DNA methylation in oligodendroglial cells during developmental myelination and in disease

Sarah Moyon\textsuperscript{a} and Patrizia Casaccia\textsuperscript{a,b,c}

\textsuperscript{a}Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA; \textsuperscript{b}Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA; \textsuperscript{c}Neuroscience Initiative Advanced Science Research Center, CUNY, New York, NY, USA

ABSTRACT

Oligodendrocyte progenitor cells (OPC) are the myelinating cells of the central nervous system (CNS). During development, they differentiate into mature oligodendrocytes (OL) and ensheath axons, providing trophic and functional support to the neurons. This process is regulated by the dynamic expression of specific transcription factors, which, in turn, is controlled by epigenetic marks such as DNA methylation. Here we discuss recent findings showing that DNA methylation levels are differentially regulated in the oligodendrocyte lineage during developmental myelination, affecting both genes expression and alternative splicing events. Based on the phenotypic characterization of mice with genetic ablation of DNA methyltransferase 1 (\textit{Dnmt1}) we conclude that DNA methylation is critical for efficient OPC expansion and for developmental myelination. Previous work suggests that in the context of diseases such as multiple sclerosis (MS) or gliomas, DNA methylation is differentially regulated in the CNS of affected individuals compared with healthy controls. In this commentary, based on the results of previous work, we propose the potential role of DNA methylation in adult oligodendroglial lineage cells in physiologic and pathological conditions, and delineate potential research approaches to be undertaken to test this hypothesis. A better understanding of this epigenetic modification in adult oligodendrocyte progenitor cells is essential, as it can potentially result in the design of new therapeutic strategies to enhance remyelination in MS patients or reduce proliferation in glioma patients.

KEYWORDS

alternative splicing; cell cycle exit; DNA methylation; gliomas; multiple sclerosis; myelination; oligodendrocyte progenitor cells; oligodendrocytes; remyelination

Introduction

Oligodendrocyte progenitor cells (OPC) are widely recognized by the expression of platelet-derived growth factor \(\alpha\) receptor (PDG\(\alpha\)R) and chondroitin sulfate proteoglycan (Cspg4 or NG2).\textsuperscript{1,2} Differentiation into mature oligodendrocytes is regulated by the successive expression of transcription factors (recently reviewed by K\"uspert et al.\textsuperscript{3}). Briefly, OPC are specified by the combined expression of \textit{Ascl1}, \textit{Dlx1/Dlx2} and \textit{Olig1/Olig2}, then \textit{Sox10} and \textit{Nkx2.2}.\textsuperscript{4-8} Upon differentiation, cells express a different set of transcription factors-\textit{Sox17}, \textit{Myt1}, \textit{Yy1}, \textit{Myrf} and \textit{Zfp191},\textsuperscript{9-13} as well as new membrane markers such as Gal-C, CNPase, MBP, PLP, MAG and MOG.\textsuperscript{4-17} The mature OLs take on a more complex morphology, forming condensed myelin sheaths around adjacent axons for metabolic support and functional conduction.

Epigenetic modifications represent another layer of regulation of OL differentiation. Genomic DNA is compacted into nucleosomes that are further assembled into a higher degree structure inside the nucleus.\textsuperscript{18} This structure can be modified by epigenetic marks that enable or prevent accessibility of transcription factors to the genome.\textsuperscript{19} We and others have shown that various epigenetic phenomena, including histone modifications, microRNAs and chromatin remodelers are required for OPC differentiation, during myelination and remyelination\textsuperscript{20-24} (recently reviewed by Moyon et al.\textsuperscript{25}). Another epigenetic mark, DNA methylation, has been shown to be particularly important for brain development and function,\textsuperscript{26-30} but its role has been studied almost exclusively in neurons and astrocytes.\textsuperscript{31,32} In particular, DNA demethylation of lineage specific genes and subsequent transcriptional activation has been proposed for neuronal, astrocytic, and Schwann cell differentiation.\textsuperscript{26,33,34} Therefore, we aimed at further characterizing the role of DNA methylation in the
oligodendroglial lineage and in associated pathologies (i.e., MS), by combining histological and bioinformatic analysis on animal models and human tissues.35,36

Here, we review several studies on the dysregulation of DNA methylation in disease-affected tissues and more recent findings describing the role of DNA methylation in neonatal oligodendrocyte progenitor cells during developmental myelination. We then propose a potential beneficial role for DNA methylation in adult oligodendroglial lineage cells, which - if better characterized - might allow the development of new therapeutic strategies for myelin regeneration in the adult brain.

