

Minireviews

Pleiotropic and Adverse Effects of Statins—Do Epigenetics Play a Role?

Stephanie C. Allen and Cyril D.S. Mamotte

School of Biomedical Sciences and Curtin Health Innovation Research Institute, Curtin University, Perth, Western Australia

Received April 10, 2017; accepted May 23, 2017

ABSTRACT

Statins are widely used to prevent major cardiovascular events by lowering serum cholesterol. There is evidence that statins have pleiotropic effects—that is, cholesterol-independent effects—that may also confer protection from cardiovascular disease and potentially numerous other pathologies, including cancer. Statins also have a number of well described adverse effects, including myopathy, rhabdomyolysis, liver damage, and type 2 diabetes. This paper examines the evidence of epigenetic modifications as a contributory factor to the pleiotropic and adverse effects of statins. In vitro and animal studies have shown that statins can inhibit histone deacetylase activity and increase histone acetylation. Similarly, there is evidence that statins may inhibit both histone and DNA methyltransferases and subsequently

demethylate histone residues and DNA, respectively. These changes have been shown to alter expression of various genes, including tumor suppressor genes and genes thought to have anti-atherosclerotic actions. Statins have also been shown to influence the expression of numerous microRNAs that suppress the translation of proteins involved in tumorigenesis and vascular function. Whether the adverse effects of statins may also have an epigenetic component has been less widely studied, although there is evidence that microRNA expression may be altered in statin-induced muscle and liver damage. As epigenetics and microRNAs influence gene expression, these changes could contribute to the pleiotropic and adverse effects of statins and have long-lasting effects on the health of statin users.

Introduction

3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, a class of cholesterol-lowering drugs commonly known as statins, are the most commonly prescribed drugs in numerous advanced economies, with approximately one-quarter of Americans over the age of 40 being prescribed statins (Gu et al., 2014). Statins are competitive inhibitors of HMG-CoA reductase, the rate-limiting enzyme in the mevalonate pathway of cholesterol synthesis. The efficacy of statins in preventing morbidity and mortality from cardiovascular disease (CVD) is well established; large-scale trials of atorvastatin (Sever et al., 2004) and pravastatin (Nakamura et al., 2006) reported 34–37% reductions in major cardiovascular events and 14–29% reductions in overall mortality. There is evidence that the beneficial actions of statins on cardiovascular health extend beyond lipid lowering; pleiotropic effects may also contribute (Zhou and Liao, 2009). Statin use has been associated with additional pleiotropic effects that may confer anticancer activity, immunomodulation, and renoprotection. However, statins also have a number of established

adverse effects, including muscle complaints, such as myopathy and rhabdomyolysis, liver damage, and type 2 diabetes (T2D). Although the lipid-lowering mechanism of statins is understood, the exact mechanisms of these pleiotropic and adverse effects are not as clear. Epigenetics, the collection of reversible modifications to cellular DNA that affect gene expression without altering the base DNA sequence, may play an important role. MicroRNAs (miRNAs), often included in discussions on epigenetic effects, may also contribute.

The switching of genes on and off through epigenetic mechanisms is required in the development of organisms and in the differentiation and function of different cell types. However, epigenetic changes have also been associated with numerous disorders, including cancer (Kanwal and Gupta, 2012), T2D (Dayeh et al., 2014), and CVD (Ordovas and Smith, 2010). Epigenetic modifications are influenced by environmental factors, including diet, exposure to pollutants and other toxins, temperature, and stress, and may be passed on to offspring (Feil and Fraga, 2012). In particular, the fetal environment is known to imprint epigenetic modifications that have lifelong effects; epidemiologic studies reported that nutrient deficiency during pregnancy, such as during the Dutch Hunger Winter in WWII,

<https://doi.org/10.1124/jpet.117.242081>.

ABBREVIATIONS: ACE2, angiotensin-converting enzyme 2; BMP2, bone morphogenetic protein 2; CCR7, CC-chemokine receptor 7; CVD, cardiovascular disease; DNMT, DNA methyltransferase; EPC, endothelial progenitor cell; EZH2, enhancer of zeste homolog 2; FOXP3, forkhead box P3; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMT, histone methyltransferase; miRNA, microRNA; SAHA, suberoylanilide hydroxamic acid; SIRT1, sirtuin 1; T2D, type 2 diabetes; TSG, tumor suppressor gene.

resulted in children at an increased risk of T2D and obesity regardless of birth weight and childhood nutrition, with differences in DNA methylation compared with unaffected same-sex siblings in adulthood (Tobi et al., 2009). Epigenetic changes also occur due to aging, and these changes have been associated with disorders such as T2D (Bacos et al., 2016). Due to the role of epigenetic modifications in disease, drugs that target epigenetics have been investigated for numerous disorders. There is an increasing body of evidence that established and widely used drugs, including statins, may also impact health through epigenetic mechanisms. This paper summarizes research on the effects of statins on epigenetics and miRNAs, with a focus on their contribution to pleiotropic and adverse effects.

