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Transcriptional signatures mediated by acetylation overlap with early-stage Alzheimer's disease

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Abstract

The mechanisms by which environmental influences lead to the development of complex neurodegenerative diseases are largely unknown. It is known, however, that epigenetic mechanisms can mediate alterations in transcription due to environmental influences. In order to identify genes susceptible to regulation in the adult cortex by one type of epigenetic mechanism, histone, and protein acetylation, we treated mice with the histone deacetylase inhibitor Trichostatin A (TSA). After 1 week of treatment with TSA, RNA was extracted from the brain cortices of mice and gene expression differences were analyzed by microarray profiling. The altered genes were then compared with genes differentially expressed in microarray studies of disease by database and literature searches. Genes regulated by TSA were found to significantly overlap with differentially expressed genes in the Alzheimer's disease (AD) brain. Several TSA-regulated genes involved in chromatin remodeling and epigenetic reprogramming including histone cluster 1, H4 h (Hist1H4 h), methionine adenosyltransferase II, alpha (Mat2a), and 5-methyltetrahydrofolate homocysteine reductase (Mtrr) overlapped with genes altered in early-stage AD in gray matter. We also show that the expression of hemoglobin, which has been shown to be altered in neurons in the AD brain, is regulated by TSA treatment. This analysis suggests involvement of epigenetic mechanisms in neurons in early stages of AD.

Keywords

Acetylation; Histone deacetylase inhibitors; Trichostatin A; Epigenetics; Alzheimer's disease; Expression microarray

Introduction

Many neurodegenerative diseases including AD are complex diseases in that, except in rare familial forms of disease, the development of disease does not follow Mendelian rules of inheritance. Susceptibility is conferred by a combination of genetic and environmental influences, and twin concordance rates suggest a strong environmental component exists for the development of AD (Gatz et al. 2006; Brickell et al. 2007). While susceptibility alleles have been identified in familial and sporadic forms of AD (Goate et al. 1991; Corder et al. 1993; Selkoe 1996; Delacourte et al. 2002; Blacker et al. 2003), the environmental factors involved in disease development and progression have not been clearly defined. Differences in environmental exposure between individuals can lead to differences in gene expression

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through epigenetic mechanisms (Szyf et al. 2008). Epigenetic modifications allow for the modulation of gene expression without any change to the primary DNA sequence and provide the basis for differences in gene expression even in identical twins. These modifications can be mitotically and sometimes meiotically heritable and include chemical modifications to histones, methylation of the cytosine bases of DNA, and the synthesis of non-coding RNA (Mehler 2008).

During the development of the nervous system, epigenetic silencing orchestrated by the neuron restrictive silencing factor, alters chromatin structure, and shuts down transcription of non-neuronal genes in neurons (Ballas and Mandel 2005). The maintenance of these epigenetic modifications provides a basis for cell memory. Even in postmitotic cells, however, some genes continue to be regulated by modifications of chromatin structure due to environmental or physiological signals. Reports have shown that histone modifications are involved in the immune response and that inflammatory signals can modulate the repression of genes to allow for wound healing (Shaw and Martin 2009). Furthermore, epigenetic mechanisms can alter transcription in response to external environmental influences such as malnutrition and exposure to toxins as well as environmental stresses within a cell such as oxidative damage (van Vliet et al. 2007; Ryu et al. 2003; Cyr and Domann 2011). One type of epigenetic modification that can modulate transcription in response to an oxidative environment is histone acetylation (Shahbazian and Grunstein 2007). The balance between histone acetylation and deacetylation is maintained by two types of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs). Acetylation of lysines on the N-terminal tail of histones by HATs eliminates the positive charge on the lysine residue and therefore reduces the affinity of the histone for DNA (Gregory et al. 2001). As a result, the chromatin is in a more open state allowing transcriptional activators and coactivators access to promoter regions, thereby enhancing transcription. While histone hyperacetylation activates transcription in general, histone hypoacetylation tends to silence gene transcription. The balance of histone and protein acetylation can be tipped toward increased acetylation in an oxidative environment due to increased protein tyrosine nitration and other redox modifications to HDACs, which lead to decreased deacetylase activity (Ito et al. 2004; Doyle and Fitzpatrick 2010).

While evidence exists for an epigenetic basis in disease susceptibility and progression in complex neurodegenerative diseases such as AD (Chouliaras et al. 2010; Mastroeni et al. 2011), not much is known concerning the genes that are susceptible to regulation by epigenetic modifications including histone and protein acetylation in the adult CNS.Inthe present study, we identify genes and transcriptional pathways susceptible to regulation by an imbalance in acetylation in the cortex by treating mice with the histone deacetylase inhibitor TSA followed by expression microarray profiling.

Methods and materials

Treatment of mice with TSA and microarray profiling

Four-week-old, male C57Bl/6 mice were treated with either TSA (7.5 mg/kg/day) or vehicle (9:1 PBS to DMSO) for 1 week via daily intraperitoneal injections. After 1 week of treatment, mice were killed by cervical dislocation, and brain cortices were harvested and pooled into groups of three mice each and placed on ice. Total RNA was immediately extracted from harvested tissue using RNeasy Lipid Tissue Mini Kit (Qiagen, Maryland, USA) according to the manufacturer's instructions and stored at −80 °C until used for hybridization to gene arrays. RNA quality was determined by formaldehyde agarose gel electrophoresis to visualize intact 28S and 18S RNA. Total RNA (100 mg) was converted to cDNA, labeled, and fragmented using an Affymetrix 3′ IVT Express Kit per manufacturer's instructions (Affymetrix, Santa Clara, CA, USA). cDNA was fragmented and hybridized

overnight at 42 °C to Affymetrix Mouse Genome 430A 2.0 arrays, which contains oligonucleotide probe sets representing 14,000 well-characterized genes. After hybridization, arrays were washed in a Genechip Fluidics Station and scanned on a GeneChip Scanner 3000 (both from Affymetrix) to generate cell intensity files (CEL files). After normalization and background correction by the Robust MultiChip Analysis (RMA) method with Affymetrix Expression Console software, statistical analysis of resulting CHP files was performed with Partek Genomics Suite 6.5 (Partek, St. Louis, MO, USA). A one-way ANOVA was performed, and genes were considered significantly changed if they had a fold change greater than ±1.2 and a p value less than 0.05.