**DNA methylation is differentially regulated in diseases**

Alterations in DNA methylation have been implicated in oligodendroglial pathologies, suggesting an important role for DNA methylation in oligodendrocyte function. In addition to neuropathy, dementia and hearing loss, patients with DNA methyltransferases (DNMT1) mutations presented slight CNS hypomyelination.37 Additionally, several studies in gliomas have described an extensive global DNA hypomethylation associated with aberrant activation of genes and non-coding regions38-40 but also site-specific DNA hypermethylation that could contribute to tumorigenesis by silencing tumor suppressor genes.41,42

Recently, our laboratory has provided the first evidence of DNA methylation changes in post-mortem tissues from patients affected by multiple sclerosis (MS), a common immune-mediated demyelinating disease.35 We performed in parallel RNA-Sequencing and whole-genome bisulfite sequencing to directly address and correlate the transcriptomic and methylomic changes occurring in MS-affected brain tissues compared with controls. Genes known to regulate oligodendrocyte survival (i.e. BCL2L2, NDRG1) were hypermethylated and downregulated, whereas genes implicated in proteolytic processing (i.e., LGMN, CTSZ) were hypomethylated and upregulated in MS tissues. Our findings suggested that DNA methylation changes in OPC and OL contributed to MS pathology, possibly by affecting demyelination. To address the role of DNA methylation on OPC and OL gene expression and function we turned to cell-specific genetic approaches.

**DNA methylation is a key regulator of oligodendrocyte differentiation during developmental myelination**

Previous studies have shown that specific mature myelin genes (e.g., Mag) were demethylated upon oligodendrocyte differentiation but that blockade of DNA methylation enzymes during rat CNS development delays myelination, which suggested a more complex role of DNA methylation in the oligodendroglial lineage.43,44 Our recent work addressed in detail the role of DNA methylation of OPC differentiation into OL during developmental myelination by combining whole-genome transcriptomic and methylomic analysis with loss-of-function experiments using conditional knockout mouse models.36

We first observed that DNA methylation levels (5-mC levels) and expression of DNA methyltransferases (e.g., DNMT1 and DNMT3A) were dynamically regulated during the transition from OPC to OL in development. We then identified genome-wide changes in DNA methylation between OPC and OL and overlapped them with transcriptomic changes, revealing a negative correlation between DNA methylation at the promoter region of genes and transcription of those genes. The most significant methylomic and transcriptomic changes between OPC and OL were detected on genes with hypermethylated promoters and decreased expression during OPC differentiation (including genes related to neuronal lineage, cell cycle regulation and proliferation) and on genes with hypomethylated promoters and increased expression during OPC differentiation (including genes related to lipid enzymes enriched in the myelin compartment and myelin components, e.g., Mag). Indeed, DNA methylation at promoter regions is mainly associated with transcriptional repression, either by directly preventing the access of transcription factors to their binding sequence or by recruiting cofactors that modulate the chromatin environment.45,46 For example, the E2F consensus motif contains two CpGs, whose methylation levels affect the affinity for E2F members including activator E2F1 that cannot bind to methylated motif and repressor E2F4 that can bind to one methylated CpG motif.47 Magri et al. have shown that E2F1 binding sites in proliferative OPC are targeted and silenced by E2F4 during their differentiation, suggesting that DNA hypermethylation at these sites could directly modulate TF binding and induce gene repression in the oligodendroglial lineage.48
To define the functional role of DNA methylation in the OL lineage in vivo, we crossed the \textit{Dnmt1}\textsuperscript{fl/fl} line with the \textit{Olig1-cre} line, to target the ablation of \textit{Dnmt1} specifically in OPC. Interestingly, this resulted in severe CNS hypomyelination in mutants, associated with tremors and decreased survival.\textsuperscript{31,34,36} OPC were able to undergo lineage specification correctly, but despite the hypomethylation of myelin genes, they did not precociously differentiate or proliferate, either in vitro or in vivo. In highly proliferative embryonic cells, ablation of \textit{Dnmt1} has been associated with activation of genotoxic stress and apoptosis, eventually leading to embryonic lethality.\textsuperscript{28,49} Here, in OPC lacking \textit{Dnmt1} we detected phosphorylated histone H2AX, a measure of genotoxic stress, but no massive apoptosis, suggesting that altered methylation in OPC was not threatening their survival. In addition, the lack of phenotype observed when crossing the \textit{Dnmt1}\textsuperscript{fl/fl} line with the \textit{Cnp-cre} line to target later stages of OL development, suggest that DNA methylation is essential to activate the first steps of OPC differentiation once they exit cell cycle.

