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Adverse Maternal Environment Alters MicroRNA-10b-5p Expression and Its Epigenetic Profile Concurrently with Impaired Hippocampal Neurogenesis in Male Mouse Hippocampus

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Keywords

Adverse maternal environment · MicroRNA-10b · Brain-derived neurotropic factor · Hippocampal neurogenesis · Epigenetics

Abstract

An adverse maternal environment (AME) predisposes adult offspring toward cognitive impairment in humans and mice. However, the underlying mechanisms remain poorly understood. Epigenetic changes in response to environmental exposure may be critical drivers of this change. Epigenetic regulators, including microRNAs, have been shown to affect cognitive function by altering hippocampal neurogenesis which is regulated in part by brain-derived neurotropic factor (BDNF). We sought to investigate the effects of AME on miR profile and their epigenetic characteristics, as well as neurogenesis and BDNF expression in mouse hippocampus. Using our mouse model of AME which is composed of maternal Western diet and prenatal environmental stress, we found that AME significantly increased hippocampal miR-10b-5p levels. We also found that AME significantly decreased DNA methylation and increased accumulations of active histone marks H3 lysine (K) 4me3, H3K14ac, and H3K36me3 at miR-10b promoter. Furthermore, AME significantly decreased hippocampal neurogenesis by decreasing cell numbers of Ki67$^+$ (proliferation marker), NeuroD1$^+$ (neuronal differentiation marker), and NeuN$^+$ (mature neuronal marker) in the dentate gyrus (DG) region concurrently with decreased hippocampal BDNF protein levels. We speculate that the changes in epigenetic profile at miR-10b promoter may contribute to upregulation of miR-10b-5p and subsequently lead to decreased BDNF levels in a model of impaired offspring hippocampal neurogenesis and cognition in mice.

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Introduction

Adverse maternal environment (AME) and the consumption of a Western diet (WD) in early childhood increases the risk for cognitive impairment later in life in both humans [1–3] and animal models [4–6]. Indeed, using our mouse model we have demonstrated that AME

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together with postweaning WD impairs learning and memory function in adult male mice [7]. Unfortunately, how early life insults affect cognition functions later in life remain poorly understood. One important mechanism is epigenetic changes which can occur in response to environmental exposure [8]. Epigenetic changes include DNA methylation, histone modifications, and microRNAs (miRs). Interestingly, miRs are key players in epigenetic regulation under stress condition [9]. Furthermore, miRs have been shown to affect cognitive function in neurodegenerative disorders [10–12].

miRs act as key regulators of different biological functions including neurogenesis. miRs can indirectly influence neurogenesis by regulating the proliferation and self-renewal of neural stem cells [13]. Importantly, decreased neurogenesis and neuronal death in the hippocampus are the characteristics of impaired cognitive function [14–17]. Furthermore, regulation of hippocampal neurogenesis depends in part upon brain-derived neurotrophic factor (BDNF) levels. BDNF is a member of the neurotrophic family of growth factors and acts to support the survival of existing neurons, promote neuronal differentiation, and maturation of newborn neurons [18, 19]. Reductions in the levels of BDNF have been shown to significantly impair the production of new neurons [20–24], whereas its direct infusion into the hippocampus has been demonstrated to increase neurogenesis [25]. In addition, BDNF protects hippocampal neurons against apoptosis [19]. Interestingly, BDNF also is a target of miRs [26].

miRs are gene expression modulators, acting at the posttranscriptional level, and fine-tuning the expression of protein-encoding genes. miRs modulate gene expression by binding to 3′ untranslated region (3′ UTR) of target mRNAs and leading to translational inhibition or mRNA degradation [27, 28]. The BDNF gene 3′ UTR region contains numerous target sites for miRNA binding, which have been confirmed for miRNA-BDNF interactions [29]. BDNF protein levels have been shown to negatively correlate with miRs expression [27–30]. For example, miR-1, miR-10b, miR-155, and miR-191 were found to directly repress BDNF through binding to their predicted sites in BDNF 3′ UTR [26]. Specifically, overexpression of miR-1 and miR-10b both suppressed BDNF protein levels while mutations of their binding sites prevented repression [29].

miRs are short single-stranded noncoding RNAs about 22 nucleotides in length. Mature miRs are generated from pre-miRs either 5′ arm or 3′ arm. The 5′ arm of the miRNA is designated miR-5p present in the forward (5'-3') position, while the 3′ arm is miR-3p located in the reverse position. Both are functional. Like protein-coding genes, regulation of miRs’ expression involves epigenetic mechanisms [31–33]. DNA hypermethylation at the promoter region leads to miR silencing [34, 35]. Histone modifications, including histone acetylation (ac) and methylation, affect gene transcription. For example, histone H3 lysine (K) 9ac, H3K14ac, and H3K4 trimethylation (me3) are often associated with gene activation, while H3K36me3 is associated with actively transcribed regions [36, 37]. Moreover, H3K9me3 and H3K27me3 are associated with gene silencing [36]. These histone marks are also vulnerable to early life insults [38, 39].

However, the effects of AME on miRs’ expression and their epigenetic profile in the hippocampus are unknown. Understanding the effects of AME on hippocampal miRs’ expression may provide mechanistic insight into the regulation of hippocampal neurogenesis and subsequent cognitive impairment. We therefore hypothesized that AME would alter the expression of miRs in the hippocampus. We further hypothesized that changes in miRs’ expression would be associated with alterations in miR epigenetic patterns, decreased hippocampal neurogenesis, and decreased BDNF levels.

