Emerging Epigenetic Maps in Atherosclerosis

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Where are we? Where do we want to go? How do we get there? And for those who come along after us, how can we share what we already know about these places?

Perhaps the simplicity of these questions, and their importance, combine to help explain the longstanding human drive to draw maps. A 14,000-year-old cave painting in Spain reportedly shows the local topography and food resources outside where it was found.1 A 9000-year-old drawing of Çatalhöyük, a Stone Age settlement in modern Turkey, is considered an aerial schematic of the village, including the nearby volcano.2 Babylonians were practicing cartography on clay tablets about 3200 BC.3 Independent of ongoing debates about what constitutes the oldest known map, the development of different kinds of maps has often offered fundamental new insight, whether they are antiquarian Greek astronomical maps of the stars or Google Earth images that bring precise details of the world to every online device. Maps have been especially instrumental in medicine and biology. Early anatomists detailed the inner structures of the human body. By simply mapping cholera deaths in London in 1854, Dr John Snow established that the disease was spread by contaminated water and not by air, as had been presumed, helping end the epidemic.4 Landmark achievements of our time include the identification of DNA as a readable blueprint map, as more fully elaborated with the sequencing of the human genome.

The recognition that distinct modifications and mechanisms, whether on the DNA itself or the histones around which DNA is coiled, could modulate gene expression, control phenotypes, influence environmental responses, and determine heritable traits—indeed, independent of any change in DNA sequence—has suggested completely new maps for understanding genetic landscapes and functional biology. Epigenetics, which includes the post-translational modifications of specific amino acids on the N-terminal tail of histones and covalent modifications of DNA bases, such as methylation, has become a burgeoning field.5 The placement of methyl groups on nucleic acids, typically in the context of a cytosine directly linked to a guanine through a phosphate group, so-called CpG, results in 5-methylcytosine.

Methylation is performed by a family of DNA methyltransferases and the symmetric pattern between cytosines and guanines usually results in dimethylation at these residues.6 The CpGs that occur in the human genome are limited, except for certain enriched locations known as CpG islands, which contain an estimated 7% of all CpGs. Non–CpG-related DNA methylation may also be important.

DNA methylation seems to exert its effect in a context-specific manner: generally repressing transcription when present in promoter regions but fostering gene expression when found in gene bodies.5 DNA methylation, as occurs in ≈28 million sites in humans, is important in many processes, including development, as underscored by its critical role in X chromosome inactivation. Interestingly, most CpGs are stable in terms of their methylation state, with only a subset undergoing dynamic transitions. Perhaps the greatest progress in clinical epigenetics to date has been in oncology, with DNA hypermethylation being found in CpG islands, as well as adjacent CpG shores, in multiple different cancers and hematologic conditions.7,8 Human cancers are also often characterized by DNA hypermethylation in gene promoter regions, which can silence gene expression, including tumor suppressor genes. DNA methylation is now implicated in multiple diseases settings, from neurodegenerative diseases9 to rheumatologic conditions10 to diabetes mellitus11 and far beyond. Some of this pace of progress derives from the advent of high throughput methodologies that allow rapid processing of multiple samples, including Whole Genome Bisulfite Sequencing, DNA Methylation microarrays, and massive parallel sequencing that enables DNA (or RNA) annotation.12

The part that epigenetics play in cardiovascular disease is undergoing impressive progress on multiple fronts.13,14 Many distinct aspects of cardiovascular disease may be influenced through epigenetic mechanisms, including hypertension, lipid disorders, inflammation, and even stem cell programming that could influence disease states many years later. DNA methylation has been implicated as mechanism contributing to the role of diet on atherosclerosis, as well as specific effects of homocysteine as a cardiovascular risk factor deriving from DNA hypomethylation.15 DNA methylation may be modulated by reactive oxidant species, for example, contributing to aging and specific changes in vascular smooth muscle cells, endothelial cells, and inflammatory cells.