**DNA methylation is modulating alternative splicing events in oligodendrocyte lineage**

Recently, DNA methylation marks have been shown to be correlated with alternative splicing regulation.\textsuperscript{50,51} According to a kinetic model, the elongation rate of RNA polymerase could be directly modulated by chromatin structure, including 5-mC marks.\textsuperscript{52,53} A recruitment model, mediated by binding partners (i.e., MeCP2, HP1 and PRMT5) with DNA methylation-dependent affinities, could also explain the link between methylation and alternative splicing.\textsuperscript{5} Yearim et al. have shown the first direct evidence that DNA methylation regulates alternative splicing, acting as a splicing enhancer or silencer by recruiting splicing factors (i.e., HP1 in their model).\textsuperscript{56} Alternative splicing events are occurring during oligodendrocyte differentiation and this mechanism is necessary for normal myelination.\textsuperscript{7,61} Indeed, further analysis of our RNA-Sequencing have shown that 831 transcripts are alternatively spliced (70% concerning skipped exons) during the transition from OPC to OL in development.\textsuperscript{36} By comparing the transcriptomic profile of control OPC and mutant OPC lacking \textit{Dnmt1}, we identified 994 downregulated and 566 upregulated genes, as well as 341 alternatively spliced transcripts (51% concerning skipped exons and 26% concerning retained introns) in mutant cells compared with control cells. The regions targeted by differential alternative splicing events were associated with massive hypomethylation at the exon-intron boundaries in mutant OPC, suggesting a link between DNA methylation and alternative splicing in oligodendroglial cells. The gene ontology of the alternative spliced transcripts was enriched for genes involved in cell cycle process and myelination which indicated that lack of DNA methylation, in addition to modulate gene expression, might alter alternative splicing events, thus directly affecting oligodendrocyte differentiation.

**Perspectives: DNA methylation a potential target of therapeutic strategies for oligodendroglial pathologies?**

We concluded that the role of DNMT1 in oligodendroglial lineage cells is more complex than originally anticipated and encompasses the regulation of the proliferative state of OPC, as well as a tight coordination between alternative splicing and protein synthesis in the generation of myelinating OL (Fig. 1). The dysregulation of DNA methylation observed in many neurologic pathologies,\textsuperscript{35,37-40,42} and the recapitulative hypothesis, suggesting that adult OPC differentiation during remyelination mimics neonatal OPC differentiation during developmental myelination,\textsuperscript{52,66} let us speculate that DNA methylation might also be essential to adult OPC proliferation and differentiation regulation in the adult CNS. However, the transcriptomic profiles of neonatal OPC and adult OPC differ,\textsuperscript{65} implying that epigenetic marks might accumulated in adult OPC and that DNA methylation might regulate slightly differently their differentiation.

Further epigenome-wide studies should be performed on adult OPC to specifically identify genomic loci that might be hypo- or hyper-methylated during their proliferation and their differentiation, in control conditions and after demyelination or in gliomas. Use of DNA methylation modulatory compounds could address the possibility to directly target and modulate specific gene expression and alternative splicing events in the oligodendroglial cells.\textsuperscript{67,68} From a therapeutic standpoint, it would open new strategies to regulate OPC proliferation or differentiation that would have beneficial implications for oligodendroglial-related pathologies such as MS or glioma.
Abbreviations

CNS Central nervous system  
DNMT DNA methyltransferase  
MS Multiple Sclerosis  
OPC Oligodendrocyte progenitor cell  
OL Oligodendrocyte

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Candice Chapouly and Kamilah Castro for their comments on the manuscript.

Funding

This work was supported by NIH-NINDS R37NS042925 and NS-R0152738 to P.C. and by postdoctoral fellowships from the Paralyzed Veterans of America (3061) and National Multiple Sclerosis Society (FG-1507–04996) to S.M.

