Age-related Changes in the Global DNA Methylation Profile of Oligodendrocyte Progenitor Cells Derived from Rat Spinal Cords*

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Summary: Demyelination of axons plays an important role in the pathology of many spinal cord diseases and injuries. Remyelination in demyelinated lesions is primarily performed by oligodendrocyte progenitor cells (OPCs), which generate oligodendrocytes in the developing and mature central nervous system. The efficiency of remyelination decreases with age. Many reports suggest that this decline in remyelination results from impaired OPC recruitment and differentiation during aging. Of the various molecular mechanisms involved in aging, changes in epigenetic modifications have received particular attention. Global DNA methylation is a major epigenetic modification that plays important roles in cellular senescence and organismal aging. Thus, we aimed to evaluate the dynamic changes in the global DNA methylation profiles of OPCs derived from rat spinal cords during the aging process. We separated and cultured OPCs from the spinal cords of neonatal, 4-month-old, and 16-month-old rats and investigated the age-related alterations of genomic DNA methylation levels by using quantitative colorimetric analysis. To determine the potential cause of dynamic changes in global DNA methylation, we further analyzed the activity of DNA methyltransferases (DNMTs) and the expression of DNMT1, DNMT3a, DNMT3b, TET1, TET2, TET3, MBD2, and MeCP2 in the OPCs from each group. Our results showed the genomic DNA methylation level and the activity of DNMTs from OPCs derived from rat spinal cords decreased gradually during aging, and OPCs from 16-month-old rats were characterized by global hypomethylation. During OPC aging, the mRNA and protein expression levels of DNMT3a, DNMT3b, and MeCP2 were significantly elevated; those of DNMT1 were significantly down-regulated; and no significant changes were observed in those for TET1, TET2, TET3, or MBD2. Our results indicated that global DNA hypomethylation in aged OPCs is correlated with DNMT1 downregulation. Together, these data provide important evidence for partly elucidating the mechanism of age-related impaired OPC recruitment and differentiation and assist in the development of new treatments for promoting efficient remyelination.
Key words: T2 mapping; Duchenne muscular dystrophy; skeletal muscle; fat infiltration

Demyelination of axons is a common feature of many spinal cord diseases and injuries that affect a large number of patients[1]. In the central nervous system (CNS), oligodendrocytes are responsible for myelinating neuronal axons and are essential for functional and efficient neuronal signaling. Oligodendrocytes are highly vulnerable to insults due to CNS injury and disease, such as inflammation, oxidative stress, and elevated glutamate levels leading to excitotoxicity[2].

Remyelination involves the generation of new mature oligodendrocytes. It is well accepted that new oligodendrocytes derive from a population of CNS/precursor cells, most often referred to as oligodendrocyte progenitor cells (OPCs), during development and in adulthood[3]. There is a pool of adult OPCs in the CNS that responds to demyelinating insults by differentiating into mature oligodendrocytes[4]. In an attempt to remyelinate axons, OPCs residing in the

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CNS are recruited to the lesion site and differentiate to oligodendrocytes for myelinating denuded axons[5]. However, endogenous OPCs are not sufficient to repair the demyelination that occurs after demyelinating insults[6]. Increasing data have revealed that recruitment and differentiation of OPCs are impaired with age, which results in the decline of remyelination efficiency and remyelination failure[7]. However, the molecular mechanisms responsible for the age-related impairment of recruitment and differentiation of OPCs are not completely understood.

Aging is a complex phenomenon associated with physiological, biochemical, and functional changes of cells and organs that involves various molecular mechanisms such as accumulation of mutations, oxidative stress, telomere-shortening, and alteration of molecular pathways[8, 9]. In particular, there is a growing perception that epigenetic modifications, including DNA methylation and histone modification, play an important role in the aging process[10]. Recent studies demonstrated that abnormal histone modulation of OPC differentiation contributed to the age-dependent decline in remyelination efficiency[11]. DNA methylation is a major epigenetic mechanism that plays an important role in various biological processes including embryonic development, imprinting, X chromosome inactivation, and stem cell differentiation[12, 13]. DNA methylation patterns mainly include global methylation and localized methylation at promoter CpG island regions of genes. Global DNA methylation has been associated with genomic stability[14, 15]. A number of studies have uncovered links between changes in global DNA methylation and cellular senescence and organismal aging[16, 17]. Therefore, age-dependent impairment of OPC recruitment and differentiation may be related to aberrations in global DNA methylation of OPCs during aging. Identifying the characteristics of dynamic changes in global DNA methylation profiles of OPCs during the aging process will help to explain the age-dependent impairment of recruitment and differentiation of OPCs.