The Nature of Epigenetic Modifications

Epigenetic changes are modifications that alter the accessibility of genes for transcription, resulting in an increase or decrease in gene expression (Fig. 1). Changes occur at two fundamental levels: 1) modifications to histone proteins that affect the packaging of DNA, and 2) modifications to DNA, most notably through methylation. Both affect the ability of genes to be transcribed. Post-transcription, miRNAs provide another level of regulation by suppressing translation.

Histone Modifications

Modifications to histones, including acetylation, methylation, phosphorylation, and ubiquitination, alter chromatin condensation. DNA is packaged into nucleosomes consisting of 146 base pairs of DNA wrapped around a histone core of two each of histone proteins H2A, H2B, H3, and H4, with H6 binding to the DNA as it enters the core, held in place by H1. Each histone protein has an amino-terminal tail extending from the nucleosome; modifications to residues on the tail act as signals for further organization of chromatin. Acetylation of histone lysine residues neutralizes the positive charge of the histone, reducing the strength of the bond with negatively charged DNA and thus allowing transcription factors access to the DNA (Bannister and Kouzarides, 2011). Conversely, deacetylation leads to chromatin compaction and decreased gene expression. Histone acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). In contrast to acetylation, histone methylation does not alter the charge of the histone and can either promote or suppress gene transcription depending on the precise location, denoted by the histone protein (e.g., H3) and the lysine (K) or arginine (R) residue which is methylated. Methylation of histone lysines H3K4, H3K36, and H3K79 is found on transcriptionally active genes, whereas methylation of H3K9, H3K27, and H4K20 is associated with gene silencing (Lara et al., 2011). Histone methylation is regulated by histone methyltransferases (HMTs) and histone demethylases.

DNA Methylation

DNA methylation occurs when a methyl group is added to cytosines in CG dinucleotides, often denoted as CpG, by DNA methyltransferases (DNMTs). Hypermethylation of DNA in CpG islands, regions of DNA with a high frequency of CpG clusters often found in the promoter regions of genes, results in repression of gene expression. At its simplest level, methylation blocks the binding of transcription factors to DNA (Bird, 2002).

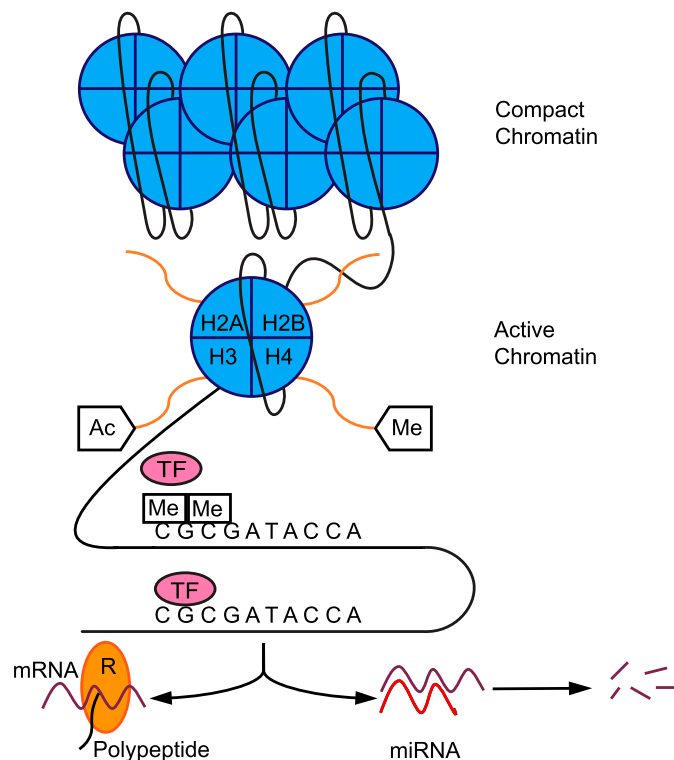


Fig. 1. Alteration of gene expression by epigenetic changes and miRNA. Chromatin consists of DNA wound around histone cores consisting of histone proteins H2A, H2B, H3, and H4. Compaction of chromatin reduces access of transcription factors and other proteins to DNA and is associated with transcriptionally silent genes. More relaxed, “active” chromatin, mediated by modifications such as acetylation (Ac) and methylation (Me) of residues on histone tails protruding from the nucleosome, allows easier access to DNA and is associated with active transcription. Methylation of cytosines in CpG dinucleotides represses gene expression by blocking transcription factors (TF) and/or interacting with histones. At the post-transcription level, miRNAs offer another mode of regulation through interaction with complementary mRNAs, blocking ribosomal (R) translation directly and/or resulting in mRNA degradation.