References

[1] Nishiyama A, Chang A, Trapp BD. NG2+ glial cells: a novel glial cell population in the adult brain. J Neuropathol Exp Neurol 1999; 58:1113-24; PMID:10560654; https://doi.org/10.1097/00005072-199911000-00001
[2] Pringle NP, Mudhar HS, Collarini EJ, Richardson WD. PDGF receptors in the rat CNS: during late neurogenesis, PDGF alpha-receptor expression appears to be restricted to glial cells of the oligodendrocyte lineage. Development 1992; 115:535-51; PMID:1425339
[3] Küspert M, Wegner M. SomethiNG 2 talk about-Transcriptional regulation in embryonic and adult oligodendrocyte precursors. Brain Res 2016; 1638:167-182
[4] He W, Ingraham C, Rising L, Goderie S, Temple S. Multipotent stem cells from the mouse basal forebrain contribute GABAergic neurons and oligodendrocytes to the cerebral cortex during embryogenesis. J Neurosci 2001; 21:8854-62; PMID:1169597
[5] Lu QR, Sun T, Zhu Z, Ma N, Garcia M, Stiles CD, Rowitch DH. Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. Cell 2002; 109:75-86; PMID:11955448; https://doi.org/10.1016/S0092-8674(02)00678-5
[6] Mei F, Wang H, Liu S, Niu J, Wang L, He Y, Etxeberria A, Chan JR, Xiao L, et al. Stage-specific deletion of Olig2 conveys opposing functions on differentiation and maturation of oligodendrocytes. J Neurosci 2013; 33:8454-62; PMID:23658182; https://doi.org/10.1523/JNEUROSCI.1503-12.2013
[7] Nakatani H, Martin E, Hassani H, Clavairoly A, Maire CL, Viadieu A, Kerninon C, Delmaseur A, Frah M, Weber M, et al. Ascl1/Mash1 Promotes Brain Oligodendrogenesis during Myelination and Remyelination. J Neurosci 2013; 33:9752-9768; PMID:23739972; https://doi.org/10.1523/JNEUROSCI.0805-13.2013
[8] Zhu Q, Zhao X, Zheng K, Li H, Huang H, Zhang Z, Mastrocchi T, Wegner M, Chen Y, Sussel L, et al. Genetic evidence that Nkx2.2 and Pdgfra are major determinants of the timing of oligodendrocyte differentiation in the developing CNS. Dev Camb Engl 2014; 141:548-555

Figure 1. During development, DNA methylation, mediated by DNMT1, regulates oligodendrocyte progenitor cell (OPC) growth arrest and differentiation into mature oligodendrocyte (OL), by modulating gene expression and alternative splicing events. After ablation of Dnmt1, OPC present DNA damage, aberrant alternative splicing and endoplasmic reticulum stress, and fail to differentiate into mature OL, resulting in severe CNS hypomyelination and early death of the mutant mice.
[9] Emery B, Agalli D, Cahoy JD, Watkins TA, Dugas JC, Mulinyawe SB, Ibrahim A, Ligon KL, Rowitch DH, Barres BA. Myelin gene regulatory factor is a critical transcriptional regulator required for CNS myelination. Cell 2009; 138:172-85; PMID:19596243; https://doi.org/10.1016/j.cell.2009.04.031

[10] He Y, Dupree J, Wang J, Sandoval J, Li J, Liu H, Shi Y, Nave KA, Casaccia-Bonnefille P. The transcription factor Yin Yang 1 is essential for oligodendrocyte progenitor differentiation. Neuron 2007; 55:217-30; PMID:17640524; https://doi.org/10.1016/j.neuron.2007.06.029

[11] Howry SY, Avila RL, Emery B, Traka M, Lin W, Watkins T, Cook S, Bronson R, Davission M, Barres BA, et al. ZFP191 is required by oligodendrocytes for CNS myelination. Genes Dev 2010; 24:301-11; PMID:2080941; https://doi.org/10.1101/gad.1864510

[12] Nielsen JA, Berndt JA, Hudson LD, Armstrong RC. Myelin transcription factor 1 (Myt1) modulates the proliferation and differentiation of oligodendrocyte lineage cells. Mol Cell Neurosci 2004; 25:111-23; PMID:14962745; https://doi.org/10.1016/j.mcn.2003.10.001

[13] Sohn J, Natale J, Chew LJ, Belachew S, Cheng Y, Aguirre A, Lytle J, Nait-Oumesmar B, Kerminon C, Kanai-Azuma M, et al. Identification of Sox17 as a transcription factor that regulates oligodendrocyte development. J Neurosci 2006; 26:9722-35; PMID:16988043; https://doi.org/10.1523/JNEUROSCI.1716-06.2006