DNA methylation is catalyzed mainly by DNA methyltransferases (DNMTs). DNMTs are responsible for maintaining methylation status in the genome. In mammals, the DNMT family comprises DNMT1, DNMT3a, and DNMT3b. DNMT1 has a high preference for hemimethylated DNA and copies pre-existing methylation patterns onto the new DNA strand during DNA replication[18]. DNMT3a and DNMT3b modify both unmethylated and hemimethylated DNA and are mainly responsible for de novo methylation[19]. DNA methylation and demethylation are two opposing epigenetic processes in genome regulation, and the balance between them may help determine global DNA methylation profiles in a given cell type. Accordingly, various enzymes involved in DNA demethylation affect dynamic regulation of DNA methylation. In the past decade, several methyl-CpG binding proteins (MBDs) have been identified, including MBD1, MBD2, MBD3, MBD4, MeCP2, and Kaiso[20]. MeCP2 and MBD2 can bind methylated DNA and suppress transcription from a methylated target gene, which seems to actively demethylate DNA[21]. Recently, the ten-eleven translocation (TET) family proteins, including Tet1, Tet2, and Tet3, were shown to convert the covalent epigenetic mark 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine and further oxidize 5-hydroxymethylcytosine to 5-formylcytosine and 5-carboxylcytosine, which could eventually be removed from the genome. This newly discovered mechanism of demethylation is important and consistent mechanism for active DNA demethylation.

In the present study, we aimed to evaluate the dynamic changes in global DNA methylation profiles of OPCs derived from rat spinal cords during the aging process. We separated and cultured OPCs from the spinal cords of neonatal, 4-month-old, and 16-month-old rats and investigated the age-related alterations of genomic DNA methylation level in OPCs using a quantitative colorimetric assay. To determine the potential cause of dynamic changes in global DNA methylation of OPCs during aging, we further analyzed the activity of DNMTs and the expression of DNMT1, DNMT3a, DNMT3b, TET1, TET2, TET3, MBD2, and MeCP2 in each group. Our results showed the genomic DNA methylation level and the activity of DNMTs from OPCs derived from rat spinal cords decreased gradually and the global DNA hypomethylation is correlated with DNMT1 downregulation. To our knowledge, this is the first study to report the dynamic changes in global DNA methylation profiles of OPCs derived from rat spinal cords during aging.

1 MATERIALS AND METHODS

1.1 Isolation and Culture of OPCs

OPCs were purified from the spinal cords of neonatal (1-day-old), 4-month-old, and 16-month-old rats by sequential immunopanning as reported previously with minor modifications[22]. All procedures were approved by the Institutional Animal Care and Use Committee at Huazhong University of Science and Technology. Briefly, spinal cords were dissected from the rats, and the meninges were carefully removed from the spinal cord dissections. All tissues were minced and dissociated with collagenase (4.43 mg/mL, 30 min at 37°C) and papain (22.5 U/mL, 45 min at 37°C). After enzymatic treatment, DNase (500 μg/mL) was added, and cells were centrifuged (5 min at 1000 r/min). Cells then were mechanically dissociated using a 21 g needle. The digestion was stopped by the addition of an equal volume of DMEM containing