In this issue of Circulation: Cardiovascular Genetics, Zaina et al16 present their global interrogation of the DNA methylation patterns in atherosclerotic versus nonatherosclerotic aorta in the same human sample and their finding of significant changes in hypermethylation in association with atherosclerosis. This hypermethylation signature, which involved 774,341 172 and 877,722,420 different reads of CpGs, held across all chromosomes although found more predominantly in CpG islands. Other patterns of hypermethylation
were also discernable through this global mapping, including assessment of methylation in intragenic versus promoter regions. To consider the suggested notion from these results that DNA hypermethylation was part of atherosclerosis, DNA methylation microarrays were performed on additional atherosclerotic and nonatherosclerotic samples in 15 individuals. Interestingly, testing these marks in other atherosclerotic versus healthy samples in an unsupervised clustering analysis was able to identify 10 of 15 of the samples correctly as having atherosclerosis, suggestive of an atherosclerosis-specific DNA methylation profile present even among these individuals with different genetic backgrounds. The functional significance of hypermethylation was supported by the fact that DNA modifications were linked to changes in proatherosclerotic gene expression. The authors’ note that this map represents the first genome-wide DNA methylation profile at the level of single nucleotides in atherosclerotic versus normal human aorta. The general findings here are consistent with a previous report of similar hypermethylation patterns in leukocytes from patients with cardiovascular disease, as well as other studies; together these results align to suggest an atherosclerotic epigenetic signature exists that contributes to, if not helps define, aspects of the complex pathologic state of atherosclerosis.

Cautious notes are warranted around this report and the rapidly moving area of science in which it is intermeshed. DNA methylation patterns vary among individuals, even when comparing monozygotic twins. Thus, although discernable hypermethylation signatures in atherosclerosis are noteworthy, the ability to apply such information to individual patients or the reproducibility in other cohorts remains to be established. Zaina et al. initially studied atherosclerotic versus normal tissues in a single individual and then analyzed samples from additional subjects using a different method (Infinium Human Methylation 450 BeadChip). As such, the broadening of this investigation by increased the number of samples under study is not a validation of the initial data but rather a second line of investigation into DNA methylation in atherosclerosis. The effect of fundamental variables, such as age, sex, medication exposure, and diet on hypermethylation, is also unclear, with limited clinical information on the subjects studied by Zaina et al.: an intriguing aspect of their report is the suggestion that age itself, among the strongest determinants of cardiovascular risk, does not seem to align with the pattern of hypermethylation seen in these atherosclerotic samples. These issues and others may preclude investigators from simply analyzing the differentially methylated regions identified here in their own cohorts. Moreover, perhaps unlike more clonal diseases, such as certain cancers, atherosclerosis involves multiple cell types, arises over decades, is induced in response to many different inputs that often exert themselves as continuous risk variables, manifests different aspects depending on the anatomic bed in which it occurs, and has distinct aspects between the pathologic condition, which includes different stages and clinical presentations. All of these issues that are relevant in terms of the clinical complications of atherosclerosis may be associated with different DNA methylation patterns, creating challenges for discerning specific atherosclerotic signatures. The application of genome-wide high throughput approaches will also inevitably raise questions around the methods used to obtain the data, the quality of the data sets themselves, and how the analyses were performed. For example, as dealt with by Zaina et al., a sufficient number of reads at a given CpG methylation site is needed before the pattern can be determined; then comparison must be made between the same relevant sites in normal versus atherosclerotic tissue. Perhaps the most salient, and appropriately circumspect aspects of the findings reported here are the general trends on DNA hypermethylation seen in atherosclerotic versus nonatherosclerotic tissue. Ultimately, the high incidence of the pathologic condition of atherosclerosis and the more relevant issue of atherosclerotic complications may be so distinct that epigenetic profiling will need to be done on samples that reflect clinically relevant disease conditions.