[14] Dubois-Dalcq M., Behar T., Hudson L, Lazzarini RA. Emergence of three myelin proteins in oligodendrocytes cultured without neurons. J Cell Biol 1986; 102:384-92; PMID:2418030; https://doi.org/10.1083/jcb.102.2.384

[15] Martini R Schachner M. Immunoelectron microscopic localization of neural cell adhesion molecules (L1, NCAM, and MAG) and their shared carbohydrate epitope and myelin basic protein in developing sciatic nerve. J Cell Biol 1986; 103:2439-48; PMID:2430983; https://doi.org/10.1083/jcb.103.6.2439

[16] Solly SK, Thomas JL, Monge M, Demeren C, Lubetzki C, Gardinier MV, Matthieu JM, Zalc B. Myelin/oligodendrocyte glycoprotein (MOG) expression is associated with myelin deposition. Glia 1996; 18:39-49; PMID:8991690; https://doi.org/10.1002/(SICI)1098-1136(199609)18:1<39::AID-GLIA4>3.0.CO;2-Z

[17] Zalc B., Monge M., Dupouey P., Hauw JJ, Baumann NA. Immunohistochemical localization of galactosyl and sulfogalactosyl ceramide in the brain of the 30-day-old mouse. Brain Res 1981; 211:341-54; PMID:7016256; https://doi.org/10.1016/0006-8993(81)90706-X

[18] Gorkin DU, Leung D, Ren B. The 3D genome in transcriptional regulation and pluripotency. Cell Stem Cell 2014; 14:762-775; PMID:24905166; https://doi.org/10.1016/j.stem.2014.05.017

[19] Chen J, Weiss WA. When deletions gain functions: commandeering epigenetic mechanisms. Cancer Cell 2014; 26:160-161; PMID:25117708; https://doi.org/10.1016/j.ccr.2014.07.021

[20] Bischof M., Weider M., Küspert M., Nave K.-A, Wegner M. Brgl1-dependent chromatin remodelling is not essentially required during oligodendroglial differentiation. J. Neurosci. Off. J Soc Neurosci 2015; 35:21-35; https://doi.org/10.1523/JNEUROSCI.1468-14.2015

[21] He D, Marie C, Zhao G, Kim B, Wang J, Deng Y, Clavairoly A, Frah M, Wang H, He X, et al. Chd7 cooperates with Sox10 and regulates the onset of CNS myelination and remyelination. Nat. Neurosci 2016; 19:678-689; PMID:26928066; https://doi.org/10.1038/nn.4258

[22] Liu J, Magri L, Zhang F, Marsh NO, Albrecht S, Huynh JL, Kaur J, Kuhlmann T, Zhang W, Slesinger PA, et al. Chromatin landscape defined by repressive histone methylation during oligodendrocyte differentiation. J Neurosci Off J Soc Neurosci 2015; 35:352-365; https://doi.org/10.1523/JNEUROSCI.2606-14.2015

[23] Shen S, Sandoval J, Swiss VA, Li J, Dupree J, Franklin RJ, Casaccia-Bonnefil P. Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. Nat Neurosci 2008; 11:1024-34; PMID:19160500; https://doi.org/10.1038/nn.2172

[24] Yu Y, Chen Y, Kim B, Wang H, Zhao C, He X, Liu L, Liu W, Wu LM, Mao M, et al. Olig2 targets chromatin remodelers to enhancers to initiate oligodendrocyte differentiation. Cell 2013; 152:248-61; PMID:23332759; https://doi.org/10.1016/j.cell.2012.12.006

[25] Moyon S, Liang J, Casaccia P. Epigenetics in NG2 glia cells. Brain Res 2016; 1638:183-198PMID:26929421; https://doi.org/10.1016/j.brainres.2015.06.009

[26] Fan G, Beard C, Chen RZ, Csankovszki G, Sun Y, Sinaia M, Biniszkwicz D, Bates B, Lee PP, Kuhn R, et al. DNA hypomethylation perturbs the function and survival of CNS neurons in postnatal animals. J Neurosci 2001; 21:788-97; PMID:11157065

[27] Hutmick LK, Golshani P, Namihira M, Xue Z, Matynia A, Yang XW, Silva AJ, Schweizer FE, Fan G. DNA hypomethylation restricted to the murine forebrain induces cortical degeneration and impairs postnatal neuronal maturation. Hum Mol Genet 2009; 18:2875-88; PMID:19433415; https://doi.org/10.1093/hmg/ddp222