Methods

Animals

All experiments were conducted according to the Public Health Services Policy on Human Care and Use of Laboratory Animals and all procedures were approved by the Medical College of Wisconsin Institutional Animal Care and Use Committee [40]. The mouse model of AME used in this study has been previously described [41]. Briefly, AME was induced in mice by exposing six-week-old C57/Bl6 female mice randomly to either a control diet (10% fat without cholesterol and sucrose, Research Diet Inc., New Brunswick, NJ, USA, Product# D14020502) or a WD (40% fat, composed of increased saturated fat, cholesterol, and sucrose, Research Diet Inc., Product# D12079B) for 5 weeks prior to pregnancy and throughout lactation. Dams in the control diet group experienced a normal environment throughout pregnancy and are designated as control (Con). Dams fed a WD experienced a “stressed” environment in the last third of pregnancy. The combination of chronic WD and gestational stress is designated as AME. The stressed environment consisted of daily random environmental changes as well as a static change in the maternal environment consisting of 1/3 of the standard amount of bedding from embryonic day (E) 13–E19. The acute random environmental changes included altered light cycles on 3 nonconsecutive days, 3 repeat cages throughout the day on E15, and the short-term introduction of a novel object in the cage for a day. The period of prenatal stress was limited to E13–E19 to minimize newborn mortality, avoid interference with implantation, and still target a period of rapid development and environmental vulnerability. Dams delivered

Table 1. Primers for pyrosequencing

	Forward	Reverse	Sequencing
Set 1	5′ GATAGTTTTAGTTTAGGGAGGTT	5′ Biosg/ATAAATAATCACAACTAATCCTCCTAAAA	5′ AGTAGTAGTTATAGTAGTAGTAGA
Set 2	5′ GGGTTTTTGTTTTTTTTTGGGAAATATT	5′ Biosg/AACTAAACAACCAAAACCTTCT	5′ GGAGATTTGGGTTTTGA
Set 3	5′ AAGAAGGTTTTGGTTGTTTAGT	5′ Biosg/CTACCAAACCTAATCTCTTCAACACA	5′ GGTGGTTTTTATTTTGGTAA
Set 4	5′ TTTGAATGTTTGTTTTGGGTAGG	5′ Biosg/ATCTATAACTATATAAATACCACACAAATT	5′ GTTTGTTTTGGGTAGGA

Table 2. Primers for ChIP assays

	Forward	Reverse	Probe
miR-10b promoter	5′ CTACCTGCACCATCTCTGAAAG	5′ TCCTACCCAGAGCAGACATT	5′ ACCTGATCTCTTCAGCACAGCCAC
Intergenic region	5′ TTTGGAACCACCATTTTCTACGT	5′ GCTCAATAGATTTGATGGGCTTACT	5′ ACACCAGGCACTATT

ChIP, chromatin immunoprecipitation; miR, microRNA.

spontaneously, and litters were culled to 6 pups. At postnatal day 21 (P21), male pups from both Con and AME groups were anesthetized and killed. Brains were quickly removed, hippocampi were dissected, flash frozen in liquid nitrogen, and stored in −80°C for molecular studies. For immunohistochemistry studies, animals were individually fixed via intracardiac perfusion with ice-cold 0.9% normal saline (VWR, Radnor, PA, USA), followed by ice-cold 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) for 5 min each for a total volume of 10–15 mL fixative. Whole brains were removed and postfixed at 4°C overnight. Brains were then transferred to 70% ethanol, paraffin embedded, and sectioned coronally at 4 μm per section. The reason for choosing to study juvenile hippocampus was because we wanted to examine immediate maternal effect on neurogenesis and its regulation in the hippocampus. A total of 40 pregnant female mice were used in the study with $N = 6$ dams per group.

RNA Isolation and Real Time RT-PCR

Total hippocampal RNA was extracted from P21 old mice using miRNeasy Micro Kit (Qiagen, Germantown, MD, USA) and quantified using the BioTek Cytation 5 microplate reader (Fisher Scientific Inc., Pittsburgh, PA, USA). RNA was treated with DNase I (Ambion, Austin, TX, USA). Gel electrophoresis was used to confirm the integrity of the samples. miR cDNAs were synthesized from total RNA using TaqMan® Advanced miRNA cDNA Synthesis Kit (Thermo Fisher Scientific). Real-time PCR for miRNA assays were performed according to TaqMan® advanced miRNA assays user guide using ABI ViiA7 system (Thermo Fisher Scientific). Relative quantification of PCR products was based on value differences between the target and internal control using the comparative C_T method (Taqman Gold RT-PCR manual, PE Biosystems, Foster City, CA, USA). Cycle parameters were 50°C, 2 min; 95°C, 10 min; and then 40 cycles at 95°C, 15 s; 60°C, 60 s. For each set of reactions, samples were run in quadruplicate. Expression levels of miRs known to affect cognitive function and BDNF levels [26, 42–44] were tested, including miR-1a-3p (mmu482914_mir), miR-10b-5p (mmu478494_mir), miR-10b-3p (mmu481501_mir), miR-155-5p (mmu480953_mir), miR-155-3p (mmu481328_mir), miR-183-5p (mmu482690_mir), miR-191-5p (mmu481584_mir), miR-191-3p (mmu480970_mir), and miR-206-3p (mmu481645_mir). Expression was calculated relative to hsa-miR-320a (478594-mir, TaqMan® advanced miRNA assays user guide, Thermo Fisher Scientific) which was used as an internal control. cDNA synthesis and real-time RT-PCR were performed as previously described [45]. mRNA expression of BDNF (Mm04230607_s1, Thermo Fisher Scientific) was calculated relative to hypoxanthine phosphoribosyltransferase 1 (HPRT1, Mm. PT.39a.22214828, Integrated DNA Technologies) which was used as an internal control.