20% fetal bovine serum. Tissues were dissociated by repeated trituration with fire-polished Pasteur pipettes and were filtered through 70 μm nylon mesh. Finally, progenitor cells were purified by immunopanning, using first negative selection on dishes coated with the Ran-2 antibody (Millipore, USA) to remove type-1 astrocytes and meningeal cells and with the anti-galactocerebroside antibody (Santa Cruz, USA) to remove oligodendrocytes. Cells were then transferred to an A2B5-coated dish (Millipore) for 45 min to select OPCs. The purified OPCs were removed from the A2B5 dish with trypsin and plated on poly-D-lysine-coated tissue culture dishes with DMEM/F12 medium (GIBCO-BRL, USA) containing 1× N2 (GIBCO-BRL) and 1× B27 (GIBCO-BRL) supplements, fibroblast growth factor 2 (20 ng/mL, eBioscience, USA), platelet-derived growth factor α (10 ng/mL, eBioscience), insulin (5 μg/mL, Sigma-Aldrich, Canada) and bovine serum albumin (BSA, 0.1%). Cells were fed on fresh growth medium every other day, and half of the medium was replaced at subsequent feedings. After culturing for 3 days, the OPCs were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 min at room temperature. The cells were then permeabilized in PBS containing 5% BSA and 0.1% Triton X-100 for 1 h at room temperature. Cells were double-labeled with mouse A2B5 antibody (1:400, Millipore) and rabbit Ran-2 antibody (1:400, Millipore) diluted in PBS containing 5% BSA and 0.1% Triton X-100 overnight at 4°C. Then, cells were incubated in fluorescein isothiocyanate-conjugated goat anti-mouse and goat anti-rabbit secondary antibodies (Santa Cruz, USA) diluted 1:300 in PBS at 37°C for 30 min in the dark and stained in DAPI for 2 min with PBS washes. As a negative control, cells were processed in the absence of the primary antibody, and no staining was observed.

1.2 DNMT Activity Assay

OPCs from various groups were harvested, and nuclear extracts were generated using the EpiQuik Nuclear Extraction Kit (Epigentek, USA) according to the manufacturer's protocol. Protein concentrations for nuclear extracts were determined using the BCA protein assay kit (Thermo Fisher Scientific/Pierce, USA). Total DNMT activity was determined using 20 μg total protein and the EpiQuik DNMTs Activity/Inhibition Assay Kit (Epigentek) as recommended by the vendor. The total DNMT activity (absorbance, A) was calculated as follows: Activity (A/h/mg) = ($A_{\text{Sample}} - A_{\text{Blank}}$)/(μg protein × initial incubation time in h) × 1000. All samples were analyzed in duplicate.

1.3 DNA Isolation

Genomic DNA was extracted from OPCs from each group using the kits from Epigentek. Cells were lysed with SDS in a nuclei lysis buffer and treated with RNase A (final 133 μg/mL) and RNase T1 (final 20 U/mL) to remove RNA. Proteins were coprecipitated with NaCl (330 μL saturated NaCl added per 1 mL solution) by centrifugation. Genomic DNA was recovered from the supernatant by precipitation with 100% ethanol, washed in 70% ethanol, and dissolved in the Tris-EDTA buffer (pH=8.0). The extracted DNA was quantified with a dsDNA quantitation kit (Epigentek) following the vendor's instructions.

1.4 Global DNA Methylation Quantification

Genomic DNA from each group was used to quantify the global DNA methylation levels using Methylamp Global DNA Methylation Quantification kit (Epigentek, USA) according to the manufacturer's protocol. The amount of 5-mC within each sample was determined by a colorimetric assay with detection by a microplate reader at 450 nm. Both a blank control and a methylated standard control were analyzed together with the DNA samples. After subtracting blank readings from the readings for both the sample and the standard, the DNA methylation value for each sample was calculated as the ratio of A_{sample} to A_{standard}. All samples were analyzed in duplicate.