In order to explore overlaps with disease states, human homologues of the changed genes were first determined using NetAffyx (www.affymetrix.com). Of the 544 probe sets differentially expressed in the mouse cortex due to TSA treatment, 369 human homologues were identified. Overlaps were computed for the human homologues with Gene Set Enrichment Analysis software (Subramanian et al. 2005) for gene sets in the C2 collection of the Molecular Signature Database (http://www.broadinstitute.org/gsea/MSigDB/index.jsp). This software determines significance of overlaps by a hypergeometric distribution test ($p < 0.05$ was considered significant), which corresponds to a one-tailed Fisher's exact test. Literature searches of microarray results were also performed and significant genes regulated by TSA were compared for overlaps in previously published microarray studies analyzing postmortem AD brain tissue (Colangelo et al. 2002; Yao et al. 2003; Ricciarelli et al. 2004; Dunckley et al. 2006; Weeraratna et al. 2007; Liang et al. 2008; Williams et al. 2009; Qin et al. 2009; Tan et al. 2010; Bossers et al. 2010; Blalock et al. 2011; Ginsberg et al. 2012).

Protein extraction and western blotting for acetylated proteins

After brain cortices were extracted, the rest of the brain was then pooled and used to prepare nuclear protein fractions. The fractions were run on 4–12 % Bis–Tris polyacrylamide gels (Invitrogen, Carlsbad, CA), transferred to nitrocellulose, and were probed with either acetyllysine (1:1,000, Upstate Biotechnology, Lake Placid, NY, USA) or lamin B1 (1:200, Novus Biologicals, Littleton, CO, USA) primary antibodies. After primary antibody hybridization, blots were hybridized with either mouse or rabbit secondary antibodies (1:10,000 each, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and developed using luminol reagent (Santa Cruz Biotechnology).

Quantitative RT-PCR to confirm differential gene expression

Quantitative RT-PCR (qRT-PCR) was performed in order to confirm changes in gene expression obtained by microarray profiling. qRT-PCR was performed on RNA isolated from the cortex of TSA-treated mice and control mice (vehicle-treated) from three groups (3 TSA treated and 3 control brains were pooled in each group). Statistical significance was determined by a Student's t test from at least two separate experiments performed in triplicate. Primers were designed to target hemoglobin β chain (Hbb1 and Hbb2), S100a8, Mat2a, and Ung transcripts. To eliminate amplification from any contaminating genomic DNA, primers were designed to span an intron or RNA was treated with Turbo DNA-free (Invitrogen, Carlsbad, CA, USA). Beta-actin (Applied Biosystems, Carlsbad, CA, USA) was used as a control to normalize Ct values. qRT-PCR was performed per manufacturer's specifications using Brilliant III Ultra-Fast SYBR Green qRT-PCR Master Mix and 2–10 nanograms of total RNA. Primers for Hbb1 were 5′ CCTGGGCA GGCTGCTGGTTG3′ and 5′ TGGCAAAGGTGCCCTTG AGGC 3′, for Hbb2, 5′ TGGGTAATCCCAAGGTGA AGGCCC 3′ and 5′ GCCCAGCACAATCACGATCGCA 3′, for S100a8, 5′ TCGAGGAGTTCCTTGCGATGGTG 3′ and 5′ GGACCCAGCCCTAGGCCA. GAA3′, for Mat2a, 5′ AACGGGCAGCTCAACGGCTTC 3′ and 5′ TGAAGGT GTGCATCA.

AGGACAGCA 3′, and for Ung, 5′ CAGCAG TTTGGTGCCTGCTGC 3′ and 5′ AACCCAGGGAGAAC CCCGCC 3′.

Results

In order to evaluate the impact of TSA on acetylation patterns in the brain, we performed western blots with protein isolated from the brains of TSA- and vehicle-treated mice with an antibody to acetyl-lysine as shown in Fig. 1. We observed that mice treated with TSA for 1 week experienced increases in general protein acetylation in nuclear fractions in the brain. To more specifically identify targets of acetylation, we performed expression microarray profiling of total RNA isolated from the cortex of TSA- and vehicle-treated mice. After statistical analysis of the microarray data by a one-way ANOVA (Partek Genomics Suite Software 6.5), 369 genes with human homologues were found to be differentially expressed between TSA-and vehicle-treated mice with a fold change >±1.2 and p <0.05 (Supplementary Table 1). These genes were then analyzed with Gene Set Enrichment Analysis software to compute overlaps with previously published genes sets in the Molecular Signatures Database (http://www.broad.mit.edu/gsea/MSigDB/index.jsp).

Interrogation of the Molecular Signatures Database with our TSA-regulated genes, we found significant overlap ($p < 0.05$) with studies which profiled gene expression in cancer (Lindgren et al. 2006), during the cell cycle (Ben-Porath et al. 2008), and in early-stage postmortem AD brain tissue from hippocampus (Blalock et al. 2004) shown in Table 1. Gene set enrichment analysis identified 65 genes of 369 TSA-regulated genes that overlapped with genes altered in AD. Overlap was also found in other microarray studies analyzing early-stage AD cortex and laser-captured hippocampal gray matter through literature searches (Bossers et al. 2010; Blalock et al., 2011). These studies analyzed early-stage disease defined by Braak stage (Braak and Braak 1991) or MiniMental Status Exam and neurofibrillary tangle density, respectively. Relative microarray expression levels, gene symbols, and gene descriptions for a total of 81 genes in our TSA microarrays found to overlap with genes altered in the AD brain in studies which analyzed early-stage disease are shown in Fig. 2. Thirty-two of the 81 genes were altered in early-stage disease denoted by an asterisk in Fig. 2, and 23 were altered in laser-dissected gray matter denoted by overlap in the Blalock et al. (2011) study. Another study examining gene expression in neocortex also showed overlap in 26 of the 81 genes shown in Fig. 2 (Tan et al. 2010), while studies that analyzed synaptoneurosomes and only later-stage disease did not show significant overlap with TSA-regulated genes (Colangelo et al. 2002; Yao et al. 2003; Ricciarelli et al. 2004; Dunckley et al. 2006; Weeraratna et al. 2007; Liang et al. 2008; Qin et al. 2009; Williams et al. 2009; Ginsberg et al. 2012).