DNA Isolation and Pyrosequencing

Hippocampal DNA was extracted from P21 old mice using DNeasy® Blood & Tissue kit (Qiagen, Cat#69504). DNA was subjected to sodium bisulfite treatment using Epitech fast DNA bisulfite kit (Qiagen, Cat#59824) as per the manufacturer's protocol to determine site-specific CpG methylation. DNA methylation of the validation-set samples was determined through PCR amplification with biotinylated primers (Intergrated DNA Technologies, Coralville, IA, USA). Primers were designed based on the genomic sequence obtained from Ensembl Genome Browser (Ensembl: ENSMUSG00000065500) using PyroMark Assay Design Software version 2.0. Amplified products were confirmed with agarose gel electrophoresis. The percent of methylation was quantified by PyroMark Q48 Autoprep Pyrosequencer (Qiagen, Valencia, CA, USA). Four primer sets (Table 1) were used to examine methylation status of 12 CpG sites in the promoter region of miR-10b, spanning from −1 to −540bp nucleotide position from transcription start site which was set as +1.

Chromatin Isolation and Chromatin Immunoprecipitation Assay

Hippocampal chromatin was isolated from P21 old mice using truChIP™ chromatin shearing tissue kit (#520237, Covaris Inc, MA, USA) and focused-ultrasonicator M220 (Covaris Inc.). Chromatin immunoprecipitation (ChIP) assays with antibodies against histone H3 lysine (K) 4 trimethylation (H3K4me3, #9751, Cell Signaling Technology), H3K9me3 (#13969, Cell Signaling Technology), H3K27me3 (#9733, Cell Signaling Technology), H3K36me3 (#4909, Cell Signaling Technology), H3K9ac (#9649, Cell Signal-

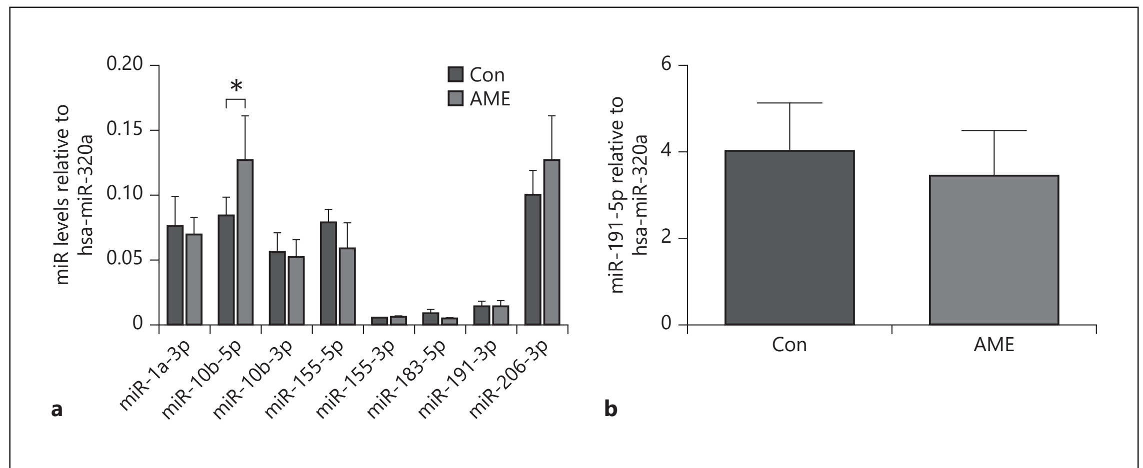

Fig. 1. AME increased miR-10b-5p levels in the hippocampus. Data are presented as mean ± SD. **a** miR levels. **b** miR-191-5p levels. $N = 6$ animals from different litters/group. $^*p < 0.05$. AME, adverse maternal environment; Con, control; miR, microRNA.

ing Technology), and H3K14ac (#7627, Cell Signaling Technology) were performed using SimpleChIP® Plus Enzymatic Chromatin IP kit (#9005, Cell Signaling Technology). Real-time PCR was used to quantitate the amount of immunoprecipitation DNA at miR-10b promoter region and an intergenic region upstream of IGF gene which was used as an internal control [46]. Primers for miR-10b promoter and the intergenic region are list in Table 2. Two control experiments were performed simultaneously with our ChIP experiments. First, we performed a "mock" ChIP that included input but did not utilize antibody. Second, we performed a ChIP that utilized an anti-rabbit secondary antibody as negative control. Two percent of input was used as loading control.