1.5 Real-time RT-PCR

Total cellular RNA was extracted by TRIzol Reagent (Invitrogen, USA). The RNA extracts were resuspended in 15 μL RNase-free water, and the RNA concentration was adjusted to 1 μg/μL and used for cDNA synthesis. The final reaction volume was 25 μL consisting of 12.5 μL Maxima SYBR Green master mix (Fermentas, Thermo Scientific, USA), 0.5 μL each specific primer (10 μmol/L), 1 μL cDNA template, and 10.5 μL nuclease-free H_2O. The cycling conditions were 5 min at 94°C followed by 40 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C. The number of cDNA molecules was normalized to that of β-Actin (ACTB). PCR amplification was carried out using the sets of primers designed by PerlPrimer: 5′-GAGGTGGGCGACTGCGTCTC-3′ and 5′-TGTG GATGTAGGAAAGTTGCA-3′ for DNMT1; 5′-CAGA ATAGCCAAGTTCAGCAAAGTGA-3′ and 5′-CTTTG CCCTGCTTTATGGAG-3′ for DNMT3a; 5′-GTTAAA GAAAGTACAGACAATAAC CAC-3′ and 5′-TCTGA TGACTGGCACACTCC-3′ for DNMT3b; 5′- CCACA ACGAATGAATGAACAG-3′ and 5′-GACCAACTCC TTGAAGACCT-3′ for MBD2; 5′-AAGCAGAGACAT CAGAAGG-3′ and 5′-TCCCTCTCCCAGTTACC-3′ for MeCP2; 5′-GTAGACGCTG GAACAAGTGCATC A-3′ and 5′-ACTTGGATGACGTTTGGGTGGTCT-3′ for TET1; 5′-TTGCCCGAATTGAATCCAGCAGCA-3′ and 5′-TAAGCCTCCCGACCTCTTCATGTT-3′ for TET2; 5′-CGCCAACCAGAAGGTCAGTAGT-3′ and 5′-TGGTGATCCTCTCGTGCTCAGT-3′ for TET3; and 5′-TGACCCAGATCATGTTTGAG-3′ and 5′-ATC TCTTGCTCGAAGTCCAG-3′ for ACTB.

1.6 Western Blotting

Cells were lysed in RIPA buffer with a protease

inhibitor cocktail (Sigma, USA). Total cell lysates were resolved by SDS-PAGE and transferred to PVDF membranes (Millipore, USA), and membranes were blocked and incubated with primary antibodies against DNMT1, DNMT3a, DNMT3b, MBD2, MeCP2, TET1, TET2, or TET3 (Santa Cruz) at 4°C overnight. Antibody binding was detected with horseradish peroxidase-conjugated antibodies (Boshide, China) and enhanced chemiluminescence (Millipore, USA). The target protein expression was normalized to GAPDH expression. Quantification of the bands was performed using Gel Analysis V2.02 software (Clin Science Instruments, China).

1.7 Statistical Analyses

All data are presented as mean±standard deviations (SD). Statistical analysis was performed using ANOVA. Differences with a *P*-value less than 0.05 were considered statistically significant. Statistical analysis was performed with SPSS 11.0 for Windows.

2 RESULTS

2.1 Age-associated Global DNA Methylation Level Changes in OPCs Isolated from Rat Spinal Cords

In this study, OPCs separated and cultured from rat spinal cords exhibited bipolar or tripolar morphology and could be subsequently expanded for multiple passages *in vitro*. Immunofluorescent staining showed that more than 90% of these cells expressed A2B5, and <10% expressed Ran-2 (fig. 1). To evaluate the age-associated global DNA methylation level changes in OPCs, we measured the amount of 5-mC in neonatal, 4-month-old, and 16-month-old rats. Data were normalized to values obtained for the methylation standard and plotted as a percentage of the standard control (fig. 2). The global DNA methylation levels decreased slightly from 34.15% for the neonatal rats to 30.46% for 4-month-old rats (P>0.05). However, the total 5-mC content significantly decreased in 16-month-old rats (20.95%) compared to the neonatal rats (P<0.05). This result demonstrated a gradual decrease in the global DNA methylation level in OPCs from the spinal cords of rats during aging. Notably, OPCs from elderly rats (16-month-old) were characterized by global hypomethylation.