Microarray expression levels, gene symbols, and descriptions for genes involved in epigenetic reprogramming which were differentially expressed ($p < 0.05$) by TSA treatment are shown in Supplemental Figure 1. This group includes 26 genes involved in chromatin remodeling, DNA repair, and methionine metabolism. Of particular interest are three genes, Mat2a, Mtrr, and Hist1H4h, which are regulated by TSA treatment, altered early in disease in gray matter in AD, and are involved in epigenetic reprogramming.

In order to confirm that the microarray expression level differences of down to 1.2-fold we detected were reliable, we confirmed differential gene expression in our TSA-treated mouse brains by qRT-PCR. We selected several genes for confirmation, which were altered at different levels by microarray from over fivefold down to 1.3-fold and overlapped in laser-captured gray matter in AD. These included S100a8 (upregulated 5.76-fold in TSA-treated mice by microarray), uracil DNA glycosylase (Ung) (upregulated 1.59-fold by microarray), and Mat2a (downregulated in TSA-treated mice 1.35-fold by microarray). By qRT-PCR, the

expression of S100a8 was increased 3.70-fold in TSA-treated mice, Ung was increased by 1.29-fold, and Mat2a was decreased by 1.21-fold ($p < 0.05$ for all three genes) as shown in Fig. 3. Also of interest, we have observed that hemoglobin β is expressed in neurons (Broadwater et al. 2011) and its expression has been shown to be regulated by histone acetylation (Fathallah et al. 2008). While we did not detect differential expression of hemoglobin β in our microarray analysis (−1.3-fold, p value of 0.11), we did detect a significant decrease in the expression of hemoglobin β chain (Hbb1 and Hbb2) transcripts (−1.40-fold for Hbb1, −1.28-fold for Hbb2, $p < 0.05$) in mRNA isolated from the cortex of TSA-treated mice compared to control mice by qRT-PCR as shown in Fig. 3.

Discussion

We created an imbalance in histone acetylation in mice by treatment with the HDAC inhibitor TSA and then analyzed gene expression in the cortex by microarray profiling with the goal of identifying genes susceptible to epigenetic regulation in the adult brain. Our analysis identified 369 genes susceptible to regulation by acetylation, which could therefore potentially be modulated by environmental influences and contribute to the development of complex disease. Our data show both increases and decreases in transcription in response to HDAC inhibition in mouse cortex. This is consistent with the fact that increased acetylation in the brain can alter gene transcription by several mechanisms. HDAC inhibition can directly alter transcription through histone acetylation at specific sites leading to a more open chromatin conformation, which would lead to upregulation of gene expression. HDAC inhibition can also result in increased acetylation of transcriptional regulators, thereby altering their activity that has been demonstrated for transcription factors including Sp1 and HNF4a (Ryu et al. 2003; Yang et al. 2009). Indirect effects of HDAC inhibition can alter transcription through upregulation of transcription factors, coactivators, or repressors. It is most likely that a combination of these mechanisms is involved in the transcriptional response observed.

TSA is an HDAC inhibitor, which will lead to increased acetylation of histones and a more open chromatin structure so it is not surprising that we detected overlap between TSA-regulated genes and genes involved in chromatin remodeling including histone deacetylase 1 (HDAC1), SWI/SNF-related, actin-dependent regulator of chromatin(Smarcd1), structural maintenance of chromosomes 4 (Smc4), and MYST histone acetyltransferase 4 (Myst4) as shown in Supplementary Figure 1. We also found overlap with genes involved in DNA repair such as Ung and thymine DNA glycosylase (Tdg) and genes involved in methionine or one carbon metabolism including Mat2A and Mtrr. Both Mat2A and Mtrr are involved in reactions that generate S-adenosyl methionine (SAM), which is the methyl donor for most methylation reactions in cells including cytosine-guanine dinucleotide (CpG) methylation. The fact that we see overlap between DNA repair and methionine metabolism genes and genes regulated by TSA treatment suggests that these genes together with genes involved in chromatin structure are a part of an overall program involved in epigenetic reprogramming.

The top two gene groups from the Molecular Signatures Database that overlapped with genes regulated by TSA in our gene set enrichment analysis were cancer and cell cycle genes (Ben-Porath et al. 2008; Lindgren et al. 2006) (Table 1). The third gene set that overlapped with our TSA microarray study was a microarray analysis of postmortem hippocampus in early-stage and late-stage AD brain (Blalock et al. 2004). We expected to see overlap with cancer and cell cycle gene sets due to previous studies, which have shown that treatment with HDAC inhibitors is beneficial in cancer, inhibiting proliferation, preventing angiogenesis, and inducing cell death pathways (Lane and Chabner 2009). Previous studies have implicated acetylation-mediated mechanisms in neurodegenerative disease as well and support our data showing overlap in AD. These include studies showing

that HDAC inhibition in mouse models of neurodegenerative disease, including Huntington's disease mice and the experimental autoimmune encephalomyelitis mouse model of multiple sclerosis, can provide neuroprotection (Ferrante et al. 2003; Gardian et al. 2005; Camelo et al. 2005). Studies have also reported that HDAC inhibition in AD mouse models reverses memory impairment (Green et al. 2008; Ricobaraza et al. 2009). An imbalance in histone acetylation in AD would be expected to lead to alterations in transcription and in fact, microarray profiling studies that have analyzed postmortem AD brain tissue have shown that transcription is altered in AD and that cortical areas are particularly vulnerable (Wang et al. 2010). Our study has identified a subset of genes altered in AD, shown in Fig. 2, which are susceptible to regulation by an imbalance in acetylation.