[28] Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell 1992; 69:915-26; PMID:1606615; https://doi.org/10.1016/0092-8674(92)90611-F

[29] Milutinovic S, Zhuang Q, Niveleau A, Szyf M. Epigenetic stress checkpoint. Mol Cell Biol 2006; 26:7575-86; PMID:17015478; https://doi.org/10.1074/mcb.M213219200

[30] Unterberger A, Andrews SD, Weaver IC, Szyf M. DNA methyltransferase 1 knockdown activates a replication stress checkpoint. Mol Cell Biol 2006; 26:7575-86; PMID:17015478; https://doi.org/10.1128/MCB.01887-05

[31] Fan G, Martinowich K, Chin MH, He F, Fouse SD, Hutnick L, Hattori D, Ge W, Shen Y, Wu H, et al. DNA
methylation controls the timing of astrogliogenesis through regulation of JAK-STAT signaling. Dev Camb Engl 2005; 132:3345-3356

[32] Wu Z, Huang K, Yu J, Le T, Namihira M, Liu Y, Zhang J, Xue Z, Cheng L, Fan G. Dnm3ta regulates both proliferation and differentiation of mouse neural stem cells. J Neurosci Res 2012; 90:1883-1891; PMID:22714992; https://doi.org/10.1002/jnr.23077

[33] Takizawa T, Nakashima K, Namihira M, Ochiai W, Uemura A, Yanagisawa M, Fujita N, Nakao M, Taga T. DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. Dev Cell 2001; 1:749-758; PMID:11740937; https://doi.org/10.1016/S1534-5807(01)00101-0

[34] Varela-Rey M, Iruarrizaga-Lejarreta M, Lozano JJ, Arañasay AM, Fernandez AF, Lavin JL, Mösén-Ansoarena D, Berdasco M, Turmaine M, Luka Z, et al. S-adenosylmethionine levels regulate the schwann cell DNA methylome. Neuron 2014; 81:1024-1039; PMID:24607226; https://doi.org/10.1016/j.neuron.2014.01.037

[35] Huynh JL, Garg P, Thin TH, Yoo S, Dutta R, Trapp BD, Haroutunian V, Zhu J, Donovan MJ, Sharp AJ, et al. Epigenome-wide differences in pathology-free regions of multiple sclerosis-affected brains. Nat Neurosci 2014; 17:121-130; PMID:24270187; https://doi.org/10.1038/nn.3588

[36] Moyon S, et al. Functional characterization of DNA Methylation in the Oligodendrocyte Lineage. Cell Rep 2016; 15:748-760; https://doi.org/10.1016/j.celrep.2016.03.060

[37] Klein CJ, Botuyan MV, Wu Y, Ward CJ, Nicholson GA, Hamsman S, Hojo K, Yamanishi H, Karpf AR, Wallace DC, et al. Mutations in DNMT1 cause hereditary sensory neuropathy with dementia and hearing loss. Nat Genet 2011; 43:595-600; PMID:21532572; https://doi.org/10.1038/ng.830

[38] Chou AP, Chowdhury R, Li S, Chen W, Kim AJ, Piccioni Klein CJ, Botuyan MV, Wu Y, Ward CJ, Nicholson GA, Hamsman S, Hojo K, Yamanishi H, Karpf AR, Wallace DC, et al. Identification of retinol binding Protein 1 promoter hypermethylation in isocitrate dehydrogenase 1 and 2 mutations in oligodendroglial tumours. Neurophathol Appl Neurobiol 2006; 32:517-524; PMID:16972885; https://doi.org/10.1111/j.1365-2990.2006.00759.x

[39] Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis 2010; 31:27-36; PMID:19752007; https://doi.org/10.1093/carcin/bgp220

[40] Felsberg J, Yan PS, Huang TH, Milde U, Schramm J, Wiestler OD, Reifenberger G, Pietsch T, Waha A. DNA methylation and allelic losses on chromosome arm 14q in oligodendrogial tumours. Neupatroph Appl Neurobiol 2006; 32:517-524; PMID:16972885; https://doi.org/10.1111/j.1365-2990.2006.00759.x

[41] Uhlmann K, Rohde K, Zeller C, Szyms J, Vogel S, Marczinke K, Thiel G, Nürnberg P, Laird PW. Distinct methylation profiles of glioma subtypes. Int J Cancer J Int Cancer 2003; 106:52-59; https://doi.org/10.1002/ijc.11175