2.2 Age-associated Total DNMT Activity Changes in OPCs Isolated from Rat Spinal Cords

We measured the total DNMT activity of OPCs from neonatal, 4-month-old, and 16-month-old rats using an activity/inhibition assay. The total DNMT activity of OPCs in neonatal rats was 9.49 A/h/mg, while that was reduced to 5.22 *A*/h/mg and 4.18 *A*/h/mg for 4-month-old and 16-month-old rats, respectively (fig. 3). There was significant difference between neonatal rats and 4-month-old rats (P<0.05) or neonatal rats and 16-month-old rats (P<0.05), but no significant difference was found between 4-month-old rats and 16-month-old rats. Our data indicated that the total activity of DNMTs in OPCs decreased gradually during aging.

2.3 Changes in the Expression Levels of DNMTs, MBDs, and TETs in OPCs during Aging

To determine the potential reason for dynamic changes in global DNA methylation of OPCs during the aging process, we further examined the expression levels of DNMT1, DNMT3a, DNMT3b, MeCP2,

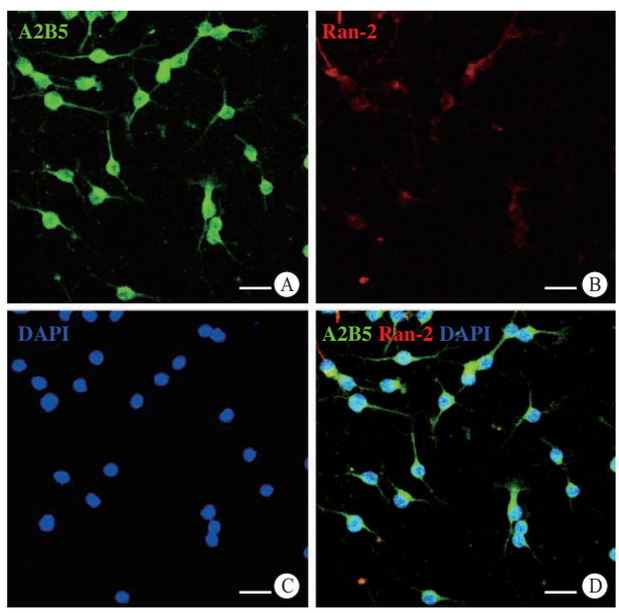

Fig. 1 Triple immunofluorescence staining for A2B5, Ran-2, and DAPI

Triple immunofluorescence staining for A2B5, Ran-2, and DAPI was performed in oligodendrocyte progenitor cells (OPCs) isolated and cultured from rat spinal cords *in vitro*. Green and red represent anti-A2B5 and anti-Ran-2 antibody immunofluorescence, respectively (A, B). The nuclei were counterstained with DAPI (C). Most purified OPCs were A2B5 positive (A) and Ran-2 negative (B). Scale bar: 20 μm

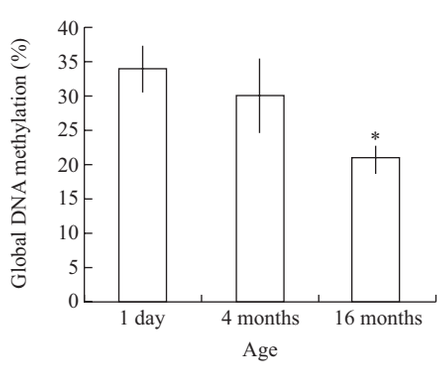

Fig. 2 The percentage of methylated CpG in neonatal (1-day-old), 4-month-old, and 16-month-old rats DNA was extracted from OPCs obtained from each group. The DNA methylation value for each sample was expressed as a ratio of A_{sample} to $A_{standard}$. Error bars represent the mean±SD; n=5; $^{*}P$<0.05 *vs.* the corresponding neonatal rats group

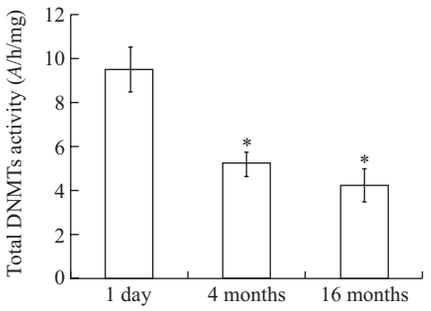

Fig. 3 Effect of aging on the total DNMT activity of OPCs
The total DNMT activity in each group was measured by the activity/inhibition assay and calculated as follows: Activity (A/h/mg) $=A_{Sample}-A_{Blank}$/(μg protein × initial incubation time in h) × 1000. Error bars represent the mean±SD; n=5, $^{*}P$<0.05 *vs.* the corresponding neonatal rats group

