Přehledový článek | Review article

DNA methylation and coronary artery disease: Brief summary of the main findings

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SOUHRN

Kardiovaskulární onemocnění (KVO), a ještě konkrétněji ischemická choroba srdeční (ICHS) jsou i nadále celosvětově hlavní příčinou úmrtí. Byla popsána řada genetických faktorů zvyšujících riziko rozvoje ICHS. Nicméně v etiologii a patofyziologii komplexních onemocnění, jako je ICHS, působí nejen genetické faktory, ale i interakce genetických a environmentálních (vnějších) faktorů. Proto dnes výzkumníci soustřeďují svoji pozornost na epigenom, což je biologické rozhraní, v němž mohou genetika a životní prostředí interagovat. Metylace DNA představuje hlavní epigenetickou formu regulace genové exprese, jíž se připisuje stále větší úloha v rozvoji KVO. Cílem tohoto krátkého přehledu je proto shrnout výsledky hlavních studií z poslední doby, zabývajících se případným příspěvkem stavu metylace DNA k rozvoji ICHS.

Keywords:
Cardiovascular disease
Coronary artery disease
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Epigenetics

ABSTRACT

Cardiovascular disease (CVD) and more specifically coronary artery disease (CAD) continue to be the leading cause of mortality worldwide. Many genetic factors have been identified to contribute to the risk of developing CAD. However, the etiology and pathophysiology of complex diseases such as CAD are driven not only by genetic factors but also by the interaction between genetic and environmental factors. Therefore, researchers are now exploring the epigenome, a biological interface at which genetics and the environment can interact. DNA methylation is the main epigenetic form of gene regulation, and it is increasingly recognized to play an important role in cardiovascular disease. Thus, we aim in this short review to summarize the main recent studies on the eventual contribution of DNA methylation status in the onset of CAD.

Introduction

Cardiovascular disease (CVD) is the major cause of mortality and morbidity worldwide and its burden has doubled these last 30 years.[1] Although, the considerable progress in the diagnosis and treatment of CVD, it is still considered as a public health issue. Coronary artery disease (CAD) is the most common form of CVD. It occurs mainly from the progressive narrowing of the lumen in the coronary arteries due to the development of atherosclerotic plaques, a process known as atherosclerosis.[2] Consequently, coronary stenosis or thrombosis can occur leading to angina pectoris and/or myocardial infarction (MI). Atherosclerosis is the main cause of CAD; it is an inflammatory disease with abundant immune competent cells in lesions producing mainly pro-inflammatory cytokines.[3] Many risk factors like advanced age, male gender, and a family history of ischemic heart disease have been identified as non-modifiable risk factors while smoking, hypertension (HTN), diabetes mellitus (DM), dyslipidemia, obesity, and a sedentary lifestyle were identified as modifiable risk factors.[4] Nevertheless, CAD is modulated by an interplay between lifestyle factors and genetics.[2] Indeed, the notion that the risk of CAD is heritable was raised since the 1950's.[5] Recently, the quantified heritability using updated genome-wide approaches estimated the heritability of CAD at 40–50%.[6] However, the variability in risk and outcomes in this disease is not fully explained by genetics or environmental risk factors individually. There are many treatment strategies for CAD such as revascularization which is the milestone in the treatment of symptomatic CAD. However, revascularization of one or two vascular lesions in chronic coronary syndrome (CCS), tend to improve the symptoms of patients with no long-term survival

