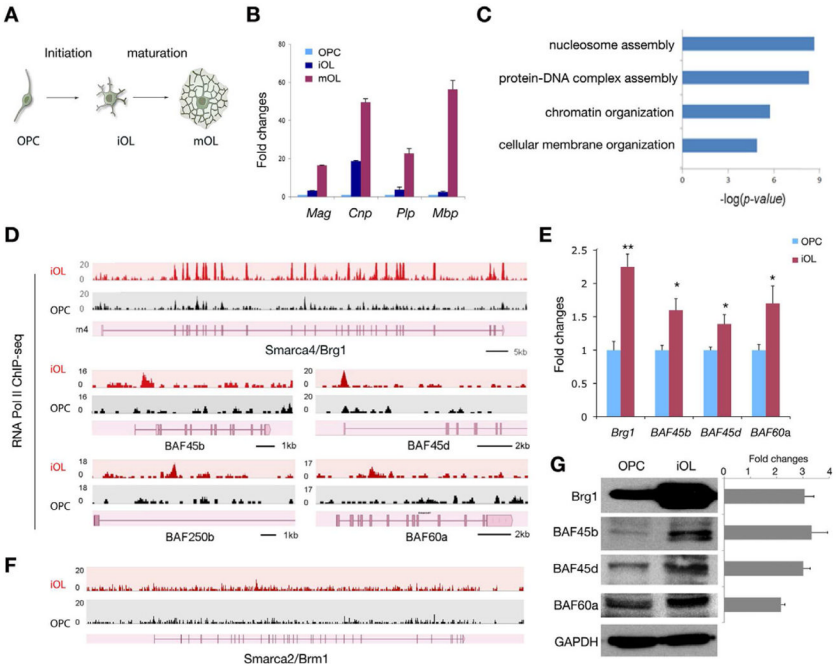

Figure 1. The Brg1 gene locus is most abundantly targeted by RNAPII at the onset of OPC differentiation

(A) Schematic overview of oligodendrocyte lineage progression.

(B) Expression of *Mag, Cnp, Plp* and *Mbp* in OPC, iOL and mOL assayed by qRT-PCR (n=3).

(C) Gene Ontology (GO) analysis of biological processes of RNAPII targeted genes in iOL.

(D) RNAPII binding on the gene loci of Brg1 and other BAF subunits in OPC and iOL.

(E) Expression of *Brg1*, *BAF45b, BAF45d* and *BAF60a* was assayed in OPC and iOL by qRT-PCR. $n \geq 3$ independent experiments. * $p < 0.05$, * * $p < 0.01$; Student's t test.

(F) RNA Pol II binding distribution at the Brm1 locus.

(G) Western blot analysis of Brg1, BAF45b, BAF45d and BAF60a. Histograms show fold changes measured by densitometry in iOL over OPCs after normalization with GAPDH (n = 3 experiments).

Data were presented as mean ± SEM. See also Figure S1 and Table S1.