MBD2, TET1, TET2, and TET3 during aging by real-time quantitative PCR and Western blotting (fig. 4). The mRNA expression of DNMT1 was significantly down-regulated in 4-month-old and 16-month-old rats compared to neonatal rats (P<0.05, P<0.01, respectively) (fig. 4A). The mRNA expression of DNMT3a in neonatal rats was similar to that in 16-month-old rats, whereas there was significant up-regulation of DNMT3a in 4-month-old rats (P<0.05) (fig. 4B). DNMT3b expression levels in 4-month-old rats and 16-month-old rats were significantly higher than those in neonatal rats (P< 0.05, P<0.01, respectively) (fig. 4C). The expression levels of TET1, TET2, and TET3 mRNAs were not significantly altered during aging (fig. 4D–4F). The mRNA expression levels of MBD2 in the 4-month-old and 16-month-

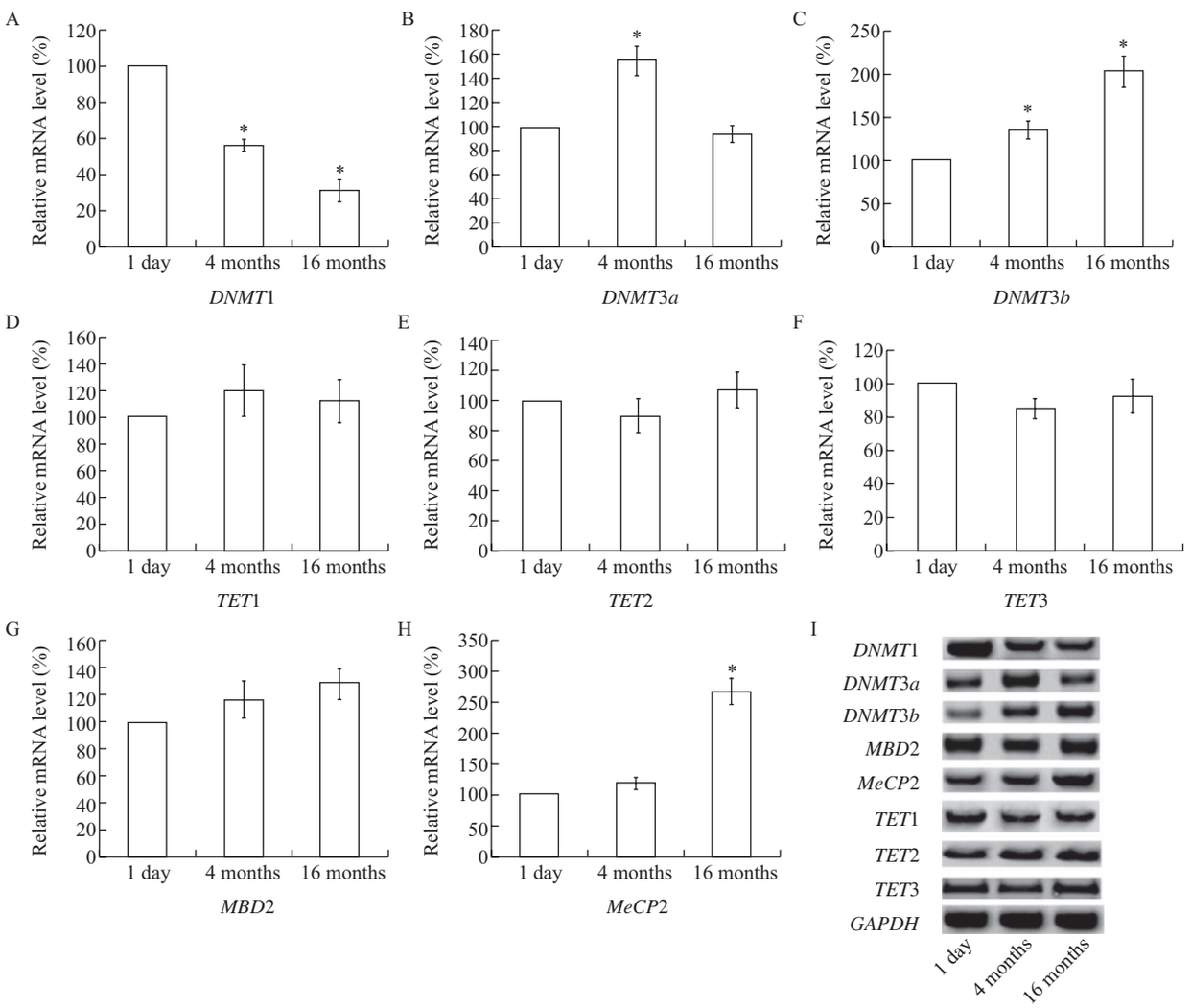

Fig. 4 Effect of aging on mRNA and protein expression levels of DNMTs, TETs, and MBDs
The mRNA expression levels of DNMTs (A, B, C), TETs (D, E, F), MBD2 (G), and MeCP2 (H) in different groups detected by quantitative RT-PCR. Data are expressed as the mean±SD from three independent experiments normalized to the ACTB expression. The fold differences were calculated relative to the levels in neonatal rats. Protein levels were detected by immunoblotting with specific antibodies against DNMT1, DNMT3a, DNMT3b, TET1, TET2, TET3, MBD2, and MeCP2 for each group. GAPDH was used as an internal control. The protein expression changes of DNMT1, DNMT3a, DNMT3b, TET1, TET2, TET3, MeCP2, and MBD2 were consistent with those at the mRNA level.

old rat groups were slightly up-regulated during aging compared to the neonatal rat group, but the difference was not significant (fig. 4G). The mRNA expression for MeCP2 was significantly up-regulated in 16-month-old rats ($P<0.01$) compared to neonatal rats; however, no significant difference was found between neonatal rats and 4-month-old rats (fig. 4H). The protein expression changes of DNMT1, DNMT3a, DNMT3b, TET1, TET2, TET3, MeCP2, and MBD2 were consistent with those at the mRNA level (fig. 4I).

In summary, our results indicate that the expression levels of DNMT3a, DNMT3b, and MeCP2 were significantly elevated; that of DNMT1 was significantly down-regulated; and there was no change in the expression levels of TET1, TET2, TET3, and MBD2 during the aging process of OPCs from rat spinal cords.