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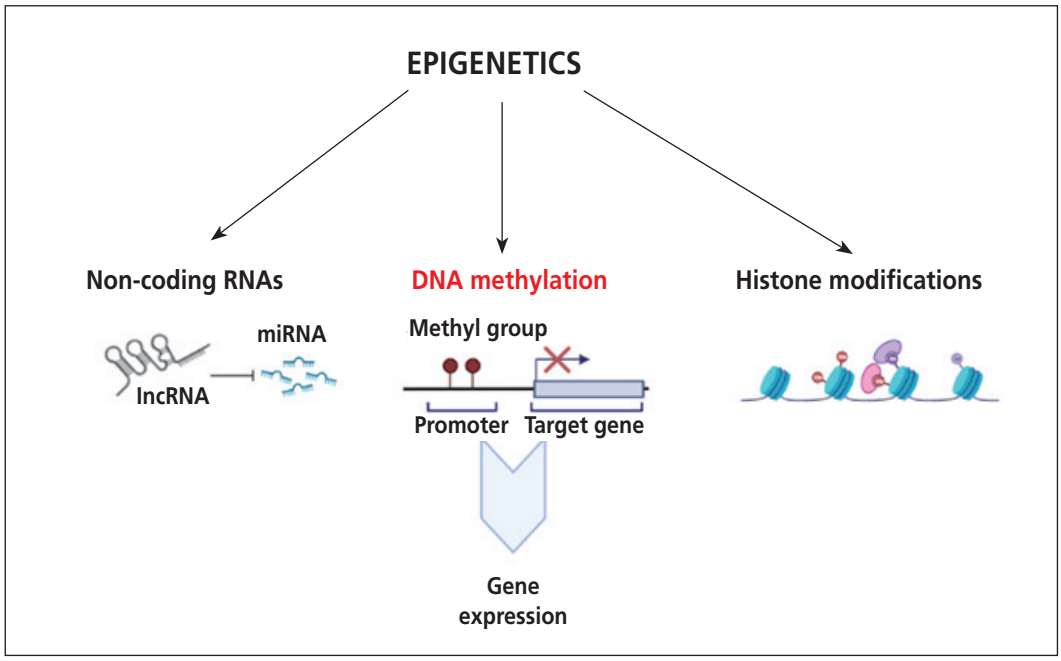

Fig. 1 – Epigenetic mechanisms. This figure was created with the aid of Biorender (https://biorender.com/).

proven benefit.[7] Moreover, many studies reported variations in drug resistance in the treatment of CAD.[8]

Due to the lack of effective treatment strategies to stop the progression of atherosclerosis and the unknown underlying pathophysiological mechanisms of CAD including gene-environmental interactions, many studies started to explore other involved aspects in the onset and development of CAD. Indeed, recent studies have reported that epigenetic mechanism is considered as an important link between genotype and phenotype variability in CVD. Epigenetic mechanisms are defined as the way that genes and the environment interact, can regulate gene expression to control a related phenotype (Fig. 1). Importantly, epigenetic modifications are measurable[9] and can be modulated at any life stage through environmental stimuli, like age, drugs, and nutrition.

Cardiovascular disease-related genes function and expression level are regulated through DNA methylation, histone modification and non-coding RNA regulation including mi-RNAs and Long non-conding RNAs.[10] Interestingly, important studies reported that epigenetic modifications can be reversed which make genes and proteins as new targets for cardiovascular disease treatment. Thus, epigenetic markers can be used as cardiovascular biomarkers for diagnosis, treatment and response prediction. In the present review, we highlight recent data about the potential contribution of epigenetic mechanisms including DNA in the development of CAD.

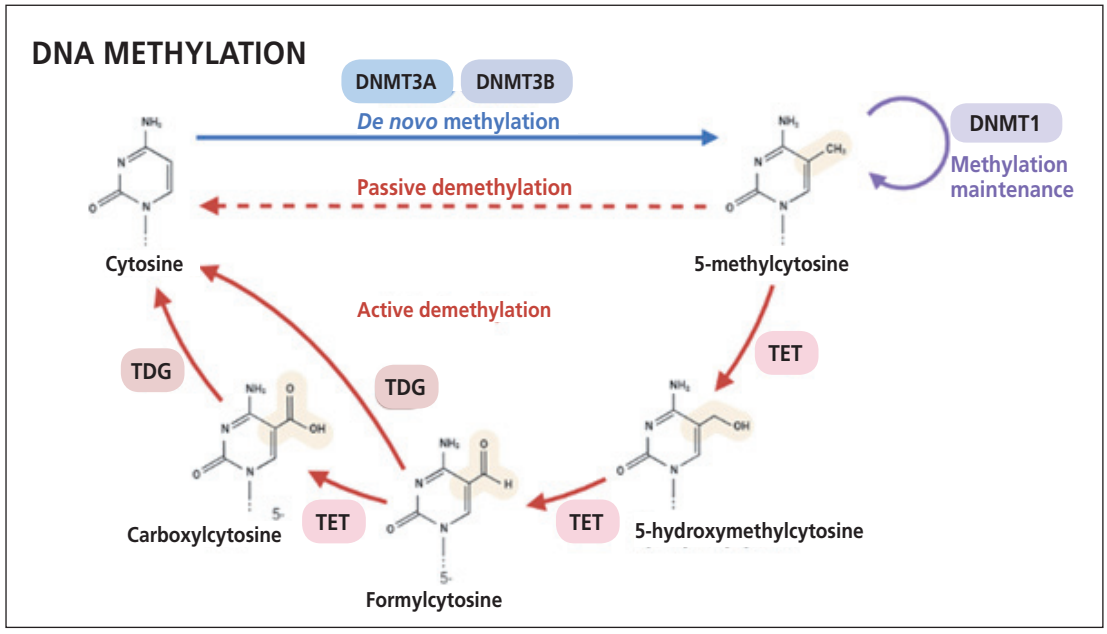

Fig. 2 – DNA methylation mechanism. This figure was created with the aid of Biorender (https://biorender.com/).