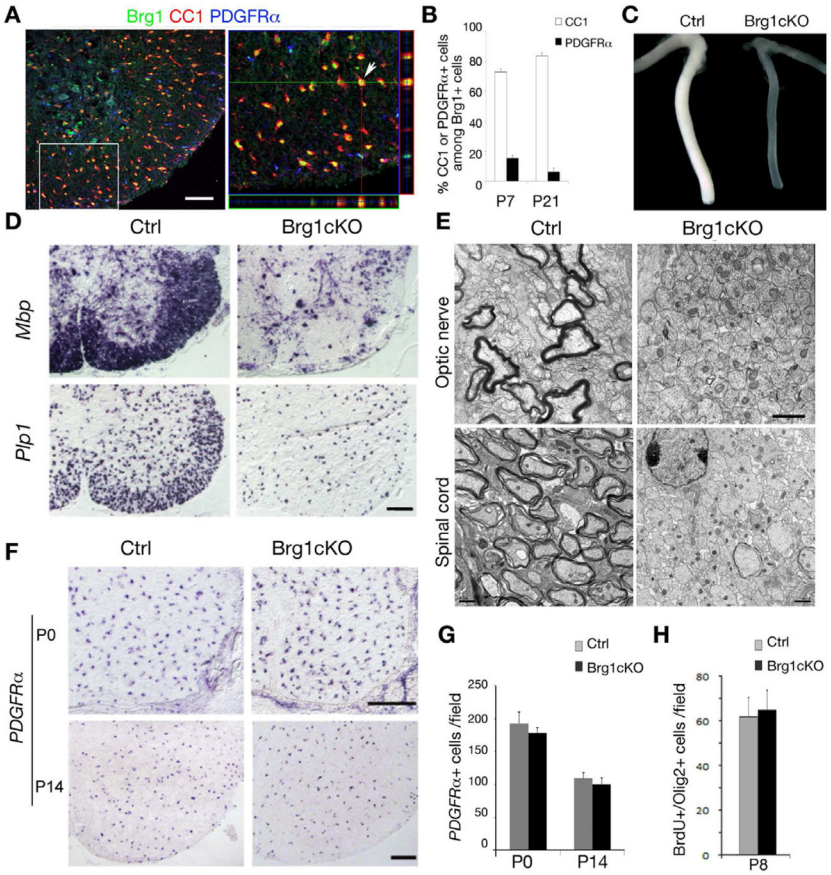

Figure 2. Brg1 ablation in oligodendrocyte lineage cells leads to myelination defects

(A) Spinal cord at P14 was immunostained with CC1, PDGFRα and Brg1. Boxed image was shown in the right with Z-axis reconstructions in side panels. Arrow indicates Brg1 co-labeling with CC1.

(B) Quantification of CC1 or PDGFRα+ cells among Brg1+ cells in the spinal white matter at P7 and P21 ($n = 3$ animals).

(C) Appearance of optic nerves from control and Brg1cKO littermates at P14.

(D) *In situ* hybridization of *Mbp* and *Plp* on the spinal cord of $Brg1^{flox/+}$;*Olig1-Cre* control (Ctrl) and $Brg1^{flox/flox}$;*Olig1-Cre* (Brg1cKO) mice at P14.

(E) Electron micrograph analysis of optic nerves and spinal cords of Ctrl and Brg1cKO at P14.

(F–G) *PDGFRα* expression in spinal cords of control and mutants at P0 and P14. The density of $PDGFR\alpha^+$ OPCs was quantified per field (0.2 mm^2)(G) ($n = 3$).

(H) Quantification of the average number of $BrdU^+$ and $Olig2^+$ co-labeled cells per area (0.7 mm^2) at P8 in cerebral cortices of control and mutants ($n = 3$).

Data were presented as mean ± SEM. Scale bars in A, 200 μm; D, F, 100 μm; E, 1 μm. See also Figures S2 and S3.