Immunofluorescence

Immunofluorescent triple labeling was used to compare neurogenesis in dentate gyrus (DG) region of hippocampus between Con and AME groups using cell proliferation marker Ki67 (#9129, Cell Signaling, Danvers, MA, USA), neuronal differentiation marker NeuroD1 (ab60704, Abcam, Cambridge, MA, USA), immature neuronal marker doublecortin (DCX, #4604, Cell Signaling, Danvers, MA, USA), and neuronal marker (NeuN, EMD Millipore, Billerica, MA, USA, Cat#MAB377). Briefly, anterior hippocampal coronal sections around bregma −1.94 area from the same brains were deparaffinized, rehydrated, and subject to antigen retrieval treatment. Sections were then blocked with Protein Block (DAKO, Carpinteria, CA, USA, Cat #x090930-2) for 30 min at room temperature (RT) and followed by incubation with either a mixture of rabbit anti-Ki67 1:200 and mouse anti-NeuN or a mixture of rabbit anti-DCX 1:200 and mouse anti-NeuroD11:750 for 60 min at RT. After washing in TBST twice, sections were then exposed to a mixture of donkey anti-rabbit AF488 1:750 and donkey anti-mouse Cy3 (Jackson ImmunoResearch) 1:750 or a mixture of donkey anti-rabbit Cy3 and donkey anti-mouse Cy51:750 for 45 min at RT. Followed by washing in TBST twice, sections were counterstained for nuclei with DAPI. Sections were washed and mounted with Prolong Gold anti-fade mounting media. Images were captured by confocal microscopy (Carl Zeiss LSM510, Jena, Germany). Positive cells for Ki67, NeuroD1, DCX, NeuN, and DAPI staining on the digital images with ×20 magnification were automated counted by ImageJ software. Two sections/pup, 5 animals from different litters per group were blindly quantified. The counts for Ki67+, NeuroD1+, and NeuN+ were normalized to DAPI count while DCX+ count was normalized to DAPI total area.

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling

Apoptosis in the DG region of hippocampus from P21 Con and AME brain was determined using DeadEnd™ Fluorometric TUNEL System (G3250, Promega, Madison, WI, USA). In brief, anterior hippocampal coronal sections around bregma −1.94 area were deparaffinized, rehydrated, and subject to terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) following the manufacturer's instructions. Images were captured by confocal microscopy (Carl Zeiss LSM510, Jena, Germany). TUNEL positive cells on the digital images with ×20 magnification were automated counted by ImageJ software and normalized by DAPI count. Two sections/pup, $n = 5$ animals from different litters per group were blindly quantified.

Protein Isolation and Immunoblot

Hippocampal tissues from P21 old mice were homogenized in ice-cold RIPA lysis buffer (Amresco LLC, Solon, OH, USA) with protease and phosphatase inhibitors. After centrifugation at 10,000 rpm at 4°C for 15 min, the supernatants were removed and stored at −80°C until use. Protein concentrations were determined by the BCA method (Pierce, Rockford, IL, USA). Antibody against BDNF (ThermoFisher Scientific, Cat# 710306) at 1:500 dilution was used to determine protein abundance and vinculin (Cell Signaling, Cat #13901) at 1:20,000 dilution was used as a loading control. Immunoblot analyses were performed by a capillary immunoblot analysis method using the ProteinSimple™ Wes system according to the manufacturer's instructions (ProteinSimple™, Santa Clara, CA, USA) as previously described [45].

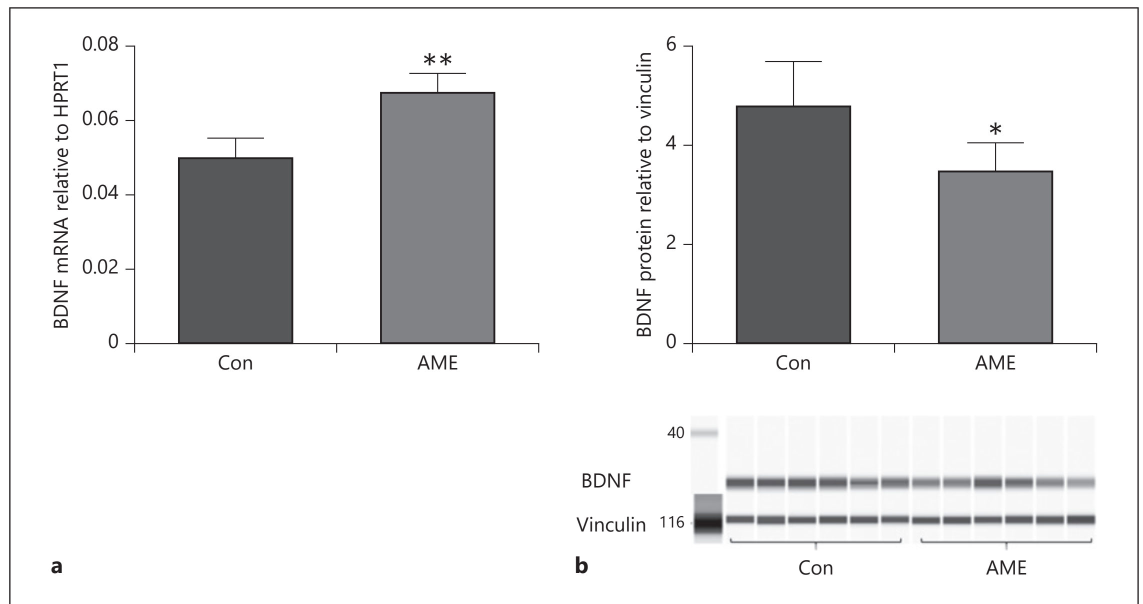

Fig. 2. AME altered BDNF expression in the hippocampus. Data are presented as mean ± SD. **a** BDNF mRNA levels in Con versus AME mice. **b** BDNF protein levels in Con versus AME mice. $N = 6$ animals from different litters/group. $^{*}p < 0.05$, $^{**}p < 0.01$. AME, adverse maternal environment; BDNF, brain-derived neurotropic factor; Con, control.