Interestingly, another study has shown that resveratrol, which activates the sirtuin class III HDACs, has been shown to slow the development of AD pathology (Anekonda and Reddy 2006). This study also suggests an imbalance in acetylation in AD. The balance between histone and protein acetylation and deacetylation may be altered in neurodegenerative diseases such as AD as a result of mitochondrial dysfunction and increased reactive oxygen species (ROS), which has been reported in the AD brain (Smith et al. 1997; Hirai et al. 2001; Moreira et al. 2008). AD is an age-related form of dementia marked by decreased neuronal and synaptic activity and cognitive impairment (Selkoe 2004). The central AD pathologies include extracellular plaques resulting from aberrant processing of amyloid precursor protein (APP) into beta-amyloid and intracellular neurofibrillary tangles containing aggregated tau protein. In the AD brain, microglia are activated by contact with fibrillar beta-amyloid in extracellular plaques. This contact activates microglial NADPH oxidase, which generates damaging ROS (Qin et al. 2006; Wilkinson and Landreth 2006). In addition, activated microglia release nitric oxide, which can react with NADPH-generated ROS to create highly reactive peroxynitrite, which damages cells by oxidizing proteins and lipids and can lead to neuronal apoptosis (Combs et al. 2001). This oxidative environment can influence both acetylation and methylation reactions in cells (Cyr and Domann 2011), leading to epigenetic reprogramming and altered transcription. In support of this notion, Drake et al. (2004) demonstrated a link between mitochondrial dysfunction, increased ROS, and altered histone modifications. In this study, 4-hydroxynonenol, which is an end product of lipid peroxidation, has been shown to be increased in the AD brain and to bind to histones. This interaction has been suggested to alter transcription by interfering with the ability of histones to bind to DNA in the AD brain. Other studies have shown a direct involvement of proteins involved in AD pathology including tau and beta-amyloid in mediating altered acetylation of histones H3 and H4, and β-tubulin (Perez et al. 2009; Lithner et al. 2009). Studies have now also directly linked alterations in acetylation of histones to gene expression involved in learning and memory and cognitive function (Stilling and Fischer 2011). Peleg et al. (2010) have reported that acetylation of histone H4 is altered in aged mice and leads to deficits in memory consolidation. Another study has provided evidence of a link between histone H4 acetylation and cognitive dysfunction more specifically related to AD (Francis et al. 2009). This study showed that levels of histone H4 acetylation were decreased in the APP/PS1 mouse model of AD compared to wild-type littermates after fear conditioning training, which is a test of associative memory. TSA treatment of the APP/PS1 mice prior to fear conditioning prevented histone H4 deacetylation and restored contextual fear behavior and hippocampal long-term potentiation.

In the present study, HDAC inhibition in mice with TSA resulted in altered expression of 81 genes, which overlapped with differentially expressed genes in the AD brain. It is important to note that in AD, the direction of the alteration of the 81 overlapping genes is not always the same as in the TSA-treated mice. For example, of the 65 genes that overlapped in the Blalock et al. (2004) study identified by gene set enrichment analysis, all 65 genes were upregulated in AD, while in TSA-treated mice, the expression of 41 of these genes was

increased and the expression of 24 was decreased. These results may explain contradictory reports with regard to HDAC activity and HDAC inhibition in the literature. In some studies, increased histone and protein acetylation or inhibition of HDACs through oxidative modifications has been implicated in inflammatory processes and in AD pathology, suggesting that treatment with HDAC inhibitors would mimic the disease and be deleterious (Ito et al. 2004; Lithner et al. 2009; Doyle and Fitzpatrick 2010). However, evidence from studies in mouse models of AD and neurodegenerative disease shows that HDAC inhibitors reverse memory impairment and provide neuroprotection (Ferrante et al. 2003; Gardian et al. 2005; Camelo et al. 2005; Green et al. 2008; Ricobaraza et al. 2009). The finding that HDAC inhibition seems to be involved in disease pathology, on one hand, but that HDAC inhibitors can be therapeutic in neurodegenerative disease mouse models can be explained by our results, which show that for a third of the overlapping genes identified in our study, the direction of transcriptional regulation by TSA was reversed compared to AD. This suggests that in AD, there exists an imbalance in acetylation due to a combination of decreased HDAC activity (or increased HAT activity) for some genes and increased HDAC activity (or decreased HAT activity) for others. These results indicate the necessity for drugs targeted to specific HDACs and HATs in the treatment for complex neurodegenerative diseases such as AD.

A potential candidate HDAC that may provide a more specific therapeutic target in AD is HDAC2. While studies have linked altered acetylation of specific histones, histone H4 in particular, to AD pathology (Francis et al. 2009), other studies point to involvement of specific HDACs including HDAC2 in cognitive impairment and in AD. HDAC2 has been shown to be involved in learning and memory by regulating the expression of genes involved in synaptic plasticity in a study by Guan et al. (2009). In this study, HDAC2 knockout mice display increased acetylation of histone H4 on lysine 12 (H4K12) in the hippocampus and enhanced learning and memory. Another study has reported that knocking down HDAC2 in the hippocampus by short hairpin RNA repairs cognitive function in an AD mouse model (Gräff et al. 2012). Another specific HDAC implicated in AD pathology is HDAC6. A study by Ding et al. (2008) reported increased HDAC6 in AD postmortem brain samples. Rather than altering transcription in the nucleus by deacetylating histones, HDAC6 acts primarily in the cytoplasm where it deacetylates β-tubulin. Aberrant HDAC6 activity has been suggested to contribute to neurodegeneration by decreasing acetylation of β-tubulin resulting in disruption of intracellular transport (Li et al. 2011). While TSA inhibits both HDAC2 and HDAC6, our study was designed to identify alterations in transcription through inhibition of HDACs that act in the nucleus, such as HDAC2, and would not have detected the effects of HDAC6 inhibition.