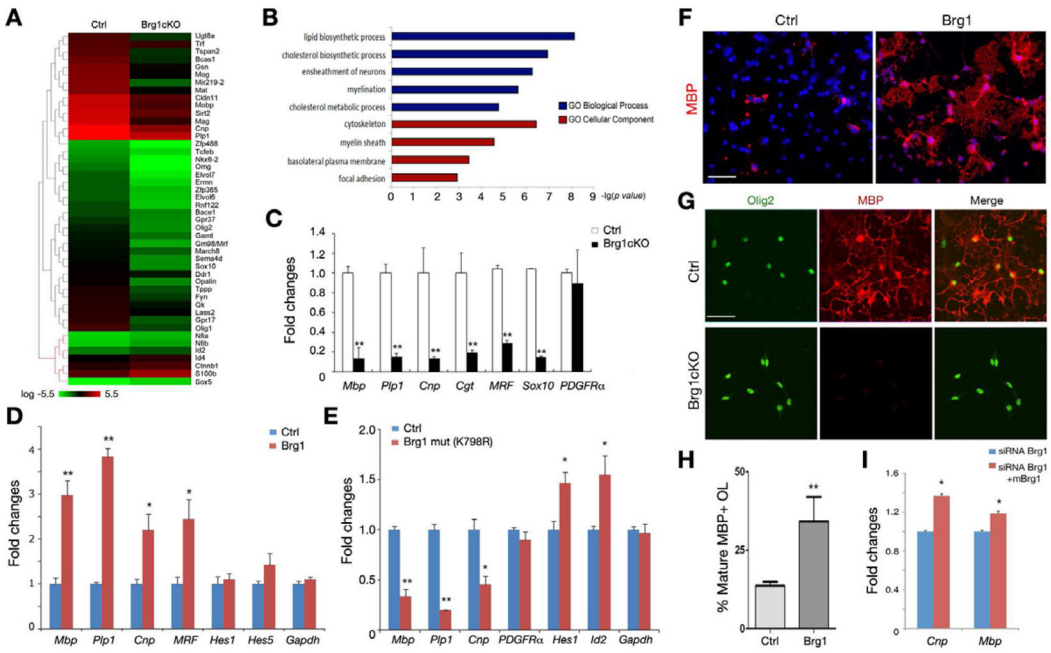

Figure 3. Downregulation of myelination-associated genes in Brg1 mutants

(A) Heatmap of representative RNA-seq data of control and mutant optic nerves at P14.

(B) GO analysis depicts biological processes (blue) and cellular components (red) overrepresented in downregulated genes in Brg1 mutants.

(C) qRT-PCR analysis of myelination-associated genes in P14 control and mutant spinal cords.

(D–E) Rat OPCs were transfected with pCAG control and pCAG-Brg1(D), or Brg1-ATPase mutant (K798R) (E), and cultured without PDGF-AA for two days. Expression of myelin-associated genes was determined by qRT-PCR. Fold changes in C–E over control are derived from at least three experiments (n ≥ 3).

(F, H) Rat OPCs were transfected with control and Brg1 expressing vectors and cultured in the growth medium supplemented with PDGF-AA for five days, and then immunostained with anti-MBP (red) (F). Panel H depicts the quantification of the percentage of MBP+ cells after transfection (n=3 experiments).

(G) OPCs isolated from cortices of control and mutants at P7 by immunopanning were cultured under differentiation condition for 3 days and immunostained with anti-MBP and anti-Olig2.

(I) Rat OPCs were treated with rat *Brg1* siRNAs for 48 hr to knockdown endogenous *Brg1*. Cells were then transfected with control vector and Brg1 expressing vector carrying mouse *Brg1*, which has a mismatching sequence to rat *Brg1* siRNA, for two days. Expression of *Cnp* and *Mbp* was quantified by qRT-PCR (n=3 experiments).

Scale bars in F, G: 25 μm. Data were presented as mean ± SEM (* $p < 0.05$, * * $p < 0.01$, Student's *t*-test). See also Figure S4 and Table S2.

