Epigenomics of Hypertension

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Summary: Multiple genes and pathways are involved in the pathogenesis of hypertension. Epigenomic studies of hypertension are beginning to emerge and hold great promise of providing novel insights into the mechanisms underlying hypertension. Epigenetic marks or mediators including DNA methylation, histone modifications, and noncoding RNA can be studied at a genome or near-genome scale using epigenomic approaches. At the single gene level, several studies have identified changes in epigenetic modifications in genes expressed in the kidney that correlate with the development of hypertension. Systematic analysis and integration of epigenetic marks at the genome-wide scale, demonstration of cellular and physiological roles of specific epigenetic modifications, and investigation of inheritance are among the major challenges and opportunities for future epigenomic and epigenetic studies of hypertension.
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Essential hypertension is a multifactorial disease involving multiple genetic and environmental factors and mediated by alterations in multiple biological pathways. Because the nongenetic mechanisms may involve epigenetic modifications, epigenomics is one of the latest concepts and approaches brought to bear on hypertension research. In this article, we briefly summarize the concepts and techniques for epigenomics, discuss the rationale for applying epigenomic approaches to study hypertension, and review the current state of this research area.

CONCEPTS AND TECHNIQUES OF EPIGENOMICS

Epigenetics is the study of molecular changes (epigenetic marks or mediators) and associated phenotypes that are mitotically or meiotically inheritable but do not involve changes in the DNA nucleotide sequence. Epigenetic marks or mediators contribute to biological regulation by influencing gene expression. Epigenomics is the study of epigenetic marks at a genome or near-genome scale. DNA methylation, histone modification, and noncoding RNA are three major types of epigenetic mediators, all of which can be studied at a genome or near-genome scale. Several examples of techniques for epigenomic analysis are listed in Table 1, and key features of these techniques are briefly discussed later. For a more detailed introduction of these techniques, refer to recent reviews published elsewhere.[1–4]

It is important to recognize that epigenomic analysis has very different requirements than genomic analysis even though epigenomic analysis often involves examination of DNA. With a few exceptions, the genomic sequence is identical across all cells in an individual and remains the same throughout an individual's lifetime. The epigenomes, however, are different between cell types and may undergo dynamic changes in response to environmental cues. Many of the variables that one typically considers in a physiological or gene expression study but might not take into account in a genomic study, such as cell type, age, disease, treatment, and so forth, should be considered in an epigenomic study.

One of the most widely studied epigenetic marks is 5-methylcytosine (5mC), which is found in cytosine-guanine dinucleotides (CpG).[5,6] Of particular interest are CpG sites located in CpG islands, which are clusters of CpG sites showing dynamic variations of methylation levels and often are colocalized with *cis* regulatory elements such as gene promoters. Increased DNA methylation (hypermethylation) in CpG islands within or close to gene promoters typically, although not always, is associated with suppression of transcriptional activity. Genomic segments of interest can be enriched by affinity purification using antibodies or other proteins that recognize methylated DNA. Alternatively, genomic segments of interest, such as segments enriched for CpG islands, can be obtained by digestion using specific endonucleases. The genomic segments then can be identified or mapped to single-base resolution using microarray hybridization or next-generation sequencing.[1,2,7] Other types of nucleotide modifications, notably 5-hydroxymethylcytosine

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Table 1. Examples of Methods for Genome-Wide Analysis of Epigenetic Marks

Epigenetic mark or mediator	Genome-wide analysis method
DNA methylation	Affinity purification and sequencing
	Endonuclease digestion and bisulfite sequencing
Histone modification	Chromatin immunoprecipitation and sequencing
	PTM-sensitive proteomics
Noncoding RNA	RNA-seq

NOTE. See text for further detail and references. PTM, post-translational modification.

(5hmC), also may be important for epigenetic regulation.[8,9] 5hmC is one of the intermediates in the process of 5mC demethylation catalyzed by the ten-eleven translocation family of dioxygenases. 5hmC could play a role in gene regulation distinct from 5mC. Methods for genome-wide analysis of 5hmC at single-base resolution have been reported recently.[10,11]

Post-translational modifications of histones, such as methylation and acetylation, are another major type of epigenetic marks. Proteomic techniques can be used to analyze a large number of histone modifications simultaneously.[12] DNA segments that bind to a histone with a specific protein modification can be examined by cross-linking DNA with histones followed by immunoprecipitation using an antibody recognizing the histone with the specific modification. The isolated DNA segments then can be identified with microarray or sequencing.[4] Histones may affect gene expression by altering chromatin structure. Several techniques are available for analyzing chromatin structure at a genome-wide scale.[13]

Regulatory, noncoding RNA may contribute importantly to epigenetic regulation.[14,15] Noncoding RNA, particularly long noncoding RNA, may play a crucial role in conferring site- or gene-specificity to epigenetic modification. Noncoding RNA can be identified by RNA-seq, sometimes with further support from identification of transcriptionally active genomic segments based on chromatin immunoprecipitation analysis.[3,16–18] Some noncoding RNA molecules do not have a poly(A) tail and can be identified only with RNA-seq methods that do not use poly(A) selection.

Association between epigenetic marks, especially DNA methylation, and a phenotype can be investigated at the genome-wide scale in epigenome-wide association studies (EWAS).[19] Similar to genome-wide association studies (GWAS), EWAS often require a sample size of several hundred or more.[19] The sample size of an EWAS is determined primarily by the magnitude of methylation rate differences among individuals, the impact of the methylation difference on the phenotype, and the number of methylation marks analyzed.

A TREE-LIKE PARADIGM FOR UNDERSTANDING HYPERTENSION

Hypertension affects 29% of the adult population in the United States and cost $93.5 billion in 2010.[20,21] Hypertension is a major risk factor for stroke, myocardial infarction, heart failure, and end-stage renal disease. About 50% of hypertensive patients in the United States do not have their blood pressure adequately controlled, in many cases owing to nonadherence and other disease management issues.[20,22] Importantly, about 5 million patients in the United States apparently are resistant to current antihypertensive treatments (hypertensive despite taking at least three antihypertensive medications).[23] Patients with apparent treatment-resistant hypertension are more likely to be black and have obesity, chronic kidney disease, and a Framingham 10-year coronary risk greater than 20%.[23]

Arterial blood pressure is the product of cardiac output and total peripheral vascular resistance. Several physiological and neurohormonal mechanisms regulate cardiac output and/or peripheral resistance and contribute to maintain the homeostasis of arterial blood pressure. These mechanisms involve several organ systems including the kidney and the cardiovascular, nervous, endocrine, and immune systems. The kidney plays a key role in this regulatory network.[24–26] Genes that underlie Mendelian forms of human hypertension typically affect tubular transport in the kidney directly or indirectly.[27] The molecular mechanisms underlying common forms of essential hypertension, however, remain poorly understood.

Although the multifactorial nature of hypertension is widely recognized, much of hypertension research has been focused on single genes or single regulatory pathways. The approach is useful despite its simplicity. Abnormalities in one gene or one pathway may very well make a measurable contribution to the development of hypertension in a specific, defined setting (a specific animal model, a well-defined subset of patients). It would be valuable to identify these abnormalities. The approach also has a strong practical appeal because numerous methods are available for analyzing and experimentally manipulating one gene or one mechanism. It often is challenging to interpret network data and difficult (if possible at all) to experimentally manipulate a regulatory network. Nonetheless, understanding molecular regulatory networks on a genome-wide scale is undoubtedly what is needed to truly understand the disease mechanism of hypertension and its associated comorbidities.