Despite these limitations in what must still be considered the early days of deciphering the epigenetic code, studying it in humans, and applying it to cardiovascular disease, some of the possibilities of where studies like this one may be pointing us are increasingly evident. Unbiased assessment of DNA methylation, as well as other epigenetic pathways, offers the opportunity to identify novel contributors to vascular disease by using completely independent means. The information contained in a map varies as a function of its resolution level. With increasing numbers of studies such as that of Zaina et al., other investigators will be able to drill more deeply into methylation marks laid out at the single nucleotide level to uncover new players in atherosclerosis. Hypermethylation maps can be overlaid on other large data sets, such as prior single nucleotide polymorphisms and gene–disease association studies, as a way of further validating and perhaps better understanding those findings. Although understandably the initial focus on mapping data focuses on the recurring patterns seen, much may be learned from the exceptions to those patterns. In this case, although ~90% of the sites were associated with hypermethylation in atherosclerosis, what about those loci in which hypomethylation was more evident? What was different about those cases with atherosclerosis whose DNA methylation pattern was not consistent with the epigenetic signature seen among the other samples? Documenting DNA hypermethylation also brings with it parallel if not higher-order issues. Given the reversible nature of DNA methylation, what is occurring in terms of demethylation in atherosclerosis and how are such dynamic changes in methylation versus demethylation related to varying inputs like different cardiovascular risk factors? Similarly, DNA methylation is only one element in what ultimate changes transcriptional activity. Modifications of DNA and histones modulate transcription outputs by influencing chromatin biology. The presence of epigenetic marks, including DNA methylation, must be placed in the context of mechanisms that read those marks and help execute the transcriptional program. As one example of many studies along these lines, we recently demonstrated that the bromodomain and extra-terminal bromodomain-containing epigenetic reader proteins, which bind to acetylated lysine residues in specific histone tails, help define the global endothelial inflammatory transcriptional program in cytokine-stimulated endothelial cells, an effect tightly coupled to nuclear factor-κB activation and p65 localization; treating mice with a bromodomain and...
extra-terminal inhibitor blocked the expression of a proatherosclerotic, proinflammatory target gene cassette and decreased atherosclerotic lesions in vivo.19 Another recent study reported the role of histone deacetylase 3 in the macrophage transcriptional program; intriguingly, histone deacetylase 3 was reported as the only deacetylase induced in ruptured human atherosclerotic plaques.20 Similar issues are relevant for DNA methylation: how does hypermethylation alter transcriptional output and functional responses on both global and specific target levels in atherosclerosis, including the specific vascular and inflammatory cells involved?

The fact that a DNA hypermethylation signature for atherosclerosis may exist across multiple DNA sites, and is reversible, raises the tantalizing prospect that perhaps one could address complex aspects of atherosclerotic complications by inhibiting methylation or promoting demethylation. Certainly in oncology, where DNA methylation is consistently pronounced and has been more extensively studied, such possibilities are being actively pursued. Whether global methylation states can be safely modulated for therapeutic purposes remains to be determined, especially given fundamental roles for methylation on DNA and histones that affect cell proliferation and other basic aspects of cell biology. Cell-specific DNA methylation patterns raise another potential hurdle although perhaps more cell-specific targeting, if achievable, might circumvent these concerns.

Realizing therapeutic opportunities by modulating DNA methylation will no doubt require better maps—validation about where DNA methylation occurs, how the dynamic balance of methylation state is achieved, how those marks drive transcription, understanding divergent responses and patterns, and how such marks connect to other sets of big data, including genetic variation, high cardiovascular risk genetic loci, and transcriptional profiling. Over millennia, we have figured out many ways to make better maps, verifying landmarks (studies in different patient samples), integrating data using different technologies (transcriptional profiling, transcription factor, and reader protein localization), and looking beyond just geography and into aspects like topology, geology, and habitat (distinct aspects of atherosclerosis involving disease states, anatomic locations, and specific risk factors). Although generating and integrating such maps may seem daunting, the fact that we even can undertake such efforts warrants recognition, the further investment of resources, and careful thinking about the most efficient approaches to leveraging this new information. It seems likely those earliest cartographers, whether they were Çatalhöyük residents drawing on walls or Babylonians tracing on clay tablets, faced similar hurdles around fundamental questions of where are we, where do we want to go and how do we get there. Like us, those ancient mapmakers must have recognized the limitations in what they knew, could apply, or even convey to others with certainty. But they apparently came to a conclusion that proves instructive as we start producing and hopefully leveraging these emerging epigenetic maps: even with their limitations and the challenges ahead, we have to start somewhere.

Disclosures
None.

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