3 DISCUSSION

The efficiency of remyelination decreases with age as with other regenerative processes[23]. Many studies suggested an association between global DNA methylation and cellular senescence and organismal aging[16, 17]. Therefore, in this study, we analyzed age-related global DNA methylation profiles in OPCs isolated from rat spinal cords. Our findings suggested a general age dependence of the global DNA methylation level of OPCs from rat spinal cords, which gradually decreased during the aging process. Specifically, OPCs from elderly rats were characterized by global hypomethylation. Our result was consistent with previous results showing that declining global DNA methylation was a hallmark of age-associated epigenetic changes in different animal and cell models[24]. The exact mechanisms underlying age-dependent global DNA hypomethylation are largely unknown. DNA methylation is largely seen as a gene silencer, although recent data suggest a more complex role for 5-mC including stimulation of gene expression[25]. Changes in global DNA methylation state affect high-level organization of genome function[26] and produce lasting effects on transcriptional regulation and chromatin folding[27]. The decline of global DNA methylation is suggested to result in increased retrotransposon activity and genomic instability, which could lead to abnormal transcriptional activation by disrupting the balance of transcription factors[28]. Thus, global DNA hypomethylation can contribute to the decline of physiological function and development of age-associated diseases[29]. This partially explains the age-associated impairment of OPC recruitment and differentiation in the spinal cord. In addition, our data showed the global DNA methylation level was decreased slightly in the middle-aged rats (4 months old) compared to neonatal rats, but there was no significant difference between the two groups. This might be explained by a limited sample size or a measurement error. An age range wider than 4 months or a larger sample size may have improved our power to detect a significant impact of aging on global DNA methylation between the two groups.