The fact that 81 genes regulated by TSA overlap in the Blalock et al. (2004, 2011) and Bossers et al. (2010) studies, which analyzed early-stage AD brains, suggests that epigenetic mechanisms may play an early role in the development of AD. Interestingly, these results also suggest that an imbalance in acetylation can lead to alterations in one carbon metabolism and potentially methylation reactions. This is shown by the downregulation of genes involved in methionine or one carbon metabolism, Mat2a and Mtrr, by TSA in our study. Mtrr reduces methionine synthase, which then catalyzes the reaction converting homocysteine to methionine. Mat2a catalyzes the next step in the one carbon cycle and converts methionine to SAM, the methyl donor for most methylation reactions in cells. The fact that these genes overlap with genes altered in early-stage disease in laser-captured gray matter suggests a role for altered DNA or histone methylation in neuronal pathology in early stages of AD. These results also reflect the complexity and crosstalk between acetylation and methylation in chromatin remodeling (Berger 2007). Our data are consistent with other studies, which have shown that alterations in transcription in AD may be mediated by epigenetic modifications involving methylation. It has been reported that the methionine

metabolism intermediate S-adenosyl homocysteine (SAH) is increased in postmortem AD brain and inhibits methyltransferases (Kennedy et al. 2004). Other studies have shown that intermediates of methionine metabolism, both SAM and SAH, can modulate the transcription of genes involved in AD pathology. In AD transgenic mice, the level of these intermediates has been manipulated by folate and vitamin B deficiency. These deficiencies were shown to lead to an accumulation of SAH, inhibition of methyltransferases, and upregulation of the expression of presenilin (PS1) and betaamyloid cleaving enzyme (Fuso et al. 2008). In a subsequent study, the expression of PS1 was found to be regulated by increased SAH due to altered levels of methylated DNA in gene regulatory regions (Fuso et al. 2011).

Our data suggest that hemoglobin may be an additional target of epigenetic reprogramming in neurons in AD. Expression of the hemoglobin β and β chains has been reported in neurons in both human and mouse brains (Biagioli et al. 2009; Richter et al., 2009; Schelshorn et al., 2009; Broadwater et al. 2011) where it has been proposed to play a role in neuronal respiration. Hemoglobin expression has been shown to be decreased in the AD brain but the significance of this observation to AD pathology is still not clear (Ferrer et al. 2011). Studies have shown that hemoglobin expression can be regulated by both histone acetylation and CpG methylation of regulatory regions in erythroid cells (Fathallah et al. 2008; Lathrop et al. 2009), but not much is known concerning its regulation in neurons. We have shown that hemoglobin β transcription is susceptible to regulation by TSA treatment in the brain providing evidence for epigenetic mechanisms in the regulation of hemoglobin expression in neurons as well.

This study has identified genes susceptible to regulation by an imbalance in acetylation in the adult CNS. It has provided evidence for epigenetic reprogramming in mediating transcriptional alterations in early stages of AD in gray matter. Future studies will be required to understand the types of environmental influences and mechanisms that initiate these processes in AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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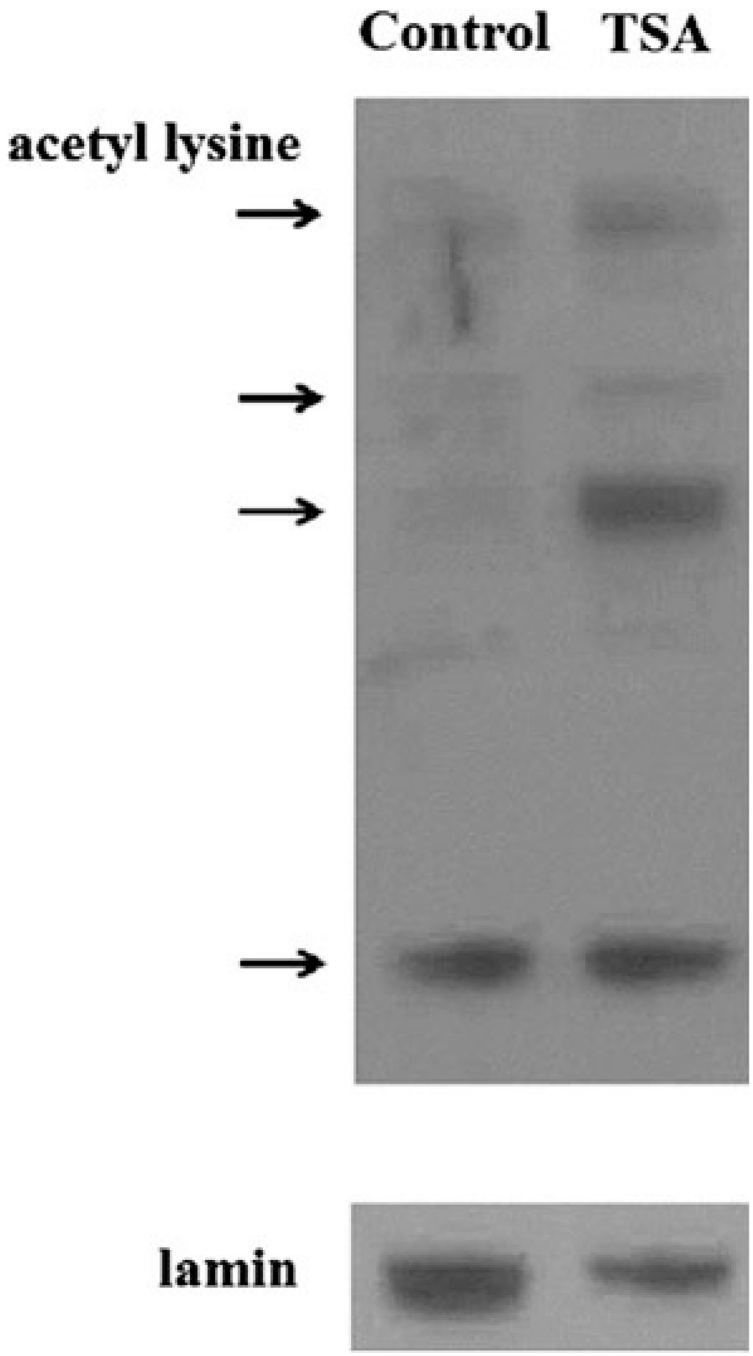
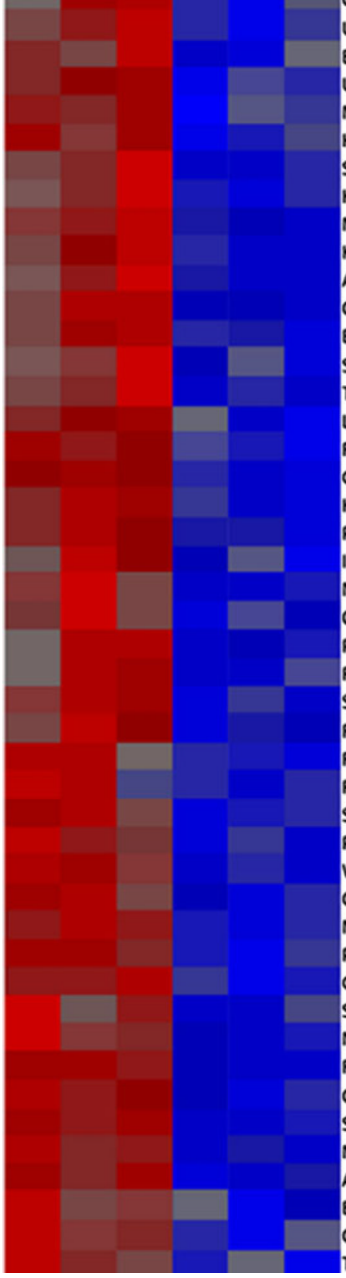