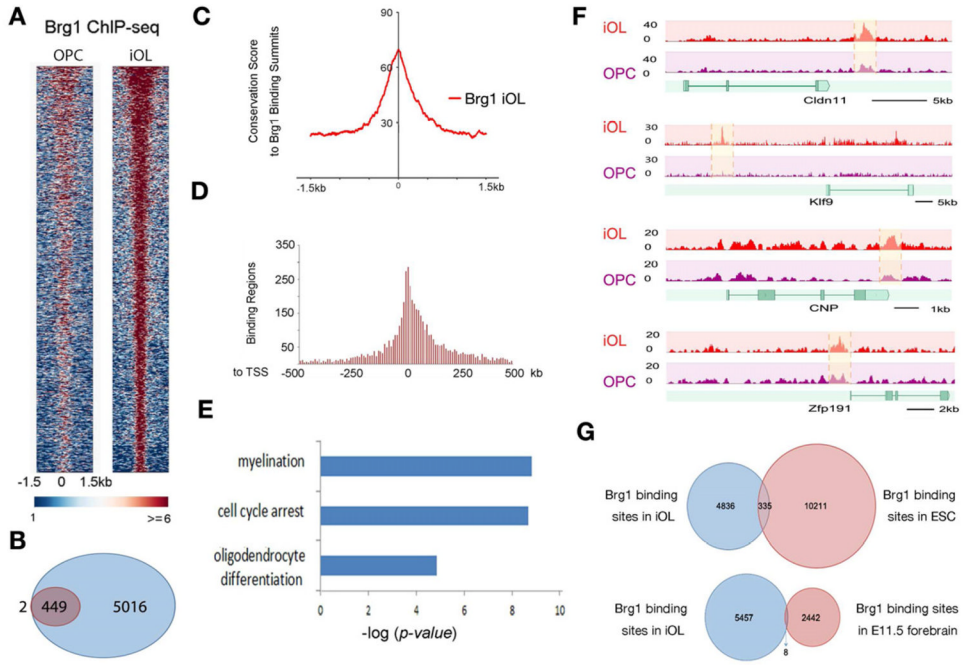

Figure 4. Brg1 targets lineage-specific regulators upon OPC differentiation

(A) Heatmap of Brg1 binding signals in OPCs (left) and iOLs (right). Each line on y-axis represents a genomic region ±1.5kb flanking Brg1 summits.

(B) Venn diagram for global Brg1 occupancy in iOLs compared to OPCs.

(C) Graph depicts the conservation score of Brg1 binding summits.

(D) The distribution pattern of Brg1 binding regions in iOLs mapped to their closest Ensembl gene transcription start site (TSS).

(E) Gene ontology analysis of Brg1 binding regions in iOLs.

(F) Genome browser view of the distribution of Brg1 on the loci of representative myelination-related genes in iOLs (red) and OPCs (purple).

(G) Venn diagram shows minimal overlap of Brg1 occupancy between iOLs and ESCs or E11.5 forebrain, respectively.

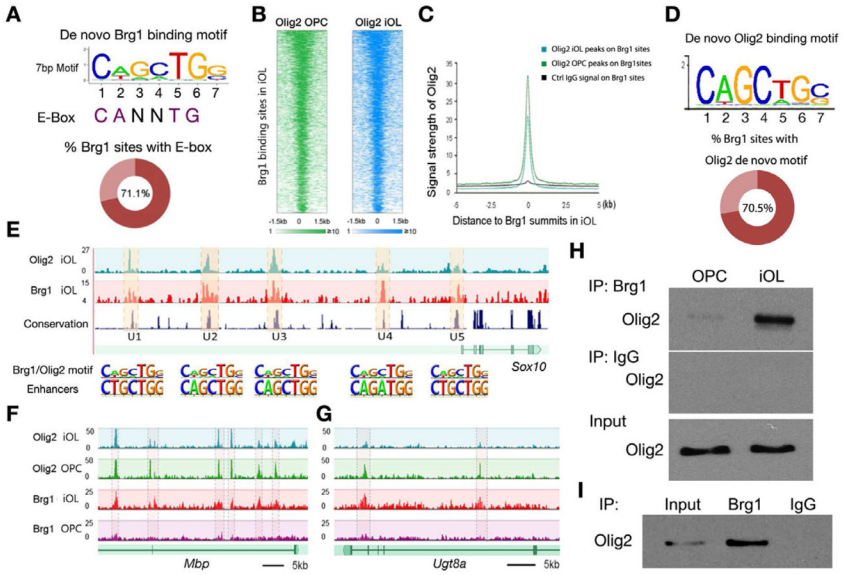

Figure 5. Brg1 recruitment to E-box containing enhancer elements pre-occupied by Olig2

(A) *De novo* motif analysis of Brg1 binding regions identified a putative E-box consensus motif (CANNTG) among ~ 71% of Brg1 ChIP-seq binding sites.

(B) Heatmap of Olig2 binding signals at OPCs (left) and iOLs (right) ±1.5kb surrounding Brg1 iOL binding summits.

(C) Binding profiles of Olig2 around Brg1 peak summits.

(D) The *de novo* binding motif derived from Olig2 binding regions. Lower panel: percentage of overlapping of Brg1 binding sites in iOL with Olig2 *de novo* motif.

(E) Brg1/Olig2 co-occupancy (as highlighted) on evolutionary conserved E-box containing enhancers (U1-U5) in the Sox10 promoter. The E-box in Sox10 enhancers and Brg1/Olig2 binding motif was shown in the lower panel.

