

## Epigenetics in Smoking-Associated Cardiovascular Diseases

Nan Hu and Jun Ren

Center for Cardiovascular Research and Alternative Medicine, School of Pharmacy, University of Wyoming  
College of Health Sciences, Laramie, WY 82071, USA

Correspondence: jren@uwyo.edu (J.R.)

*Hu N and Ren J. Reactive Oxygen Species 2(6):396–403, 2016; ©2016 Cell Med Press*

*<http://dx.doi.org/10.20455/ros.2016.871>*

*(Received: August 31, 2016; Revised: October 4, 2016; Accepted: October 4, 2016)*

**ABSTRACT** | Inhaled tobacco products cause ever rising cardiovascular morbidity and mortality and impose a global healthcare crisis. Earlier studies from our laboratory and others have indicated that inhaled toxicants of cigarette smoke are independent risk factors for a wide variety of cardiovascular diseases. Nonetheless, the precise mechanism behind cigarette smoking-induced cardiovascular sequelae remains somewhat poorly understood. Here, we updated the potential modulations of epigenetics in cigarette smoke-induced challenge to gene expression, which may sequentially affect cardiac structure and function. With the recent advances in new techniques such as high-throughput sequencing and genome-wide chromatin assays, epigenetic aspects of health and disease have exploded in many epigenome-wide approaches. These techniques have the potential to provide diverse opportunities for clinical phenotype screening and exploring novel therapeutic interventions.

**KEYWORDS** | Cardiovascular diseases; Epigenetics; Tobacco smoke

**ABBREVIATIONS** | ABCA1, ATP-binding cassette A1; AhR, aryl hydrocarbon receptor; CHD, coronary heart disease; CSE, cigarette smoke exposure; CVD, cardiovascular diseases; DNMT, DNA methyltransferase; ERK, extracellular signal-regulated kinase; F2RL3, factor II receptor-like 3; FGF2, fibroblast growth factor 2; GALNT2, polypeptide *N*-acetylgalactosaminyltransferase 2; HDAC, histone deacetylase; HDL, high-density lipoprotein; MAPK, mitogen-activated protein kinase; MOAB, monoamine oxidase-B; ncRNA, non-coding RNAs; NNK, nicotine-derived nitrosamine ketone; PKC $\epsilon$ , protein kinase C epsilon; ROS, reactive oxygen species; SHS, secondhand smoke; SRF, serum response factor

### CONTENTS

1. Overview
2. A New Era of Epigenetics
3. Epigenetics and Development of Cardiovascular Diseases
4. Epigenetics and Smoking-Related Diseases
5. DNA Methylation in Smoking-Related Cardiovascular Diseases
6. Histone Modifications in Smoking-Related Cardiovascular Diseases
7. MicroRNAs in Smoking-Related Cardiovascular Diseases
8. Perspectives of Epigenetics of Smoking-Related Cardiovascular Diseases

## 1. OVERVIEW

Among various risk factors for cardiovascular diseases (CVD), cigarette smoking is considered a primary modifiable independent one for the ever-rising cardiovascular morbidity and mortality [1]. It is well perceived that lung injuries predominantly occur in smokers accompanied with vascular remodeling, involving chronic inflammation and release of cytokines and chemokines, en route to the initiation and progression of atherosclerotic injury [2]. Lung inflammation, the major driver of both lung cancer and chronic obstructive pulmonary disease, is also linked to the increased risk of CVD. It is considered that lung inflammation, or even acute lung injury, can further lead to the onset and progression of vascular dysfunction and heart diseases [3]. A number of theories have been postulated for cigarette smoking-induced CVD including accumulation of reactive oxygen species (ROS), oxidative stress and inflammation, DNA damage, and vascular and platelet dysfunction [4]. Nonetheless, the possible contribution of epigenetics to cigarette smoking-induced pathological changes remains poorly understood.