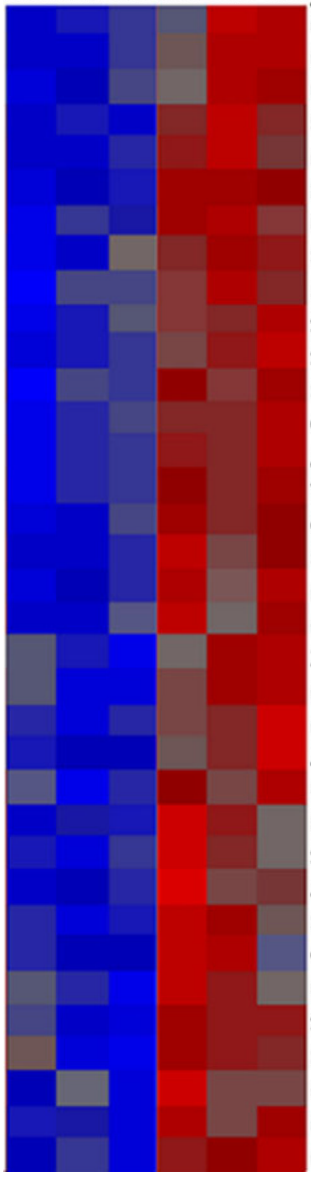
Fig. 1.
Representative western blot showing increased acetyl-lysine immunoreactive proteins (denoted by *arrows*) in nuclear extracts isolated from the brains of TSA-treated compared to control (vehicle-treated) mice

A



TSA1	TSA2	TSA3	VEH1	VEH2	VEH3	Symbol	Probe ID	Description	FC
						Cdkn2c	1416868_at	Cyclin-dependent kinase inhibitor 2C a, b, d	1.23
						Usp3	1425023_at	Ubiquitin specific peptidase 3 a	1.30
						Bmpr1a	1425493_at	Bone morphogenetic protein receptor, type 1A a *	1.27
						Ung	1425753_a_at	Uracil DNA glycosylase a, b, d	1.59
						Myo1e	1420160_s_at	Myosin IE a, b *	1.35
						Hdac1	1448246_at	Histone deacetylase 1 a, d	1.28
						S100a8	1419394_s_at	S100 calcium binding protein A8 (calgranulin A) b	5.76
						Hist1h1c	1416101_a_at	Histone cluster 1, h1c a	1.22
						Mcm7	1416030_a_at	Minichromosome maintenance deficient 7 (S. Cerevisiae) a, d	1.31
						Kpnb1	1434357_a_at	Karyopherin (importin) beta 1 a, b	1.22
						Arg2	1438841_s_at	Arginase type II a	1.62
						Cbfb	1460716_a_at	Core binding factor beta a, d	1.25
						Btg3	1449007_at	B-cell translocation gene 3 a, b	1.51
						Socs3	1456212_x_at	Suppressor of cytokine signaling 3 a, b, d	1.47
						Tspan6	1448501_at	Tetraspanin 6 a	1.21
						Lpp	1425673_at	LIM domain containing preferred translocation partner a, d	1.29
						Fgf9	1420795_at	Fibroblast growth factor 9 b, c, d *	1.33
						Gtpbp3	1450980_at	GTP binding protein 3 a	1.25
						Hs2st1	1450729_at	Heparan sulfate 2-o-sulfotransferase 1 a, b *	1.21
						Pawr	1426910_at	PRKC, apoptosis, WT1, regulator a, b *	2.68
						Il17ra	1420905_at	Interleukin 17 receptor A a, d	1.32
						Nfkbie	1431843_a_at	Nuclear factor of kappa light polypeptide in b-cells inhibitor, ep c *	1.32
						Ctbp2	1434705_at	C-terminal binding protein 2 a, b, d	1.21
						Polr1d	1448287_at	Polymerase (RNA) I polypeptide D a	1.22
						Fbn1	1460208_at	Fibrillin 1 a, d *	1.72
						Sord	1438183_x_at	Sorbitol dehydrogenase a	1.36
						Rreb1	1428657_at	Ras responsive element binding protein 1 a, d	1.26
						Fam60a	1448126_at	Family with sequence similarity 60, member A a	1.20
						Fgl1	1424599_at	Fibrinogen-like protein 1 a, d	1.31
						Smc4	1452197_at	Structural maintenance of chromosomes 4 a, d	1.42
						Pvrl3	1423331_a_at	Poliovirus receptor-related 3 c *	1.30
						Wwtr1	1417818_at	WW domain containing transcription regulator 1 a, d	1.29
						Ckap4	1426755_at	Cytoskeleton-associated protein 4 a, b	1.31
						Mitf	1455214_at	Microphthalmia-associated transcription factor a, c, d *	1.66
						Phf21a	1418391_at	PHD finger protein 21A a, b	1.31
						Gmnn	1417506_at	Geminin a	1.49
						Sfrp1	1448395_at	Secreted frizzled-related protein 1 a, b *	1.23
						Nup43	1432188_s_at	Nucleoporin 43 a	1.30
						Rod1	1455819_at	ROD1 regulator of differentiation 1 (S. Pombe) a, d *	1.30
						Cdt1	1424143_a_at	Chromatin licensing and DNA replication factor 1 c, d *	1.78
						Scarb1	1437378_x_at	Scavenger receptor class B, member 1 a	1.36
						Mafb	1451715_at	V-maf musculoaponeurotic fibrosarcoma oncogene family a, d	1.39
						Abcb6	1422524_at	ATP-binding cassette, sub-family B (MDR/TAP), member 6 a	1.22
						Eif5b	1434605_at	Eukaryotic translation initiation factor 5B a	1.23
						Casp3	1449839_at	Caspase 3 a, c *	1.34
						Trove2	1423433_at	TROVE domain family, member 2 a	1.20