[42] Watanabe Y, Maekawa M. Methylation of DNA in cancer. Adv Clin Chem 2010; 52:145-167; PMID:21275343; https://doi.org/10.1016/S0065-2423(10)52006-7

[43] Grubinska B, Laszkiewicz I, Royland J, Wiggins RC, Konat GW. Differentiation-specific demethylation of myelin associated glycoprotein gene in cultured oligodendrocytes. J Neurosci Res 1994; 39:233-242; PMID:7532722; https://doi.org/10.1002/jnr.490390302

[44] Ransom BR, Yamate CL, Black JA, Waxman SG. Rat optic nerve: disruption of gliogenesis with 5-azacytidine during early postnatal development. Brain Res 1985; 337:41-49; PMID:2408709; https://doi.org/10.1016/0006-8993(85)91607-5

[45] Schüeber D. Function and information content of DNA methylation. Nature 2015; 517:321-326; PMID:25592537; https://doi.org/10.1038/nature14192

[46] Smith ZD, Meissner A. DNA methylation: roles in mammalian development. Nat Rev Genet 2013; 14:204-220; PMID:23400093; https://doi.org/10.1038/nrg3354

[47] Campanero MR, Armstrong MI, Flemington EK. CpG methylation as a mechanism for the regulation of E2F activity. Proc Natl Acad Sci USA 2000; 97:6481-6486; PMID:10823896; https://doi.org/10.1073/pnas.100340697

[48] Magri L, Swiss VA, Jablonska B, Lei L, Pedre X, Walsh M, Zhang W, Gallo V, Canoll P, Casaccia P. E2F1 coregulates cell cycle genes and chromatin components during the transition of oligodendrocyte progenitors from proliferation to differentiation. J Neurosci Off Soc Neurosci 2014; 34:1481-1493; https://doi.org/10.1523/JNEUROSCI.2840-13.2014

[49] Unterberger A, Andrews SD, Weaver IC, Szyf M. DNA methyltransferase 1 knockdown activates a replication stress checkpoint. Mol Cell Biol 2006; 26:7575-86; PMID:17005458; https://doi.org/10.1128/MCB.01887-05

[50] Gelfman S., Cohen N., Yearim A, Ast G. DNA-methylation effect on cotranscriptional splicing is dependent on GC architecture of the exon-intron structure. Genome Res 2013; 23:789-799; PMID:23502848; https://doi.org/10.1101/gr.143503.112

[51] Wan J, Oliver VF, Zhu H, Zack DJ, Qian J, Merbs SL. Integrative analysis of tissue-specific methylation and alternative splicing identifies conserved transcription factor binding motifs. Nucleic Acids Res 2013; 41:8503-8514; PMID:23887936; https://doi.org/10.1093/nar/gkt652

[52] Jimeno-González S, Payán-Bravo L, Muñoz-Cabello AM, Guijo M, Gutierrez G, Prado F, Reyes JC. Defective histone supply causes changes in RNA polymerase II elongation rate and cotranscriptional pre-mRNA splicing. Proc Natl Acad Sci USA 2015; 112:14840-14845; https://doi.org/10.1073/pnas.1506760112

[53] Saint-André V, Batsché E, Racche C, Muchardt C. Histone H3 lysine 9 trimethylation and HP1α favor inclusion of alternative exons. Nat Struct Mol Biol 2011; 18:337-344; https://doi.org/10.1038/nsmb.1995

[54] Bezzi M, Teo SX, Muller J, Mok WC, Sahu SK, Vardy LA, Bonday ZQ, Guccione E. Regulation of constitutive and alternative splicing by PRMT5 reveals a role for Mdm4 pre-mRNA in sensing defects in the spliceosomal machinery. Genes Dev 2013; 27:1903-1916; PMID:24013503; https://doi.org/10.1101/gad.219899.113
[55] Maunakea AK, Chepelev I, Cui K, Zhao K. Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. Cell Res 2013; 23:1256-1269; PMID:23938295; https://doi.org/10.1038/cr.2013.110

[56] Yearim A, Gelfman S, Shayevitch R, Melcer S, Glaich O, Mallm JP, Nissim-Rafinia M, Cohen AH, Rippe K, Meshorer E, et al. HP1 is involved in regulating the global impact of DNA methylation on alternative splicing. Cell Rep 2015; 10:1122-1134; PMID:25704815; https://doi.org/10.1016/j.celrep.2015.01.038