Statistics

GraphPad Prism 6 (GraphPad Software, San Diego, CA) was used to perform all analyses. All data presented are expressed as mean ± SD. ANOVA (Fisher's protected least significant difference) and Mann-Whitney test determined statistical significance. Significance was set as $p < 0.05$.

Results

AME Increased Hippocampal miR-10b-5p Levels

We first examined the effect of AME on miRs' profile. Interestingly, AME significantly increased hippocampal miR-10b-5p levels but not other miRs that were examined (shown in Fig. 1a, b). Given that BDNF is a direct target of miR-10b-5p, as well as a critical player in neuronal function, we then next determined BDNF expression in the hippocampus.

AME Decreased Hippocampal BDNF Protein Abundance

AME significantly decreased hippocampal BDNF protein abundance (shown in Fig. 2b) though AME increased BDNF mRNA levels (shown in Fig. 2a). However, the contrary results between BDNF protein and mRNA levels suggest that miR-driven posttranscriptional regulation may be involved in BDNF protein production. Specifically, increased miR-10b-5p may contribute to reduced BDNF protein levels in our model.

AME Decreased Neurogenesis and Increased Apoptosis in Hippocampal DG Region

Both miRs and BDNF are key regulators of neurogenesis [13]. Importantly, impaired hippocampal neurogenesis and apoptosis are characteristics of cognitive impairment [16, 17]. Furthermore, hippocampal neurogenesis postnatally occurs at the subgranular zone in the DG region of hippocampus [47, 48]. We then examined the effect of AME on neurogenesis in hippocampal DG region. We found that AME significantly decreased neurogenesis as indicated by decreased staining of Ki67^{+} (shown in Fig. 3a, b), NeuroD1^{+} (Fig. 3c, d), NeuN^{+} (shown in Fig. 3g, h) and trending to decrease DCX^{+} staining (shown in Fig. 3e, f) in the DG region. We next determined the effect of AME on apoptosis in the hippocampus using TUNEL assay. We found that AME significantly increased apoptotic cell numbers in the DG region of hippocampus compared to Con (shown in Fig. 3i, j).

AME Altered Epigenetic Characteristics at miR-10b Promoter in the Hippocampus

Like a protein-coding gene, miR expression can be epigenetically regulated [31–33]. Additionally, measurable