B



Symbol	Probe ID	Description	FC
Ntrk3	1426003_at	Neurotrophic tyrosine kinase, receptor, type 3 [a]	-1.23
Mtrr	1452110_at	5-methyltetrahydrofolate-homocysteine methyltransferase red . [b] *	-1.29
Deaf1	1448446_at	Deformed epidermal autoregulatory factor 1 (drosophila) [a] *	-1.22
Per1	1449851_at	Period homolog 1 (drosophila) [a, d] *	-1.41
Myo10	1422544_at	Myosin X [a, d] *	-1.23
Hmbs	1436930_x_at	Predicted gene 6453 /// hydroxymethylbilane synthase [a]	-1.27
Larp7	1428310_at	La ribonucleoprotein domain family, member 7 [a]	-1.25
Hist1h4h	1428014_at	Histone cluster 1, h4h [a, b, d] *	-1.27
Epc1	1418850_at	Enhancer of polycomb homolog 1 (drosophila) [c] *	-1.21
Shmt2	1426423_at	Serine hydroxymethyltransferase 2 (mitochondrial) [a]	-1.39
Sspn	1417644_at	Sarcospan [a] *	-1.25
Rpl18	1456447_at	Ribosomal protein L18 [a, b]	-1.28
Cdc6	1417019_a_at	Cell division cycle 6 homolog (S. Cerevisiae) [a]	-1.24
Actr8	1423385_at	ARP8 actin-related protein 8 homolog (S. Cerevisiae) [a]	-1.27
Tdg	1435715_x_at	Thymine DNA glycosylase [a] *	-1.27
Cog4	1419808_at	Component of oligomeric golgi complex 4 [a] *	-1.24
Rev3l	1451960_a_at	Rev3-like, catalytic subunit of DNA polymerase zeta RAD54 like [c] *	-1.22
Mrgprf	1425894_at	Mas-related GPR, member F [c, d] *	-1.20
Myo15b	1435979_a_at	Myosin XVB [a, d]	-1.21
Zic3	1423424_at	Zinc finger protein of the cerebellum 3 [c] *	-1.27
Myst4	1423508_at	MYST histone acetyltransferase monocytic leukemia 4 [a]	-1.32
Mzf1	1420738_at	Myeloid zinc finger 1 [a]	-1.38
Anln	1433543_at	Anillin, actin binding protein [b, c] *	-1.21
Erf	1435561_at	Ets2 repressor factor [a, b, d]	-1.34
Il7r	1448575_at	Interleukin 7 receptor [b] *	-1.27
Smarcd1	1420240_at	SWI/SNF related, actin dependent regulator of chromatin [a]	-1.30
Angptl2	1421002_at	Angiopoietin-like 2 [a] *	-1.21
Hif3a	1425428_at	Hypoxia inducible factor 3, alpha subunit [b, c, d] *	-1.24
Gpr146	1451060_at	G protein-coupled receptor 146 [c] *	-1.30
Lhx3	1425041_at	LIM homeobox protein 3 [a, d]	-1.27
Stag1	1421939_a_at	Stromal antigen 1 [a, b]	-1.25
Pop5	1431373_at	Processing of precursor 5, ribonuclease P/MRP family [a]	-1.23
Mtch2	1438842_at	Mitochondrial carrier homolog 2 (C. Elegans) [a, d] *	-1.22
Nfatc1	1425761_a_at	Nuclear factor of activated t-cells, calcineurin-dependent 1 [a, d]	-1.27
Mat2a	1456702_x_at	Methionine adenosyltransferase II, alpha [a, b, c] *	-1.35

Fig. 2.

