Published in final edited form as:
Arterioscler Thromb Vasc Biol. 2014 September ; 34(9): 1798–1799. doi:10.1161/ATVBAHA.114.304295.

New role for histone deacetlyase 9 in atherosclerosis and inflammation

Jonathan D. Smith
Dept. of Cellular & Molecular Medicine, The Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, Phone: 216-444-2248

Jonathan D. Smith: smithj4@ccf.org

Epigenetic regulation of gene expression is mediated by DNA methylation as well as by modification of nucleosome structure and co-factor recruitment through histone modifications. Specific methylation and acetlylation of histone lysine residues, which comprise the so called “histone code”, are marks of different chromatin states related to the expression of neighboring genes and the activity of promoters and enhancers. Histone acetylation and methylation, along with their role in regulating transcription has been known since the 1960s. The enzymes that make these modifications can be thought of as “chromosome writers”, while the enzymes that remove these marks can be referred to as “chromosome erasers”. Another set of proteins sense these marks and alter gene expression, which can be termed “chromosome readers”. Histone acetylation is performed by members of the histone acetyltransferase (HAT) gene family, which use acetyl-CoA as a cofactor to modify specific ε-amino group of lysine residues on different histone proteins. This modification eliminates lysine’s positive charge and reduces the affinity of the histone for DNA, which can result in a more relaxed chromatin conformation that can facilitate the recruitment of DNA binding effector proteins. Thus, histone acetylation is most often associated with increased transcriptional activity, and in the most extreme case, acetylation of lysine 16 on histone H4 (H4K16ac) is involved in the switch from inactive heterochromatin to euchromatin[1]. Histone acetylation is reversible, allowing for environmental and developmental gene regulation, usually repression; and this process is carried out by members of the histone deacetylase (HDAC) gene family. There are five phylogeneic classes of HDACs in mammalian cells comprising 18 genes in humans. The classical HDAC genes in classes I, IIA, IIB, and IV are zinc dependent enzymes, while the class III genes are the sirtuins, which are NAD+ dependent enzymes[2]. Acetylation is widespread in cell protiens, and HDACs can deacetylate other proteins aside from histones. Since protein acetylation controls many features of cell physiology and proliferation, there are many HDAC and sirtuin inhibitiors in use in the lab as well as in the clinical pipeline, with two drugs, Vorinostat and Romidepsin, approved for oncology, and a host of other drugs in clinical trials for a range of diseases[3]. Completing the regulatory circuit, acetylated histones are “read” by a large family of bromodomain and extra-terminal domain (BET) proteins, which directly bind to acetylated lysine residues on histones. BRD4 is one well studied BET protein which has several activities, including acting as a transcription factor, bookmarking the chromatin during mitosis to activate specific genes, and acting as a scaffold to recruit other factors to the chromatin that play roles in RNA transcription, elongation, or splicing[4].

In this issue of *ATVB*, a team led by Mishra from Wake Forest School of Medicine demonstrated a role for HDAC9, a class IIA enzyme, in mouse macrophage polarization, cholesterol efflux, and atherosclerosis[5]. Class IIA HDAC proteins contain an N-terminal domain that regulate nuclear-cytoplasmic shuttling, which is modulated by phosphorylation and subsequent binding of 14-3-3 proteins[2]. One impetus for the study of HDAC9 is that various common SNPs in HDAC9 introns have been associated with stroke (two independent studies), retinopathy, obesity related traits, ulcerative colitis, and male pattern baldness in genome wide association studies (www.genome.gov/gwastudies, accessed 7/17/14); however the functional mechanisms by which these SNPs alter these traits has not been described. HDAC9 is expressed highly in muscle and brain, and in addition to histone deacetylation and its role in epigenetics, HDAC9 binds to and represses the activity of the transcription factor MEF2[6], and it also binds to TRIM29, altering its association with p53 and regulating cell proliferation[7]. HDAC9 knockout mice were created in the Olson lab, and although fertile and normal at birth, these mice develop cardiac hypertrophy upon aging, and are sensitized to pressure overload[8]. Others have shown that HDAC9 deficient mice exhibit polydactyly, resistance to colitis, and resistance to obesity and glucose intolerance upon high fat diet feeding[9–11]. In the present study, Cao et al. crossed the HDAC9 deficient mice from the Olson lab to LDLr deficient mice to create the double knockout (DKO) mice[5]. After feeding a high carbohydrate diet with 0.1% cholesterol, the DKO mice, compared to the single LDLr knockout (SKO), had decreased lesions in the aortic root, arch, and descending aorta, which was associated with a modest shift of plasma cholesterol from VLDL to LDL and a modest reduction in plasma triglycerides. Targeted gene expression studies in the aortic roots showed that ABCA1, ABG1, and arginase-1 were induced in the DKO, while IL-1b and MCP1 were repressed. In order to see if these effects were mediated by HDAC9 expression in leukocytes, they performed transplantation of SKO or DKO bone marrow cells into irradiated LDLr-deficient hosts, which confirmed the same anti-atherogenic effect for the mice receiving the DKO donor cells. Through *in vitro* studies, HDAC9-deficient vs. wild type (WT) macrophages were found to have increased ABCA1 and ABCG1 expression, as well as cholesterol efflux to apoA1 (ABCA1-dependent) and HDL. By the use of chromatin immunoprecipitation followed by PCR, the increased macrophage expression of ABCA1 and ABCG1 in the HDAC9 knockout cells was associated with increased H3 and H4 acetylation at their respective promoters, without any histone acetylation changes observed for the scavenger receptor A (SRA) promoter, a gene whose expression was not altered by HDAC9 deficiency. Macrophages from the HDAC9 deficient mice were also less responsive to LPS stimulation in their release of pro-inflammatory cytokines; and, target gene qPCR showed that these HDAC9 deficient cells had increased expression of markers for alternatively activated M2 macrophages, and decreased expression of M1 markers. Thus, HDAC9 deficiency results in macrophages that are polarized for promoting inflammation resolution and reverse cholesterol transport, which can put the brakes on atherosclerosis progression and promote lesion regression.

Previously, via the use of various inhibitors and gene knockouts, HDACs have been found to have both positive and negative effects on toll like receptor (TLR) signaling and innate immunity[12]. The current study demonstrates clearly that HDAC9 has a positive effect on TLR signaling, as the HCAD9 deficient macrophages are less sensitive to LPS. In contrast

to the current study, a prior study found that chronic i.p. injection of the HDAC inhibitor trichostatin (TSA), which inhibits HDACs in class I, IIA, and IIB, led to increased aortic root lesions in LDLr-deficient mice fed a high cholesterol diet[13]. It is easy to reconcile this difference, since HDAC9 deficiency is quite specific compared to the TSA mediated inhibition of many HDACs. TSA has also been shown to up regulate the expression of the macrophage scavenger receptors SRA and CD36, and increase OxLDL uptake[13, 14], whereas HDAC9 deficiency had no effect on macrophage expression of SRA[5]. One of the remaining questions about the current study is whether the anti-inflammatory and anti-atherogenic effects of HDAC9 deficiency are mediated primarily through histone modification, or via decaetylation of other effector proteins. In conclusion, Cao et al demonstrated the potential to use HDAC9 inhibition to prevent inflammation and atherosclerosis, and as more specific HDAC9 inhibitors are developed, these will be attractive drugs to evaluate.

Acknowledgments

Sources of Funding.

This work is supported by NIH grants R01 HL098193 and PO1 HL098055

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