DNA methylation can also indirectly affect gene expression—for example, by altering the binding of proteins to histones (Bannister and Kouzarides, 2011).

MicroRNAs

miRNAs are small, noncoding RNAs involved in post-transcriptional regulation of gene expression. miRNAs bind imperfectly to target mRNAs, which can number in the hundreds for each individual miRNA, resulting in blocking of translation and/or mRNA degradation (Jonas and Izaurralde, 2015). miRNAs are often included in discussions on epigenetic effects because they alter gene expression without altering DNA sequence, although they do not directly interact with DNA.

Epigenetic Effects of Statins

Effects of Statins on Histone Acetylation

Cell culture and animal studies have found that statin treatment can influence gene expression by promoting histone acetylation, primarily of histone H3 (Lin et al., 2008; Feig et al., 2011; Tikoo et al., 2015; Singh et al., 2016). Increased acetylation of histone H4 has also been reported (Feig et al., 2011; Singh et al., 2016). There are several postulated mechanisms by which

this may occur (Fig. 2). Statin use has been shown to increase the recruitment of the HAT p300 to specific loci (Feig et al., 2011). Cooney (2010) proposed that, with less acetyl-CoA used in cholesterol biosynthesis, increased amounts are available to act as acetate donors to acetyltransferases; however, this mechanism has not been investigated in the reported studies. Conversely, multiple studies have shown that statins can inhibit the deacetylation activity of class I and II HDAC enzymes (Lin et al., 2008; Feig et al., 2011; Karlic et al., 2015; Singh et al., 2016). This may be through direct inhibition; computational modeling showed lovastatin may bind the active site of HDAC2 in a manner similar to the HDAC inhibitor trichostatin A (Lin et al., 2008). Additionally, statins have been found to downregulate the expression of HDAC mRNA (Karlic et al., 2015).

In contrast to the effects on the zinc-dependent class I and II HDACs, numerous cell and animal based studies have reported that statins upregulate the expression of sirtuin 1 (SIRT1), a NAD⁺-dependent class III HDAC (Ota et al., 2010; Tabuchi et al., 2012; de las Heras et al., 2013; Kawai et al., 2013; Kok et al., 2013; Du et al., 2014; Gong et al., 2014). SIRT1 has a broad substrate specificity: in addition to histone residues H1K26, H3K9, H3K14, and H4K16 (Zhang and Kraus, 2010), it can deacetylate numerous other proteins, including p53, FOXO, and PGC1 α (Lavu et al., 2008). While it is an HDAC, SIRT1 can also influence methylation-dependent epigenetic modifications. Among the proteins deacetylated and thus activated by SIRT1 is the histone methyltransferase SUV39H1, which acts to suppress gene expression through H3K9 methylation (Vaquero et al., 2007). Additionally, SIRT1 has been associated with DNA hypermethylation and has been shown to recruit DNMT3B to CpG islands (Zhang and Kraus, 2010). Whether these epigenetic changes occur as a result of statin-induced SIRT1 expression has not been examined.

Effect of Statins on Histone and DNA Methylation

Studies on the effects of statins on histone and DNA methylation are fewer in number and have been studied in different

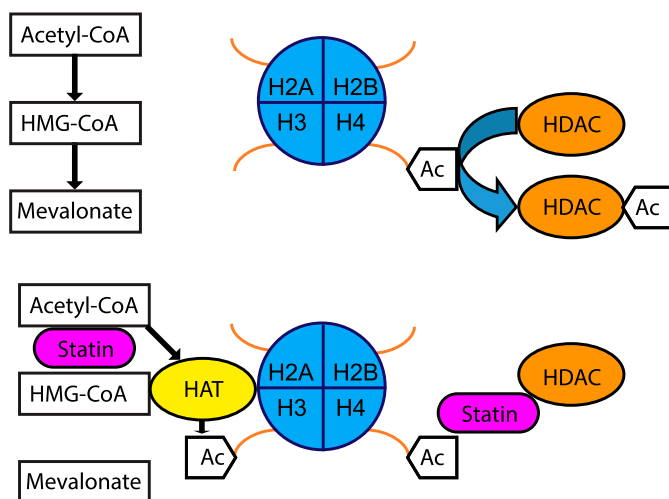


Fig. 2. Postulated mechanisms by which statins may impact histone acetylation. Without statin treatment (top), acetyl-CoA is used in the mevalonate pathway in the synthesis of cholesterol and other biomolecules. Statins, HMG-CoA reductase inhibitors, block the reduction of HMG-CoA into mevalonate, providing additional acetyl-CoA for HATs to add acetyl groups (Ac) to histone tails (bottom). Statins may also increase the recruitment of HATs to target loci. Histone deacetylases remove acetyl groups from histone tails (top); statins can prevent this through HDAC inhibition (bottom).