(F, G) Visualization of Brg1 and Olig2 binding profiles in OPCs and iOLs on representative myelin gene loci (F, *Mbp*; G, *Ugt8*).

(H–I) Lysates from rat OPCs and iOLs (H) and P10 forebrain tissues (I) were immunoprecipitated with anti-Brg1 and IgG control and blotted by anti-Olig2.

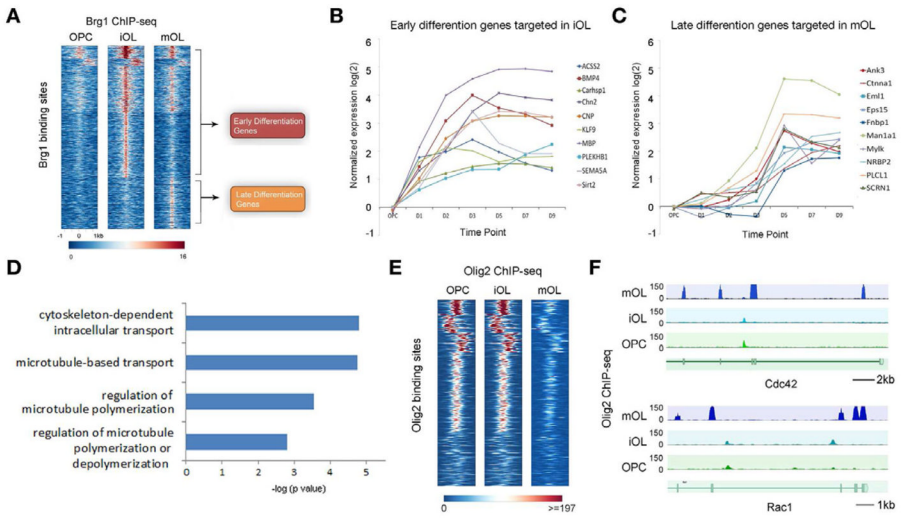

Figure 6. Brg1 and Olig2 target distinct transcriptional programs at different stages of oligodendrocyte differentiation

(A) Heatmap of Brg1 binding signals at OPCs, iOLs and mOLs ± 1kb surrounding Brg1 binding summits, showing early and late differentiation gene groups.

(B, C) Temporal expression patterns of representative Brg1-targeted genes in iOL (B) and mOL (C) were plotted against an oligodendrocyte transcriptome database (Dugas et al., 2006) at indicated stages.

(D) GO analysis of Brg1 binding targets in mOL.

(E) Heatmap of Olig2 binding signals ± 600 bp surrounding Olig2 binding summits.

(F) Genome browser visualization of Olig2 distribution on Cdc42 and Rac1 loci. See also Figure S5.