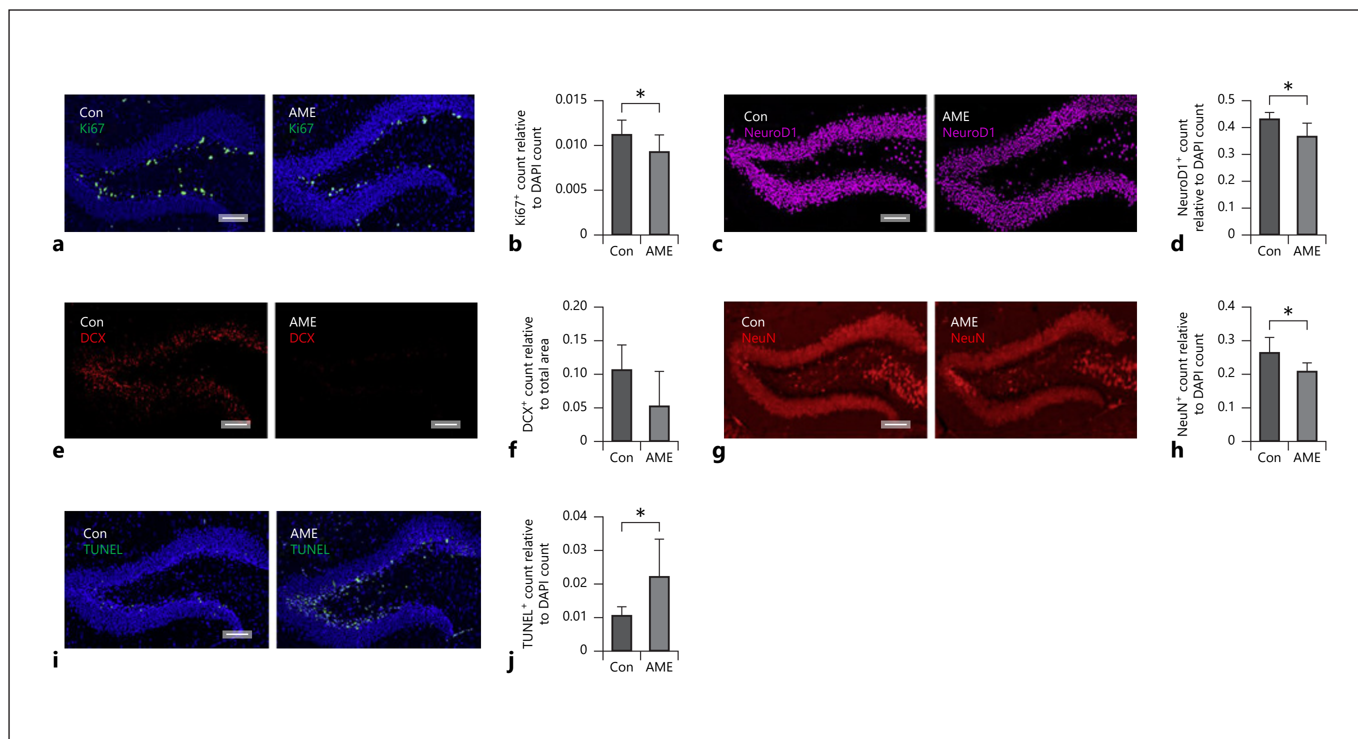

Fig. 3. AME decreased neurogenesis and increased apoptosis in hippocampal DG region. **a** Cell proliferation marker Ki67 staining (green) in DG of Con (left panel) and AME (right panel) mice. **b** Quantitative analysis of Ki67$^+$ cells in Con versus. AME mice. **c** Neuronal differentiation marker NeuroD1 staining (pink) in DG of Con (left panel) and AME (right panel) mice. **d** Quantitative analysis of NeuroD1$^+$ cells in Con versus. AME mice. **e** Immature neuronal marker DCX staining (red) in DG of Con (left panel) and AME (right panel) mice. **f** Quantitative analysis of DCX$^+$ cells in Con versus. AME mice. **g** Neuronal marker NeuN staining (red) in DG of Con (left panel) and AME (right panel) mice. **h** Quantitative analysis of NeuN$^+$ cells in Con versus. AME mice. **i** TUNEL assay showing apoptotic cells (green) in hippocampal DG region of Con (left panel) and AME (right panel) mice. **j** Quantitative analysis of apoptotic cells in Con versus. AME mice. Data are presented as mean ± SD, $n = 5$ animals from different litters/group. $^*p < 0.05$, Scale bar = 100 μm. AME, adverse maternal environment; DG, dentate gyrus; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; Con, control.

epigenetic changes often occur in effected genes following an adverse early life environment [41]. We then determined the effect of AME on DNA methylation status and histone code at miR-10b promoter.

AME significantly decreased CpG methylation at 5 out of 12 sites (−9, −20, −175, −496, and −511 relative to transcription start site) examined at miR-10b promoter compared to Con (shown in Fig. 4). Furthermore, AME significantly increased the densities of active histone marks H3K4me3, H3K14ac, and H3K36me3 at miR-10b promoter compared to Con with no effect on the densities of H3K9ac, H3K9me3, and H3K27me3 (shown in Fig. 5a, b). These data suggest that DNA methylation and histone H3 modifications are involved in miR-10b-5p upregulation.

Discussion

The novel findings of this study are that AME increased miR-10b-5p levels concurrently with decreased BDNF protein abundance and impaired neurogenesis in mouse hippocampus. In addition, increased miR-10b-5p levels were associated with altered epigenetic profile at its promoter. These findings suggest that early life stress and diet endured by the mother affect miR-10b-5p expression via epigenetic mechanism and subsequently alter BDNF levels in the hippocampus, which in turn affect hippocampal neurogenesis and lead to cognitive impairment later in life. To our knowledge, this is the first report that AME affects the epigenetic profile and expression of miR-10b-5p concurrently with neurogenesis impairment in offspring hippocampus.