To determine whether DNMTs account for the observed changes in global DNA methylation levels during the aging process, we tested the DNMT activity in each group. Our results showed the total DNMT activity of OPCs in neonatal rats was significantly higher than that of middle-aged rats and elderly rats, but no significant difference was found between the latter two groups. This result indicated that the total DNMT activity of OPCs decreased gradually during aging, which was consistent with the decreasing global DNA methylation level. However, the observed decrease in DNMT activity herein reflects an decrease in total DNMT activity and does not distinguish the relative contributions of the maintenance methyltransferase (DNMT1) or de novo methyltransferases (DNMT3a and DNMT3b). Therefore, we further measured the mRNA and protein expression levels of DNMT1, DNMT3a, and DNMT3b. Our results indicated that DNMT1 expression was significantly down-regulated in middle-aged rats and elderly rats compared to neonatal rats. DNMT3b levels in middle-aged rats and elderly rats were significantly higher than those of neonatal rats, however, DNMT3a levels only increased from neonatal rats to middle-aged rats. A similar result was observed in human embryonic lung fibroblasts[30] and mouse T cells[31]. The changes of DNMT1 expression were consistent with those of the total DNMT activity during OPC aging, indicating that DNMT1 is the predominant methyltransferase and plays a major role in the establishment and maintenance of methylation patterns in OPCs.

DNA demethylation is regulated by various enzymes, which affect dynamic regulation of DNA methylation. Therefore, we examined the expression of various enzymatic and non-enzymatic components of DNA demethylating complexes. We first detected the expression changes of TET family members TET1, TET2, and TET3 in OPCs during aging. The expression levels of TET enzymes are influenced by developmental stage. It was previously suggested that TET1 is mainly expressed in embryonic stem cells and that TET3 is the most abundant TET enzyme in oocytes and zygotes[32]. We detected the expression of TET1, TET2, and TET3 in OPCs derived from rat spinal cords, but we saw no aging-dependent changes in their expression levels. A similar result was reported in mouse hippocampus[33]. Subsequently, we evaluated the expression changes of MeCP2 and MBD2, MBDs associated with demethylation, in OPCs during the aging process. Our data revealed that the expression of

MeCP2 was significantly up-regulated in elderly rats compared to neonatal rats.

However, there was no significant change in MBD2 expression in OPCs during aging. These data suggested that MeCP2, but not MBD2, plays a role in OPC senescence. Notably, Zhang *et al* found that MBD2 significantly increased in the middle-aged stage during replicative senescence of human embryonic lung fibroblasts[30]. These different results can most likely be attributed to the different cell models and different senescence models used in both studies.

In summary, this study provided a comprehensive analysis of the dynamic changes in global DNA methylation profiles during the aging process of OPCs derived from rat spinal cords. Our results showed gradual decreases in the genomic DNA methylation level and the total DNMT activity in OPCs during aging. Compared to neonatal rats, DNMT1 was significantly down-regulated and DNMT3b was significantly up-regulated in middle-aged rats and elderly rats, and DNMT3a was significantly up-regulated in the middle-aged group. Furthermore, MeCP2 was significantly up-regulated in elderly rats when compared to neonatal rats. Based on these results, we conclude that DNMT1 plays an important role in global DNA methylation of OPCs during the aging process. To our knowledge, this is the first study to report age-related changes in the global DNA methylation profile in OPCs derived from rat spinal cords. A growing number of reports have identified loci-specific hypermethylation of the promoter regions of various genes associated with normal aging[34]. Thus, further research is needed to identify specific epigenetic changes of loci genes that are associated with recruitment and differentiation of OPCs in the spinal cord during aging. Such work may provide important evidence for elucidating the mechanism of age-related impaired OPC recruitment and differentiation and assist in the development of new treatments for promoting efficient remyelination in the spinal cord.

Conflict of Interest Statement

The authors declared that they have no conflicts of interest to this work.

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