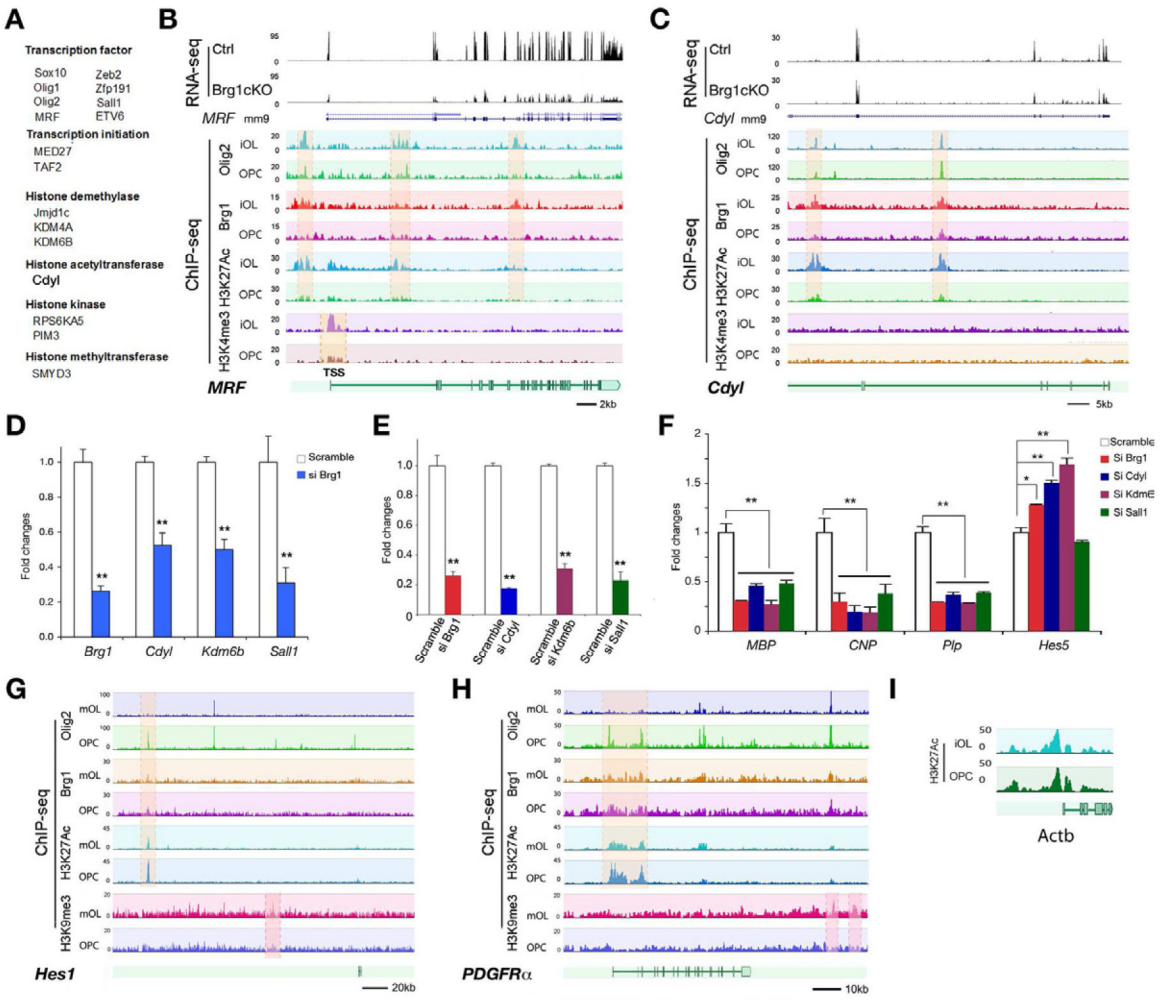

Figure 7. Brg1 and Olig2 co-occupancy coupled with epigenetic marks to modulate expression of key oligodendrocyte differentiation regulatory genes

(A) Representative targeting genes associated with transcriptional regulation predicted by Brg1/Olig2 co-occupancy and active histone marks.

(B, C) Genome browser view of genetic and epigenetic landscape surrounding the *MRF* (B) or *Cdyl* (C) gene locus. Upper panel: RNA-seq peak visualization of gene expression from Ctrl and mutants. Lower panel: ChIP-seq with indicated antibodies in rat OPCs and iOLs.

(D) Rat OPCs were treated with scrambled control and Brg1 siRNA for 48 hr. Gene expression was examined by qRT-PCR.

(E) Efficiency of *Brg1, Cdyl, Kdm6b* and *Sall1* knockdown in OPCs was examined by qRT-PCR.

(F) Expression of *Mbp, Cnp, Plp* and *Hes5* was measured in OPCs with *Brg1, Cdyl, Kdm6b* or *Sall1* siRNA knockdown as indicated.

(G–I) Genome browser visualization of H3K27Ac, H3K9me3, Olig2 or Brg1 targeting sites on the gene loci of differentiation inhibitors *Hes1* (G), *PDGFR*α (H) and β-actin (Actb) (I) during OPC differentiation.

Fold changes in D, E and F over control are derived from three experiments (n = 3). All the data were presented as mean ± SEM. * $p < 0.01$, * * $p < 0.001$; Student *t*-test (D, E); one-way ANOVA (F). See also Figures S6 and S7.