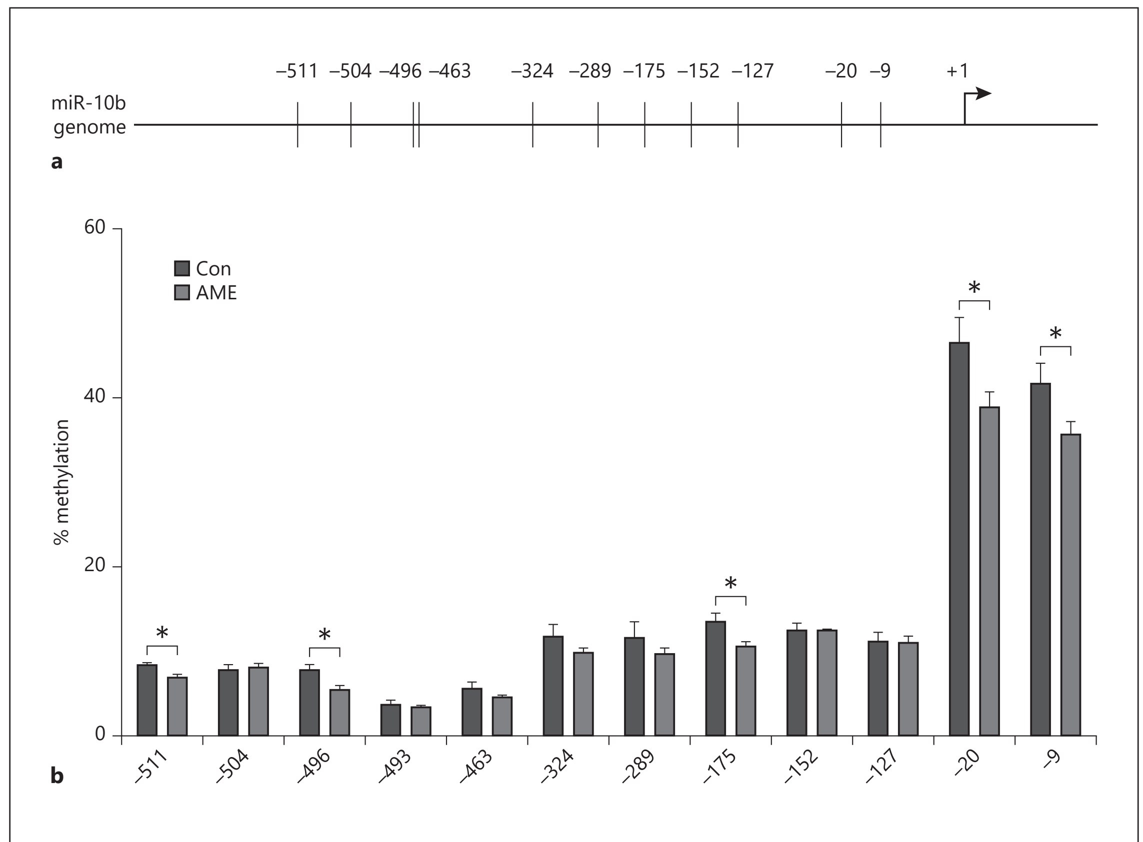

Fig. 4. AME decreased CpG methylation at miR-10b promoter in the hippocampus. **a** Schematic representation of mouse miR-10b proximal promoter. Vertical lines indicate the location of the CpG sites examined relative to translation start site set as +1. **b** Percent of methylation at 12 CpG sites examined. Data are presented as mean ± SD. $N = 6$ animals from different litters/group. $^*p < 0.05$. AME, adverse maternal environment; miR, microRNA; Con, control.

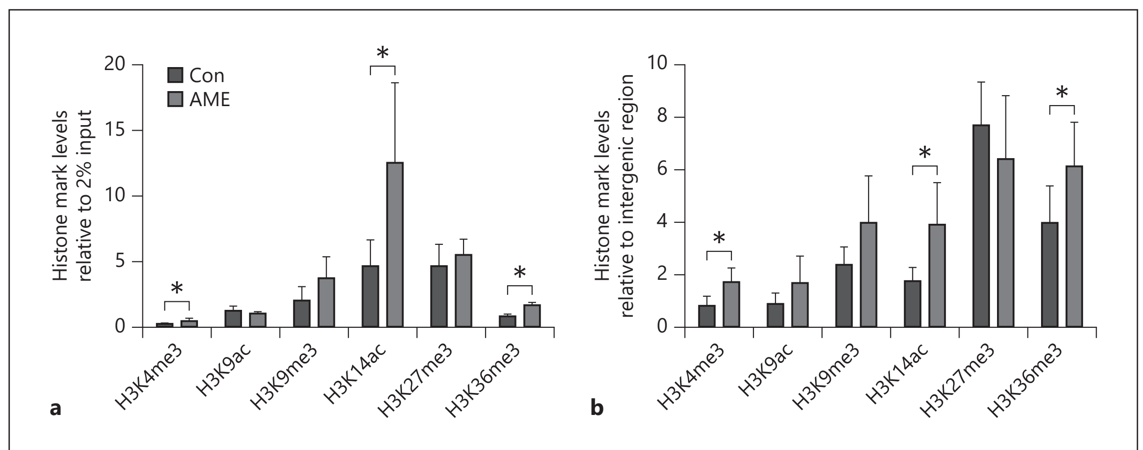

Fig. 5. AME increased active histone marks at miR-10b promoter. **a** The densities of H3K4me3, H3K9ac, H3K9me3, H3K14ac, H3K27me3, and H3K36me3 were quantified relative to 2% input by ChIP assay. **b** The densities of H3K4me3, H3K9ac, H3K9me3, H3K14ac, H3K27me3, and H3K36me3 relative to intergenic region. Data are presented as mean ± SD $n = 6$ animals from different litters/group. $^*p < 0.05$. AME, adverse maternal environment; ChIP, chromatin immunoprecipitation; K, lysine; me3, trimethylation; ac, acetylation.

AME has a profound impact on hippocampal structure and function that subsequently leads to cognitive impairment through epigenetic mechanisms [49]. miRs are key players in epigenetic regulations which often occur in response to environmental exposure and under stress condition [8, 9, 50]. Altered miR expression has been found in the key brain regions related to cognitive function including hippocampus following exposure to early life stress in both humans and animals [9, 50–52]. However, no studies have reported the relationship between early life stress and miR-10b in the hippocampus. In current study, we found that miR-10b-5p levels were significantly increased in the hippocampus of pups exposed to AME. Our data suggest that upregulated miR-10b-5p expression may play a role in the cognitive impairment previously reported in our model [41].

miRs are gene expression modulators and act at the posttranscriptional level to control protein production of target genes [27, 28]. miR-10b targets the cognition network gene BDNF and is negatively correlated with BDNF levels [53, 54]. For example, Jiang and Zhu [55] have demonstrated that BDNF is a direct target of miR-10b and BDNF expression negatively correlates with the expression of miR-10b in rat hippocampus under chronic stress depression induced by sleep deprivation. Furthermore, BDNF levels have been shown to be posttranscriptionally controlled by upregulated miR-10b-5p in human prefrontal cortex of Huntington disease brain [54]. Whereas no studies have reported the relationship between miR-10b-5p and BDNF in the hippocampus exposed to early life stress. In this study, we found that AME decreased BDNF protein abundance in line with increased miR-10b-5p levels in the hippocampus, suggesting that miR-10b-5p may target BDNF and decrease BDNF protein levels in our model.