contexts. However, all have shown an effect consistent with demethylation. Simvastatin was shown to downregulate mRNA and protein levels of enhancer of zeste homolog 2 (EZH2), an HMT that suppresses gene expression through the methylation of H3K27 (Ishikawa et al., 2014).

Similarly, statins may promote demethylation of DNA through inhibition of DNMTs. Reductions in both DNMT mRNA expression (Karlic et al., 2015) and protein activity (Kodach et al., 2011) have been reported with statin treatment, as has the demethylation of gene promoter regions of certain genes (Kim et al., 2010; Kodach et al., 2011).

Effect of Statins on miRNAs

Statins have been found to have varying influences on the expression of numerous miRNAs, i.e., to upregulate some miRNAs and downregulate others. In one study, Karlic et al. (2015) found simvastatin treatment altered expression of over 400 miRNAs in various cancer cell lines. As individual miRNAs can potentially bind to hundreds of mRNAs (Jonas and Izaurralde, 2015), the potential downstream effects are immense. One miRNA, miR-34a, has been found to be downregulated by statin treatment in multiple contexts (Tabuchi et al., 2012; Karlic et al., 2015). miR-34a regulates the expression of HDAC1, HDAC7, and SIRT1, highlighting the interaction between miRNAs and other epigenetic modifications.

Epigenetics as a Mechanism for the Pleiotropic Effects of Statins

Cancer

The potential anticancer activities of statins have generated much research and discussion. Although statins have been shown to have anticancer activity in some in vitro models, and some clinical trials and epidemiologic studies have found lower rates of some cancers in statin users (Osmak, 2012), meta-analyses of controlled clinical trials have failed to find any effect on cancer rates (Kuoppala et al., 2008; Sun et al., 2015).

Silencing of regulatory and tumor suppressor genes (TSGs) is a common feature of many cancers, and epigenetic influences are considered an important contributory factor. As a result, epigenetic drugs, including several HDAC inhibitors and DNMT inhibitors, have been approved for use as cancer therapeutics (Nervi et al., 2015). Given that statins have potential epigenetic effects and are better tolerated than other approaches, as discussed for HDAC inhibitors later, numerous studies have searched for epigenetic effects in cancer (Table 1). In fact, although the basis for statin use is to reduce the risk of cardiovascular events, there are more studies on their potential epigenetic effects in the context of cancer than in the context of CVD.

Effect of Statins on Histone Acetylation in Cancer. HDACs are upregulated in many cancers (Marks, 2010), and two studies have reported HDAC inhibition by statins in cancer cell lines. Lin et al. (2008) found lovastatin treatment resulted in hyperacetylation of histone H3 in several lung carcinoma cell lines through direct inhibition of HDAC1, HDAC2, and HDAC3 enzymatic activity, with no effect on HDAC protein levels. Specifically, there was decreased HDAC1 and HDAC2 association with histones and increased histone H3 acetylation at the promoter of the TSG *p21*. Consequently, expression of *p21* increased and cell proliferation decreased.

TABLE 1
How epigenetic modifications may contribute to pleiotropic and adverse effects of statins

Epigenetic Modification	Downstream Effect	Reference
Cancer		
Increased histone acetylation	Increased expression of TSGs (<i>p21</i>)	Lin et al. (2008)
HMT inhibition	Increased expression of TSGs (<i>p27</i>)	Ishikawa et al. (2014)
Decreased DNA methylation	Increased expression of TSGs (<i>BMP2</i>)	Kodach et al. (2011)
Upregulation of miR-612	Increased cancer cell differentiation and chemosensitivity	Karlic et al. (2015)
Upregulation of miR-33b	Downregulation of oncogene <i>c-Myc</i>	Takwi et al. (2012)
Upregulation of miR-182	Downregulation of antiapoptotic Bcl-2	Peng et al. (2013)
Downregulation of miR-34a	Upregulation of SIRT1, HDAC1, and HDAC7	Karlic et al. (2015)
	Possible increased cancer cell proliferation and invasion	
Atherosclerosis		
Increased histone acetylation	Increased expression of antiatherosclerotic genes (<i>CCR7</i> and <i>ACE2</i>)	Feig et al. (2011)
Downregulation of miR-34a	Upregulation of SIRT1, HDAC1, and HDAC7	Tikoo et al. (2015)
	Increased blood EPC counts	Tabuchi et al. (2012)
Downregulation of miR-221 and miR-222	Increased blood EPC counts	Minami et al. (2009)
Immunomodulation		
Decreased DNA methylation	Increased FOXP3+ regulatory T cells	Kim et al. (2010)
Diabetic nephropathy		
Increased histone methylation	Increased expression of antifibrotic genes (E-cadherin)	Singh et al. (2016)
Myopathy		
Upregulation of miR-499-5p	Correlated with creatinine kinase levels	Min et al. (2016)
Liver damage		
Upregulation of miR-192	Decreased ATP production	Pek et al. (2016)

