Genetic and Epigenetic Marks Weave Intricate Connections in Cardiac Disease

Daniel Vaiman

With the new era of genome sequencing, the possibility to obtain affordable comprehensive DNA sequencing of a given human being can be foreseen in a relatively near future. Although the sequencing by itself is not any more a challenging technical problem, the relevance of the sequence to the phenotype, especially in terms of medicine, remains a complex conundrum, and specific approaches are proposed to solve it.

One important issue in this respect is the integration of the sequence information per se, in particular, with the mechanisms that regulate gene expression and can be qualified in a broad sense, as epigenetic mechanisms. Among these epigenetic mechanisms, one of the most studied is DNA methylation for which several high throughput techniques exist. Heart function and dysfunction have clearly a genetic component, but the links with epigenetic regulation are insufficiently studied.

In human heart tissue, changes in DNA methylation have been recently evidenced in cardiac pathology (dilated cardiomyopathy). However, genetic diversity in humans makes it difficult to delineate for this type of complex diseases what is due to epigenetic changes, and what is due to DNA sequence variation. To this respect, mouse strains represent a unique opportunity to dissect away the genetic of heart disease phenotypes, on the one hand, and the epigenetic part of these phenotypes, on the other hand. Indeed, using inbred mouse strains is an efficient way of dissecting genetic and epigenetic effects in the study of various phenotypes. Mouse strains have been generated by brother–sister crosses during hundreds of generations, which result in a definitive allele fixation and thus a genome that is completely homozygous at every locus in these strains.

In the study of Chen et al., two inbred strains of mice, BALB/cJ and BUB/BnJ were used for a methylomic analysis of heart tissue after isoproterenol-induced cardiac pathology, using the reduced representation bisulphite sequencing strategy that limits the sequencing costs (Figure). Isoproterenol treatment (a β adrenergic agonist) is used to mimic human heart disease in rodents. It was administered during 3 weeks using subcutaneous pumps.

The 2 chosen strains have different responses to isoproterenol treatment, the BALB being sensitive, whereas BUB seems resistant. This is illustrated by the mass of the left ventricle that was dramatically increased (=from 70 to 120 mg) in the BALB/c mice treated, whereas the mass of the ventricle was not significantly changed in BUB/BnJ mice. In terms of molecular analysis, the reduced representation bisulphite sequencing procedure allowed to identify 337027 fragments of which 1509 were differentially methylated after the treatment when the 2 strains were considered. Several interesting observations of this study could be made: (1) overall, the differential methylation induced by the isoproterenol treatment of the strains concerns much less DNA fragments than the differences present between the 2 strains untreated; (2) the treatment-induced differences are shared by a limited number of fragments (31), showing that the genetic differences in the 2 mouse strains drive specific epigenomic responses (as also shown by common genes between fragments); and (3) when the differential fragments are analyzed separately in the 2 strains, the clustering in terms of gene ontology is different. Ingenuity Pathway Analysis suggested that the differentially methylated in the sensitive BALB/cJ strain are close to genes involved in heart weight and size, whereas the differentially methylated in the resistant BUB/BnJ strain are close to genes involved in muscle contractility.

The study also analyzed the possible links between methylation and gene expression levels on a genome-wide basis, a question for which the dogma still is association between high methylation and gene expression repression. As in many recent whole-genome studies, the Chen et al. article concludes on an absence of correlation when the data are taken as whole. However, a more thorough analysis of the profiles interestingly suggests the existence of specific features shared by highly expressed genes in terms of methylation: their transcription start site tends to be systematically demethylated in those, whereas this is less conspicuous in lowly expressed genes. Inside the gene, it seems also that the gene body is slightly less methylated in highly expressed than in lowly expressed genes. Importantly, methylation inside the gene body has been reported to prevent the initiation of transcription from internal alternative promoters. The results of this study on heart tissue can be put in perspective with a recent study analyzing together gene expression and DNA methylation in human placentas, which showed that overall, high gene expression levels correlate well with high methylation levels inside the gene body, whereas promoter methylation does not seem to correlate with gene expression.
This could indicate that regulation of expression and methylation subtly depends on the tissue under scrutiny. Another recent article showed that promoters themselves can be highly methylated and associated with a high level of gene expression in male germ cells. In conclusion, the results of the Chen et al study substantiate the recent paradigm shift leading to the conclusion that gene methylation association with gene expression is variable and context dependent.

This dependence could be linked to the increasing list of tissue- or time-specific synthesis of transcription factors that bind differentially to methylated or demethylated genome sequences, such as methyl-CpG-binding domain proteins or Zn-finger proteins of the Zinc Finger and BTB (Broad-Complex, Tramtrack and Bric-a-brac) family, with striking consequences on gene expression.

Connections with other chromatin marks were studied as well for fragments modified by isoproterenol treatment. The authors used a subtle procedure of genome analysis: they grouped DNA fragments, which responded similarly to isoproterenol in terms of methylation, that they called modules. They found

Figure. A summary of the experiment of Chen et al: DNA from hearts of the 2 strains of mice treated or not by isoproterenol were used to evaluate DNA methylation. BALB is isoproterenol sensitive, whereas BUB is isoproterenol resistant. The 2 strains have different responses in terms of abnormal methylation of heart cells, showing that the epigenetic response depends strongly on the genetic background. The study shows also that methylation alterations correlate with a specific histone mark (H3K4Me1). The number of genes was computed from the data of online-only Data Supplement Table I.
groups of genes that responded similarly in the 2 strains, whereas some responded oppositely. Analysis of the ENCODE database allowed to correlate the response to the status of specific chromatin post-translational histone marks or other chromatin-associated genes. They discovered a significant overlap between H3K4me1 mark and modules of fragments that are demethylated in both strains after isoproterenol treatment (Figure).

One limit of the study is the fact that the analyzed histone marks were obtained from ENCODE published marks of mouse heart tissue13 but of course not under isoproterenol treatment, and it is not known how these marks are modified by the treatment. Another epigenetic question can be raised for the small RNAs that are not analyzed in the article of Chen et al5 despite their recognized function in epigenetic regulation. Finally, a last question is that of the egg and hen: which, between CpG methylation and histone marks, is the primeval signal fostering chromatin activity?

Acknowledgments

I acknowledge the help of his colleagues Julie Cocquet, Sandrine Barbaux, Céline Méhats, and Francisco Miralles, who critically read the article.

References


Key Words: Editorials ■ isoproterenol ■ phenotype
Genetic and Epigenetic Marks Weave Intricate Connections in Cardiac Disease
Daniel Vaiman

Circ Res. 2016;118:773-775
doi: 10.1161/CIRCRESAHA.116.308339
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/118/5/773

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/