BDNF regulates neuronal survival and differentiation [56]. Reduced BDNF expression is associated with neuronal dysfunction and death [48, 49, 57, 58]. Here, we found that AME-induced hippocampal BDNF protein reduction coincides with impaired neurogenesis by decreasing cell proliferation, neuronal differentiation, and mature neuron numbers, as well as increased apoptosis in the hippocampus. Similar findings have been reported in the hippocampus of offspring exposed to other early life stress in animal models [43, 48, 57, 58]. Although maternal WD alone has been shown to have negative effects on BDNF expression and neurogenesis in offspring hippocampus [59, 60], our AME model consists a maternal WD component and did not affect cognitive function in adult animals [7]. We suggest that BDNF protein reduction and neurogenesis impairment seen in our model may result from the effects of the combination of maternal WD and prenatal stress.

miRs' expression can be regulated epigenetically [61]. DNA hypermethylation at the promoter region has been shown to lead to miR silencing [34, 35]. Specifically, DNA CpG methylation in a transcription factor binding site of miR-10b leads to downregulation in a human cervical cancer cell line [62]. Inversely, in our study, we found that AME decreased DNA methylation at limited CpG sites of miR-10b promoter in the hippocampus concurrently with increased miR-10b-5p expression. Interestingly, the promoter region examined in this current study contains a Sp1 binding element at the −324 site examined here. Sp1 is typically a transcriptional activator [63] and enhances transcription by RNA polymerase II [64]. miR genes are transcribed by RNA polymerase II [65]. We speculate that decreased CpG methylation at miR-10b promoter around Sp1 binding site may facilitate Sp1 binding and RNA polymerase II transcriptional machinery assembling and subsequently lead to miR-10b-5p transcriptional activation. Future studies are warranted to explore the role of TFs including Sp1 on miR-10b transcription.

Interestingly, we also found that AME significantly increased accumulations of active histone marks H3K4me3, H3K14ac, and H3K36me3 at the promoter region of miR-10b. Our data suggest that multiple histone modifications are involved in the regulation of miR-10b-5p expression. Though early epigenetic studies focused on the consequences of single-site histone modifications, it is now evident that modifications at different sites influence their effect on transcription, which involves simultaneously reading multiple histone markers [66]. Different histone marks may interact with each other to affect gene transcription. For example, H3K4me3 has been shown to intricately link acetylation at H3K14 by recruiting Ybg1 PHD finger binding and stabilizing the interaction of the scNuA3 HAT leading to hyperacetylation of its substrate [67]. In addition, while H3K36me3 has been observed to be more enriched at body regions of active genes and related to transcriptional elongation [68, 69], H3K36me3 has also been shown to be involved in transcriptional initiation [69, 70]. Specifically, Zhang et al. [70] have shown that H3K36me3 mediated by its methyltransferase Setd2 was enriched at a distal promoter region of a gene of interest and led to transcriptional initiation. Taken together, our data suggest that accumulations of H3K4me3, H3K14ac, and H3K36me3 to miR-10b promoter may work together to favor miR-10b-5p transcription.

Our study has limitations. Whether AME-induced upregulation of miR-10b-5p directly affects BDNF protein production and subsequently impairs neurogenesis in developing hippocampal DG requires further investigations. Moreover, maternal WD or prenatal stress alone may affect epigenetic profile of miR-10b promoter and subsequently alter mir-10b-5p expression, which have not been examined in this study. Additionally, how prenatal maternal stress affects postnatal maternal care behavior and subsequent offspring DG development, as well as how epigenetic changes are maintained during neural stem cell proliferation have not been revealed. All molecular analysis performed in this study utilized whole homogenized hippocampus, thus limiting our ability to understand the contribution of hippocampal subfields but allowed for a comprehensive hippocampal investigation. Last, we recognize that we do not know whether AME-induced upregulation of miR-10b-5p occurs in female offspring, who do not suffer similar cognitive impairment relative to the male counterparts. If AME did not induce upregulation of miR-10b-5p in females, this finding would add circumstantial support to our speculation.

In this study, we have identified that AME increased miR-10b-5p levels concurrently with decreased BDNF protein levels and neurogenesis as well as increased apoptosis in juvenile mouse hippocampus. We speculate that AME increases miR-10b-5p levels by altering epigenetic profile at the promoter region of miR-10b. Increased miR-10b-5p subsequently acts on BDNF to reduce BDNF protein production and leads to decreased neurogenesis in juvenile mouse hippocampus.

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Statement of Ethics

All experiments were conducted according to the Public Health Services Policy on Human Care and Use of Laboratory Animals and all procedures were approved by the Medical College of Wisconsin Institutional Animal Care and Use Committee.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

X.K., Y.H., Q.F., and A.M. performed experiments; X.K. analyzed data and prepared figures; X.K., R.H.L., and A.M. interpreted results of experiments; X.K. drafted the manuscript; R.H.L. and A.M. edited and revised the manuscript; R.H.L. and A.M. approved the final version of manuscript; A.M., R.H.L., and X.K. conceived and designed research.

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