The ability of lovastatin to directly inhibit HDAC activity was confirmed through docking simulation showing binding of lovastatin to the active site of HDAC2. Karlic et al. (2015) found reduced levels of HDAC1, HDAC2, HDAC3, HDAC7, and HDAC8 mRNA in osteosarcoma, breast cancer, and prostate cancer cell lines treated with simvastatin. It could be postulated that this could increase histone acetylation, but there have been no confirmatory studies to measure the effect on HDAC activity, protein expression, or histone acetylation.

Although numerous HDAC inhibitors have been approved by the Food and Drug Administration for use in cancer treatment, statins are generally well tolerated and would offer advantages over traditional HDAC inhibitors, which have severe adverse effects that have caused treatment-related deaths in clinical trials (Mottamal et al., 2015). However, as the studies showing HDAC inhibition in cancer cells by statins have been conducted on cell lines in vitro, it is unknown if statin use at safe human doses could inhibit HDAC activity at the required level to have anticancer actions.

Due to the potential anticancer activities of statins and HDAC inhibitors, the use of dual HDAC and HMG-CoA reductase inhibitors has been explored. Combination therapy with a statin and HDAC inhibitor—namely, mevastatin and trichostatin A, respectively—was shown to have a synergistic effect in inducing cancer cell apoptosis in an in vitro study (Gan et al., 2008). In a subsequent study (Chen et al., 2013), a series of compounds based on coupling of HMG-CoA reductase inhibitors and the hydroxamate group of the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) were synthesized, including rosuvastatin, lovastatin, simvastatin, and atorvastatin hydroxamic acids. In vitro studies showed the latter-four dual-action compounds inhibited cancer cell proliferation more selectively than either statins or SAHA in isolation (Chen et al., 2013). Lovastatin hydroxamate (compound JMF3086; (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8*aR*)-Hexahydro-2,6-dimethyl-8-[(2-methylbutyryl)oxy]naphthalenyl]-3,5-dihydroxy-*N*-hydroxyheptanamide) reduced the number and size of tumors and metastases in mouse models of colorectal

cancer (Wei et al., 2016a) and reduced colonic inflammation and the stem cell–like characteristics of cancer cells to a greater degree than a combination of lovastatin and SAHA (Wei et al., 2016b). Theoretically, this dual inhibition may enhance the anticancer activity of statins and HDAC inhibitors while reducing the toxic side effects of traditional HDAC inhibitors.

Effects of Statins on Histone and DNA Methylation in Cancer. The HMT EZH2 has been associated with the silencing of TSGs involved in cell cycle regulation, including *p27*. Ishikawa et al. (2014) found simvastatin downregulated EZH2 expression, induced *p27* expression, and decreased cell proliferation in colorectal cancer cell lines. These effects were apparent for the lipophilic simvastatin but, interestingly, not for the hydrophilic pravastatin.

Overexpression of DNMTs and hypermethylation of the promoter regions of TSGs are seen in many cancers (Subramaniam et al., 2014). The TSG bone morphogenetic protein 2 (*BMP2*) is a differentiation factor for numerous cell types, and Kodach et al. (2011) reported that lovastatin treatment downregulated DNMT activity, decreased *BMP2* promoter hypermethylation, and increased *BMP2* expression in colorectal cancer cell lines. This was accompanied by a change from a stem cell–like phenotype to a differentiated state that was more amenable to treatment. The reduction in DNMT activity was not accompanied by decreased DNMT mRNA or protein expression, and was therefore postulated to be a direct effect on DNMT enzymatic activity. Similar to the studies on HDAC inhibition, this is in contrast to the findings of Karlic et al. (2015), who reported a reduction of DNMT1 mRNA in cancer cell lines in response to simvastatin.