DNA methylation

DNA methylation is the main epigenetic form of gene expression regulation in mammals. It is a type of epigenetic process where a methyl group is added from the S-adenyl methionine (SAM) to the 5′-position of a cytosine, forming 5-methylcytosine. It is found mostly in regions containing a large number of cytosine 5′ to guanine dinucleotides (CpGs) in promoters. Importantly, 70% of all CpG dinucleotides in the genome are methylated. As a result, gene transcription could be turned off if the CpG is methylated. The methylation status of genes is maintained by enzymes such as DNA methyltransferases (DNMTs) and DNA demethylases, whose expressions are regulated at the transcriptional and post-transcriptional levels. There are three major (DNMTs) such as DNMT1 and DNMT3 with two major isoforms, DNMT3A and DNMT3B[11] (Fig. 2) DNMT1 has a large regulatory N-terminal domain (NTD), which covers two-thirds of the molecule, and a large C-terminal catalytic domain (CD), which contains all essential motifs of active C5 DNA methyltransferases. Notably, it plays an important role in the reparation of DNA methylation besides the mimic of the original methylation pattern before the replication.[12] Regarding the two isoforms of DNMT3 which are also known as *de novo* DNMT, they add methyl groups to cytosine at unmethylated DNA and can make a new methylation pattern for the unchanged DNA.

DNA methylation and CAD

Several studies have reported that DNA methylation plays an important role in coronary artery disease. In fact, the evaluation of the abnormal methylation status of candidate genes may be used as an important biomarker to assess cardiovascular disease progression. DNA methylation can be detected thanks to the advances of new technologies like micro-arrays and bisulfite sequencing approaches, where it is possible to assess hundreds of thousands of CpGs along the genome in the population. Indeed, methylations at specific cytosine- phosphate- guanine (CpG) sites have been associated with several diseases in epigenome wide association studies (EWAS). EWAS are used to examine genome-wide epigenetic variants (predominantly DNA methylation at CpGs), to detect differences that are statistically associated with phenotypes of interest. Several studies have reported that DNA methylation is associated with CVD and with cardiometabolic risk factors.[13–15]

Notably, some previous studies have assessed the association between incident coronary heart disease (iCHD) and DNA methylation levels in the genome where they reported that Long Interspersed Nuclear Elements-1 (LINEs-1) hypomethylation in blood is associated with iCHD.[16,17] Similarly, Kim et al.[18] have explored the DNA methylation level of the ALU and Satellite 2 (AS) repetitive element. However, these authors have found that AS methylation in peripheral blood leukocytes was higher in males with iCHD.

In the same aspect, Agha et al.[19] reported among a sample of 11 461 individuals across 9 population-based cohorts from the United States and Europe, differences in blood leukocyte DNA methylation at 52 CpG loci were robustly associated with iCHD. Moreover, Westerman et al.[20] reported in 2023 discovery female subjects of several ancestries including 1009 iCHD cases and 2587 replication subjects of European ancestry where 55% were female and 305 had iCHD, several epigenetic associations with incident CVD including DNA methylation in three regions *SLC9A1, SLC1A5,* and *TNRC6C* genes. The authors have found after Mendelian randomization analysis at 4 CpGs with methylation quantitative trait loci (meQTLs) in these regions that one CpG in *SLC1A5* is related to incident coronary artery disease.[20] Similarly, Kennel et al.[21] showed that *SLC1A5*, an important carrier for glutamine and other neutral AAs, is decreased in heart failure and is influenced by inflammatory signals. In addition, a recent EWAS on iCHD, Navas-Acien et al.[22] reported that blood DNAm was associated with CHD beyond traditional factors associated with cardiovascular disease, with a complex epigenomic signature across populations. Furthermore, Palou-Marquez et al.[23] reported that four independent latent factors (9, 19, 21 – only in women and 27), driven by DNA methylation, were associated with cardiovascular disease independently of classical risk factors. They have also found that three of the genes included in factor 27 were also present in a factor identified to be associated with myocardial infarction (*CDC42BPB*, *MAN2A2,* and *RPTOR*). Interestingly, recently, Luo et al.[24] performed an integrative analysis of DNA methylation and mRNA expression data-sets deriving from AMI mouse models at series of time points to identify key epigenetic alterations in AMI.