[57] de Ferra F, Engh H, Hudson L, Kamholz J, Puckett C, Molineaux S, Lazzarini RA. Alternative splicing accounts for the four forms of myelin basic protein. Cell 1985; 43:721-727; PMID:2416470; https://doi.org/10.1016/0092-8674(85)90245-4

[58] Jordan CA, Friedrich VL, Jr, de Ferra F, Weismiller DG, Holmes KV, Dubois-Dalcq M. Differential exon expression in myelin basic protein transcripts during central nervous system (CNS) remyelination. Cell Mol Neurobiol 1990; 10:3-18; PMID:1692262; https://doi.org/10.1007/BF00736361

[59] Kevelam SH, Taube JR, van Spaendonk RM, Bertini E, Sperle K, Tarnopolsky M, Tonduti D, Valente EM, Travaglini L, Sistermans EA, et al. Altered PLP1 splicing causes hypomyelination of early myelinating structures. Ann Clin Transl Neurol 2015; 2:648-661; PMID:26152040; https://doi.org/10.1002/acn3.203

[60] Nave KA, Lai C, Bloom FE, Milner RJ. Splice site selection in the proteolipid protein (PLP) gene transcript and primary structure of the DM-20 protein of central nervous system myelin. Proc Natl Acad Sci USA 1987; 84:5665-9; PMID:2441390; https://doi.org/10.1073/pnas.84.16.5665

[61] Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O’Keefe S, Phatnani HP, Guarnieri P, Caneda C, Rudersch N, et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci Off J Soc Neurosci 2014; 34:11929-11947; https://doi.org/10.1523/JNEUROSCI.1860-14.2014

[62] Fancy SP, Zhao C, Franklin RJ. Increased expression of Nkx2.2 and Olig2 identifies reactive oligodendrocyte progenitor cells responding to demyelination in the adult CNS. Mol Cell Neurosci 2004; 27:247-54; PMID:15519240; https://doi.org/10.1016/j.mcn.2004.06.015

[63] Fancy SP, Baranzini SE, Zhao C, Yuk DI, Irvine KA, Kaing S, Sanai N, Franklin RJ, Rowitch DH, et al. Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. Genes Dev 2009; 23:1571-85; PMID:19515974; https://doi.org/10.1101/gad.1806309

[64] Koennig M, Jackson S, Hay CM, Faux C, Kilpatrick TJ, Willingham M, Emery B, et al. Myelin gene regulatory factor is required for maintenance of myelin and mature oligodendrocyte identity in the adult CNS. J Neurosci 2012; 32:12528-42; PMID:22956843; https://doi.org/10.1523/JNEUROSCI.1069-12.2012

[65] Moyon S, Dubessy AL, Aigrot MS, Trotter M, Huang JK, Dauphinot L, Potier MC, Kerninon C, Melik Parsaadiantz S, Franklin RJ, et al. Demyelination causes adult CNS progenitors to revert to an immature state and express immune cues that support their migration. J Neurosci Off J Soc Neurosci 2015; 35:4-20; https://doi.org/10.1523/JNEUROSCI.0849-14.2015

[66] Zhao C, Ma D, Zawadzka M, Fancy SP, Elis-Williams L, Bouvier G, Stockley JH, de Castro GM, Wang B, Jacobs S, et al. Sox2 Sustains recruitment of oligodendrocyte progenitor cells following CNS demyelination and primes them for differentiation during remyelination. J Neurosci 2015; 35:11482-11499; PMID:26290228; https://doi.org/10.1523/JNEUROSCI.3655-14.2015

[67] Choudhury SR, Cui Y, Lubecka K, Stefanska B, Iru-dayaraj J. CRISPR-dCas9 mediated TET1 targeting for selective DNA demethylation at BRCA1 promoter. Oncotarget 2016; 7(29):46545-46556; PMID:25261375; https://doi.org/10.18632/oncotarget.10234

[68] McDonald JI, Celik H, Rois LE, Fishberger G, Fowler T, Rees R, Kramer A, Martens A, Edwards JR, Challen GA. Reprogrammable CRISPR/Cas9-based system for inducing site-specific DNA methylation. Biol Open 2016; 5(6):866-74; PMID:27170255; https://doi.org/10.1242/bio.019067