Effects of Statins on miRNAs in Cancer. Deregulation of miRNAs is a common feature of cancer, with some miRNAs over or underexpressed in many cancer cell types and others used to differentiate cancer subtypes (Iorio and Croce, 2012). Different miRNAs can act as oncogenes or TSGs by targeting mRNAs involved in proliferation, epithelial–mesenchymal transition, angiogenesis, cell-cycle arrest, and apoptosis. Statin

treatment has been found to alter numerous miRNAs that have been associated with cancer. Karlic et al. (2015) found simvastatin altered expression of over 400 miRNAs in various cancer cell lines. miRNAs with anticancer activities found to be upregulated with statin treatment include miR-612, involved in promoting cancer cell differentiation and thus increasing the chemosensitivity of cancer cells (Karlic et al., 2015); miR-33b, a negative regulator of the oncogene *c-Myc* (Takwi et al., 2012); and miR-182, which may promote apoptosis through downregulation of the antiapoptotic Bcl-2 protein (Peng et al., 2013). On the other hand, it has been shown that statins downregulate miR-34a (Karlic et al., 2015), which is thought to act as a tumor suppressor (Misso et al., 2014). Silencing of miR-34a has been observed in multiple types of cancer (Lodygin et al., 2008), and it has been found to inhibit cancer cell proliferation and invasion in vitro (Liu et al., 2011; Liang et al., 2015; Adams et al., 2016).

Overall Epigenetic Effects on Cancer in Context. The aforementioned studies show that statins may mediate multiple epigenetic modifications that influence numerous pathways conferring pro- or anticancer activities. As each study only examined very specific aspects and was conducted in vitro, it is difficult to extrapolate the overall effect on oncogenesis in vivo. Furthermore, different statins have been shown to have differing epigenetic effects (Table 2); lovastatin inhibited HDAC and DNMT activity without altering expression, whereas simvastatin decreased HDAC and DNMT expression; simvastatin downregulated EZH2 expression, whereas pravastatin had no effect. This suggests the epigenetic effects of statins in cancer may not be solely a result of HMG-CoA reductase inhibition, although few studies have attempted to examine the exact mechanisms by which statins influence epigenetic-modifying enzymes. Thus, more detailed studies, including animal and human studies, will be required to determine if and how statin-induced epigenetic changes affect cancer risk in humans.

Atherosclerosis

Due to the well established ability of statins to reduce major cardiovascular events, the epigenetic effects of statins have also been studied in the context of atherosclerosis. Whereas lipid lowering is thought to play a major role in the antiatherosclerotic effects of statins, subgroup analysis of clinical trials found that statin users have a significantly lower risk of cardiac events compared with non-statin users with comparable serum cholesterol (Zhou and Liao, 2009), indicating that pleiotropic effects may also contribute. Postulated antiatherosclerotic pleiotropic effects of statins include increased endothelial nitric oxide production, proliferation of endothelial and vascular smooth muscle cells, decreased platelet activation, and clearance of inflammatory cells from atherosclerotic plaques (Wang et al., 2008; Zhou and Liao, 2009).

Effect of Statins on Histone Acetylation in Atherosclerosis. Macrophages have a central role in the atherosclerotic process, promoting inflammation and lipid accumulation in fatty plaques, and there is evidence that a key regulator of macrophage emigration, CC-chemokine receptor 7 (*CCR7*), may be influenced by statin-induced epigenetic modifications. Feig et al. (2011) found rosuvastatin increased H3 and H4 acetylation in proximity of the *CCR7* gene promoter region in a macrophage cell line, associated with decreased HDAC6 and HDAC7 and increased HAT p300 content at the same locus.

TABLE 2
Comparison of the epigenetic effects of different statins

	Simvastatin	Atorvastatin	Lovastatin	Pravastatin	Rosuvastatin
Histone acetylation	Decreased HDAC expression (Karlic et al., 2015)	Inhibited HDAC activity without altering expression. Increased H3 and H4 acetylation (Singh et al., 2016)	Inhibited HDAC activity without altering expression. Increased H3 acetylation (Lin et al., 2008)	Not reported	Increased H3 and H4 acetylation. p300 recruitment (Feig et al., 2011)
Histone methylation	Decreased EZH2 expression (Ishikawa et al., 2014)	Not reported	Not reported	No effect on EZH2 expression (Ishikawa et al., 2014)	Not reported
DNA methylation	Decreased DNMT expression (Karlic et al., 2015) Promoter demethylation (Kim et al., 2010)	Not reported	Inhibited DNMT activity without altering expression. Promoter demethylation (Kodach et al., 2011)	Not reported	Not reported

Consequently, they found increased CCR7 expression and decreased macrophage content in atherosclerotic plaques of statin-treated mice.