TSA-regulated genes overlap with genes differentially expressed in AD. Overlaps were identified by a combination of gene set enrichment analysis and literature searches. Heat maps show relative gene expression levels for TSA-regulated genes by expression microarray profiling. *Red* denotes increased expression and *blue* denotes decreased expression of genes in TSA-treated mice compared to controls (vehicle-treated, veh.). a Heat map of genes upregulated by TSA, which overlap with genes altered in AD. b Heat map of genes downregulated by TSA treatment, which overlap with genes altered in AD. [a]Overlap identified by gene set enrichment analysis (Blalock et al. 2004, which analyzed early and later stages of AD), [b]overlap in *gray* matter (Blalock et al. 2011, which analyzed early-stage disease in laser-captured hippocampal gray matter), [c]overlap with Bossers et al. (2010), which analyzed cortical gene expression in early-stage AD, [d]overlap with Tan et al. (2010),

which analyzed neocortex. *TSA-regulated genes that overlap in early-stage AD. *FC* indicates fold change, *TSA* treated relative to control

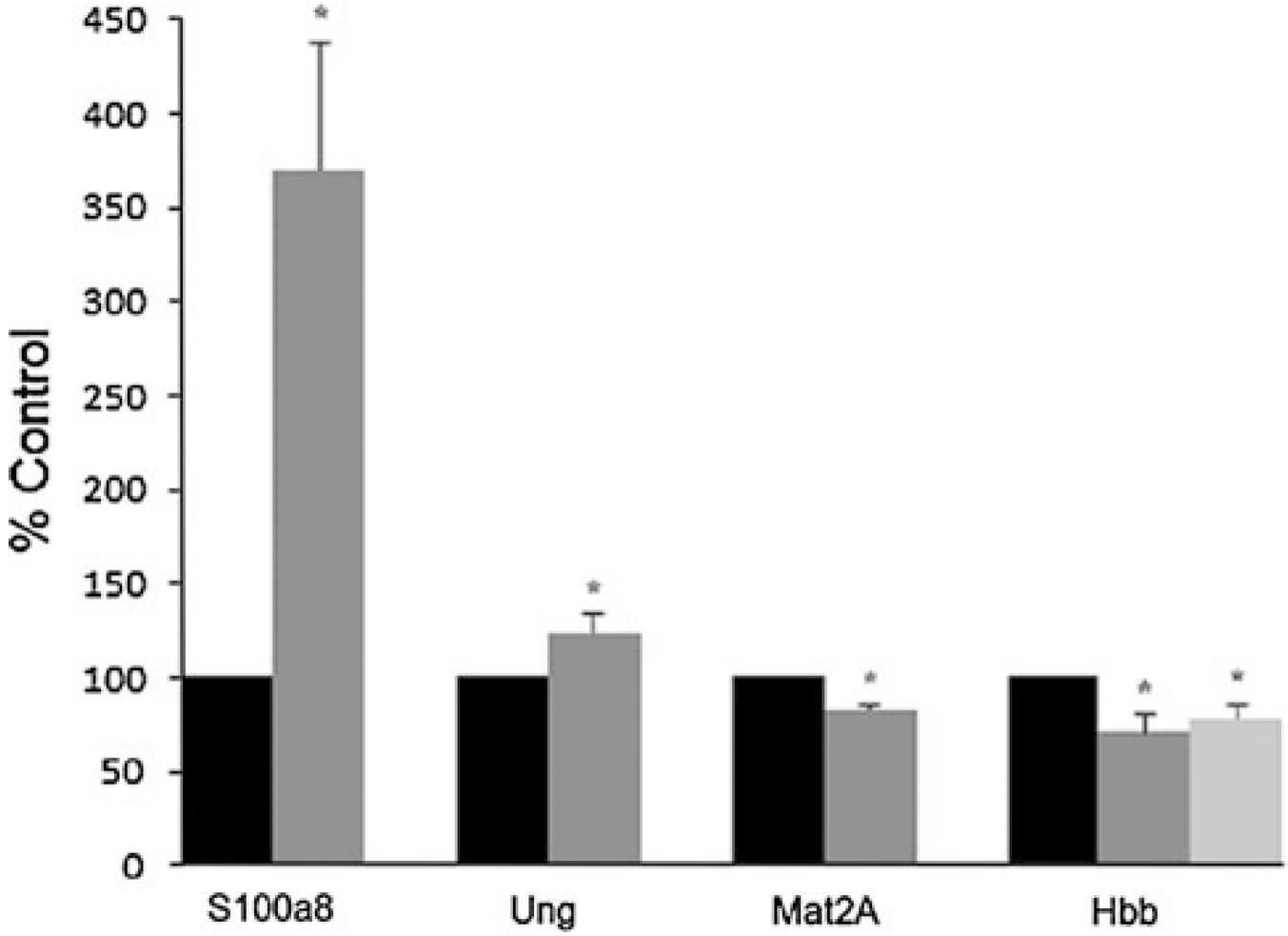


Fig. 3.

Quantitative RT-PCR confirms altered expression of genes found to be differentially expressed due to TSA treatment by microarray profiling. By qRT-PCR of RNA isolated from the cortex of TSA-treated and control mice, the expression of S100a8 was increased 3.70-fold, Ung was increased 1.29-fold, Mat2a was decreased 1.21-fold, and the hemoglobin β1 and β2 transcripts (Hbb1 and Hbb2) were decreased by 1.40- and 1.28-fold, respectively. *Black bars* represent gene expression levels from control mice, and *gray bars* represent gene expression levels from TSA-treated mice. For Hbb, the *dark gray bar* represents Hbb1 expression and the *lighter gray bar* represents Hbb2 expression. Gene expression levels in TSA-treated mice are shown as percent of control from at least two experiments performed in triplicate. *Error bars* represent SEM, $*p < 0.05$

Table 1

Gene set enrichment analysis

Gene set	# of genes in gene set	Description	# of genes in overlap	*p* value
BENPORATH_CYCLING_GENES	648	Genes showing cell cycle stage-specific expression	39	5.71 E–12
LINDGREN_BLADDER_CANCER_CLUSTER_3_UP	325	Genes whose expression profile is specific to cluster III of urothelial cell carcinoma (UCC) tumors	25	2.96 E–10
BLALOCK_ALZHEIMERS_DISEASE_UP	1,720	Genes upregulated in brain from patients with Alzheimer's disease	65	4.62 E–10

Gene set enrichment analysis was performed on previously published microarray studies in the Molecular Signatures Database (www.broadinstitute.org/gsea/MSigDB/index.jsp). The top three gene sets, ordered by significance of p values, overlapping with TSA-regulated genes include cell cycle genes (Ben-Porath et al. 2008), cancer genes (Lindgren et al. 2006), and AD genes (Blalock et al. 2004)