The authors found a correlation between the development of AMI stage and the modification in a large number of methylation sites during this stage. Additionally, they identified five candidate genes (*Ptpn6*, *Csf1r*, *Col6a1*, *Cyba,* and *Map3k14*) to be associated with MI through the regulation of DNA methylation.

DNA methylation therapy for CAD

DNA methyltransferase inhibitors (DNMTIs) or epi-drugs play a crucial role in the treatment of CAD by regulating targeted gene methylation status (Table 1). DNA methylation has emerged as a potential therapeutic target for

Table 1 – Epigenetic drugs for CHD treatment

Diseases	Epigenetic drugs	Targeted genes	References
Atherosclerosis, coronary heart disease	RG108	*DNMT1,DNMT3a*	25, 26
Atherosclerosis, coronary heart disease	5-AZa-2-deoxycytidine	*ERa,ERb,COL15A1*	27, 28
Atherosclerosis, coronary heart disease	Acetylsalicylic acid	*ABCA1*	29

coronary artery disease (CAD). Several studies have demonstrated that DNA methylation inhibitors can improve the development and progression of atherosclerosis, the underlying cause of CAD.

One of the most commonly used DNA methylation inhibitors is 5-aza-2′-deoxycytidine (5-Aza). A study by Wang et al. (2019) found that 5-Aza reduced atherosclerotic plaque size and improved endothelial function in a mouse model of atherosclerosis. The study also found that 5-Aza reduced the expression of inflammatory cytokines and improved lipid metabolism in the mice, suggesting that DNA methylation plays a role in regulating these processes. These findings suggest that 5-Aza could be a promising therapeutic strategy for CAD.

In the same aspect, Wang et al. (2020) investigated the effect of the DNA methyltransferase inhibitor, decitabine, on the development of atherosclerosis in mice. The study found that decitabine reduced atherosclerotic plaque size, improved endothelial function, and reduced inflammatory cytokine expression in the mice. These findings suggest that decitabine could be another potential therapeutic strategy for CAD. Another study by Huang et al. (2020) investigated the effect of the DNA methyltransferase inhibitor, SGI-1027, on the development of atherosclerosis in a mouse model. The study found that SGI-1027 reduced atherosclerotic plaque size and improved endothelial function in the mice. The study also found that SGI-1027 reduced the expression of genes involved in smooth muscle cell proliferation and migration, suggesting that DNA methylation plays a role in regulating these processes.

Another DNA methylation inhibitor that has shown promise for the treatment of CAD is RG108. A study by Zhang et al. (2021) found that RG108 reduced atherosclerotic plaque size and improved endothelial function in a mouse model of atherosclerosis. The study also found that RG108 reduced the expression of genes involved in smooth muscle cell proliferation and migration, suggesting that DNA methylation plays a role in regulating these processes.

However, it is important to note that the use of DNA methylation inhibitors as a therapeutic strategy for CAD is still in the early stages of development and requires further investigation. For instance, it is not clear which specific genes or pathways are regulated by DNA methylation and how these processes contribute to the development and progression of CAD. Moreover, the long-term safety and efficacy of DNA methylation inhibitors for the treatment of CAD remain to be determined.

Despite these limitations, the potential of DNA methylation inhibitors for the treatment of CAD is a promising area of research. Future studies should aim to elucidate the specific genes and pathways regulated by DNA methylation in CAD, as well as to determine the long-term safety and efficacy of DNA methylation inhibitors in preclinical and clinical studies. If successful, DNA methylation inhibitors could offer a novel and effective therapeutic strategy for the treatment of CAD, a leading cause of morbidity and mortality worldwide.

Conclusion

Epigenetic studies are a promising way to understand molecular mechanisms of diseases. However, there is a lack of information about the potential link between DNA methylation and coronary heart disease. Thus, we tried in this short review to focus on the main recent findings about the potential implication of DNA methylation in CHD etiology. We have also summarized some epigenetic drugs used in the treatment of CHD. Further studies on the role of other aspects of epigenetics like histone methylation and acetylation, miRNA, and long non coding RNA are needed to unravel the epigenomic signatures linked to CHD which could contribute to better understanding of their mechanisms and to the definition of new therapeutic targets and preventive strategies.

Conflict of interest
The authors declare that they have no conflict of interest.

Ethical statement
Not applicable.

Informed consent
Not applicable.

Consent for publication
Not applicable.

Data availability
The analyzed data sets generated during the study are available from the corresponding author on a reasonable request.

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