Increased histone acetylation may also modify the expression of angiotensin-converting enzyme 2 (ACE2), which has been found to play a protective role in atherosclerosis. In a rabbit model of atherosclerosis, atorvastatin treatment increased acetylation of H3 globally and specifically in the *ACE2* gene promoter region in the hearts of rabbits fed a high-cholesterol diet (Tikoo et al., 2015). ACE2 expression was subsequently increased and the contractile response of aortic rings *ex vivo* was normalized compared with rabbits on the high-cholesterol diet without statin treatment.

Effects of Statins on miRNAs in Atherosclerosis. Three studies have investigated miRNAs as an effector of the antiatherosclerotic effects of statins, two focusing on specific miRNAs thought to influence endothelial progenitor cells (EPCs) and the third examining statin effects on numerous miRNAs using an array approach. EPCs play an important role in vascular vessel formation and repair through the release of cytokines and by their differentiation into endothelial cells at sites of ischemia or vascular damage. Numerous studies have shown impairment of EPCs in vascular diseases, including peripheral and coronary artery disease (Fadini et al., 2005, 2006; Kunz et al., 2006; Morishita et al., 2012), with blood EPC counts being reduced by nearly 50% in patients with CVD (Vasa et al., 2001). Multiple miRNAs have been linked to the regulation of EPC proliferation. In a trial of CVD patients, 8 months of atorvastatin treatment resulted in the downregulation of miR-34a, with subsequent upregulation of SIRT1 and increased peripheral blood EPC counts (Tabuchi et al., 2012). The effects of atorvastatin on miR-34a and SIRT1 expression were confirmed *in vitro* on cultured EPCs. In a similar study, Minami et al. (2009) found 12 months of atorvastatin therapy in CVD patients resulted in decreased miR-221 and miR-222 expression and increased blood EPC counts. Interestingly, the study showed pravastatin had no such effect, although this may be related to relative potencies at the doses used, as cholesterol lowering was less in pravastatin-treated subjects.

Finally, using a low-density array, Li et al. (2015) found statin use upregulated the expression of 19 miRNAs in plasma and 22 in whole blood in patients with unstable angina. The nature or type of statins used was not specified. Integrated analysis using multiple bioinformatics resources identified the coagulation and Rho GTPase pathways as two important pathways regulated by these miRNAs. Specifically, the authors suggest the miRNAs act to inhibit coagulation through regulation of vascular wall–cell surface interactions and platelet activation and aggregation. Rho signaling proteins have been implicated in atherosclerosis, primarily through regulation of the actin cytoskeleton. Statin use is known to inhibit the Rho pathway as geranylgeranyl pyrophosphate, an intermediate in the mevalonate pathway, isoprenylates Rho and results in the activation of its downstream effectors (Cai et al., 2015). Li et al. (2015) speculate that statin-upregulated miRNAs may also inhibit the Rho pathway.

Immunomodulation

Multiple clinical trials have found that statins improve outcomes of heart transplantation, including the reduction of hemodynamically severe graft rejection (Kobashigawa et al., 1995; Wenke et al., 1997; Stojanovic et al., 2005). As a result,

the use of statins as immunosuppressive agents has been investigated for other organ transplants; clinical trials reported improved outcomes and lower rates of rejection with statin treatment following renal (Katznelson et al., 1996; Tuncer et al., 2000) and lung transplantation (Johnson et al., 2003). It has also been reported that statins modulate the proliferation, activation, and cytokine production of T cells *in vitro* (Jameel et al., 2013).

DNA methylation has been investigated as a contributing factor to the immunomodulatory activity of statins. The immunosuppressive regulatory T cells express the transcription factor forkhead box P3 (FOXP3), and Kim et al. (2010) found demethylation of the *FOXP3* gene promoter in T cells of mice treated with simvastatin. Subsequently, FOXP3 expression increased, as did the proportion of regulatory T cells. The authors suggest this mechanism may contribute to the immunosuppressive actions of statins.

Diabetic Nephropathy

Statins are among the most commonly prescribed drugs, including among patients with type 2 diabetes. However, whereas there are numerous studies on epigenetic changes in diabetes, there are surprisingly few studies on the epigenetic effects of statins in diabetes. Only one study is available, and only in the limited context of diabetic nephropathy (Singh et al., 2016). The study rationale was based in part on the postulated beneficial effects of statins. A recent meta-analysis showed that patients with diabetes who are on statins have better renal function than those on placebo (Shen et al., 2016). A previous study also showed that rosuvastatin conferred renoprotection in mice resistant to the lipid-lowering actions of statins (Giunti et al., 2010), again suggesting pleiotropic effects may play a role.

The study by Singh et al. (2016) reported HDAC inhibition in the renal cortex of diabetic mice treated with atorvastatin. Atorvastatin significantly decreased HDAC activity without altering HDAC protein levels, similar to the findings with lovastatin by Lin et al. (2008) mentioned previously in the context of cancer. H3 and H4 acetylation was increased globally and specifically at the promoter of the antifibrotic gene E-cadherin, with subsequent increased E-cadherin and decreased fibrosis. The effects on HDAC activity and kidney pathology were greater for atorvastatin than the nonstatin lipid-lowering drug ezetimibe, despite similar reductions in serum cholesterol. These findings suggest that direct HDAC inhibition contributes to the renoprotective actions of statins independent of lipid lowering.

Epigenetics in Liver and Muscle Toxicity

The epigenetic effects of statins in the context of adverse effects have been less widely studied. Negative effects associated with histone modifications or altered DNA methylation as a result of statin treatment do not appear to have been reported. However, two studies have observed that miRNAs expressed by damaged liver or muscle tissue may serve as biomarkers for statin-induced damage.

Statin-Induced Myopathy

Min et al. (2016) compared muscle-specific miRNAs in the blood of statin users running in the Boston marathon to

a matched control group of non-statin users. They found increased levels of miR-499-5p in the serum of statin users 24 hours after the race. miR-499 is produced by both cardiac and skeletal muscle cells, and increased levels of miR-499 have been found in patients following acute myocardial infarction (Corsten et al., 2010) and in rats following notexin-induced muscle damage (Siracusa et al., 2016). Levels of this miRNA correlated with creatinine kinase levels, a well established marker of muscle damage. The authors suggest that miR-499-5p could serve as a biomarker for statin-related exercise-induced muscle damage. However, the possibility that statin users had raised levels due to underlying metabolic or vascular disorders that necessitate statin treatment cannot be ruled out.

Statin-Induced Liver Damage

Pek et al. (2016) examined changes in miRNA expression in the context of statin-induced liver injury. They compared miRNA expression in 20 hypercholesterolemic patients before and after 12 weeks of simvastatin treatment and found 13 downregulated and 28 upregulated miRNAs. Increases in miR-192 and miR-21 correlated with changes in alanine aminotransferase, a common marker of hepatocellular damage, and were also seen in simvastatin-treated HepG2 hepatocellular carcinoma cells. Transfection of HepG2 cells with miR-192, but not miR-21, resulted in a reduction of ATP production, signaling a reduction in cell metabolism. Elevations of miR-192 have been reported in patients with acetaminophen-induced liver damage (Starkey Lewis et al., 2011) and in nonalcoholic fatty liver disease (Pirola et al., 2015). Pek et al. (2016) suggest miR-192 could be an early biomarker for statin-induced liver damage.

Conclusion

There is a growing body of evidence that statins, the most widely prescribed drugs in developed countries, may have wide-ranging effects beyond lowering serum cholesterol, both beneficial and deleterious. This review has shown that statins can alter epigenetic processes and miRNA expression in various cell types and contexts. However, this is a new area of research, and as such, studies have been few. The reported studies mostly examine only specific genes or pathways in particular contexts. As a result, it is difficult to extrapolate overall effects on human users. Furthermore, differing epigenetic effects have been observed with different statins, e.g., atorvastatin and lovastatin were reported to inhibit HDAC activity without lowering HDAC expression, whereas simvastatin was reported to reduce HDAC expression, and most studies have not examined detailed mechanisms by which statins interact with epigenetic-modifying enzymes and miRNAs. Finally, investigations into the epigenetics of the adverse effects of statins are lacking. More studies are clearly needed. Even so, there are some consistent effects that have been observed in numerous contexts—namely, increased histone acetylation and demethylation of both histone lysines and DNA.

As both epigenetics and miRNAs affect gene expression, these changes may contribute to the primary, pleiotropic, and adverse effects of statins, as summarized in Table 1. This may impact the long-term health of individuals and their offspring. Furthermore, drugs that alter epigenetics, e.g., HDAC inhibitors, have been investigated for numerous

disorders, most widely for cancer but also for diabetes, liver disease, and autoimmune disorders, among others (Nebbio et al., 2012). If statins can act as epigenetic agents, this may lead to novel indications for the commonly used drugs, or the development of new drugs as seen with the dual HMG-CoA reductase and HDAC inhibitors.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Allen, Mamotte.

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Address correspondence to: Cyril Mamotte, School of Biomedical Sciences and Curtin Health Innovation Research Institute, Curtin University, GPO Box U1987, Perth, Western Australia, 6845. E-mail: C.Mamotte@curtin.edu.au