## 2. A NEW ERA OF EPIGENETICS

With the recent advance in genome-wide association approach, epigenetic contribution to the health and disease has drawn much attention in many epigenome-wide approaches. In order to better understand the tie between smoking and heart diseases, epigenetic modulation including DNA methylation at CpG residues, histone modification, and small non-coding RNAs (ncRNAs) has been carefully examined in the settings of cigarette smoking or secondhand smoke (SHS) exposure [5].

Epigenetics is the study of stable, yet impermanent and not necessarily heritable, regulators that provide structural and regulatory mechanisms to modulate the gene expression. Epigenetic regulation is distinct from variation in the DNA sequence of genome. Generally speaking, epigenetic changes influence the expression of genes, as evaluated by RNA transcription and protein production, which sequentially affect cellular structure and function [6]. Changes in DNA methylation may be one of the essential mechanisms governing cigarette smoking-induced adverse health outcomes [7]. Up-to-date, the best-known epi-

genetic mechanisms are represented by the covalent binding of a methyl group to the C5 position of cytosine residues at cytosine ring neighbored by guanine sites in CpG dinucleotide sequences and various modifications of histone residues across eukaryotic kingdoms, such as acetylation or ubiquitination. The ncRNA such as microRNA generally appears to occur at the post-transcriptional level, but a subset closely interacts with the aforementioned epigenetic mechanisms [8, 9].

Epigenetic variations represent an additional layer of information transmission to explain various individual phenotypes to particular pathological changes. There is also a vital interplay between the oxidative environmental exposure and the genome background in the pathogenesis of such human diseases that can be influenced by the gene-environment interaction [10].

## 3. EPIGENETICS AND DEVELOPMENT OF CARDIOVASCULAR DISEASES

How epigenetics manages early CVD risk remains to be determined. A possible mediator at the cellular level is epigenetic suppression of gene expression. However, an adverse intrauterine environment and impaired fetal growth are well confirmed to trigger the early development of atherosclerosis, with a long incubation period between potential exposures and adult CVD [11]. Taking the low-birth-weight children as an example, poor nutritional states of these fetuses during their fetal period may adapt metabolic system to poor nutrition using epigenetic modifications. Thus, these children may be programmed before birth to reserve as much energy as possible. In consequence, they tend to be affected by obesity, elevated blood pressure, metabolic syndrome, and early-life pathogenesis of CVD during early childhood and later in adulthood [12].

The above consequences may result from a combination of environment and epigenetics. For example, a study showed that hypoxia induced in vivo to pregnant rats resulted in a significant decrease in protein kinase C epsilon (PKC $\epsilon$ ) protein and mRNA abundance in fetal hearts, which was associated with a significant increase in CpG methylation of the SP1-binding sites at the PKC $\epsilon$  promoter [13]. This study suggested that hypoxia might repress PKC $\epsilon$  gene through an ROS-mediated epigenetic approach in fe-

tal hearts, thereby making myocardial system vulnerable to ischemic challenge [13].

DNA methylation can occur during the entire lifespan, although a large part of epigenome is established during initiation of embryo and early development of fetus. Epigenome changes that are relatively stable and heritable are also sensitive to environmental factors [14]. Even though several recent articles have reviewed the role of epigenetics in the pathogenesis of CVD, how these epigenetic marks, including DNA methylation, histone modification, and RNA, interfere with numerous CVD risk factors remains to be clueless.

#### 4. EPIGENETICS AND SMOKING-RELATED DISEASES

One third of smoking-associated mortalities are secondary to CVD and 11.1% of these deaths occur in the SHS population. Extensive epidemiological research on SHS spanning several decades supports a strong causal relationship with a 25% to 30% increase in coronary heart disease (CHD) [4]. Cigarette smoke exposure (CSE) has a significant dose-dependent impact on CVD, and the relative risk is raised even among people smoking less than 5 cigarettes daily and those only with exposure to SHS [15]. The unfavorable impacts of CSE and SHS are regulated by the particulate phase and the gas phase, which both contain a huge number of free radical species resulting in oxidative stress in the blood, and such effects are able to cause vascular endothelial cell activation, damage, and injury [16].

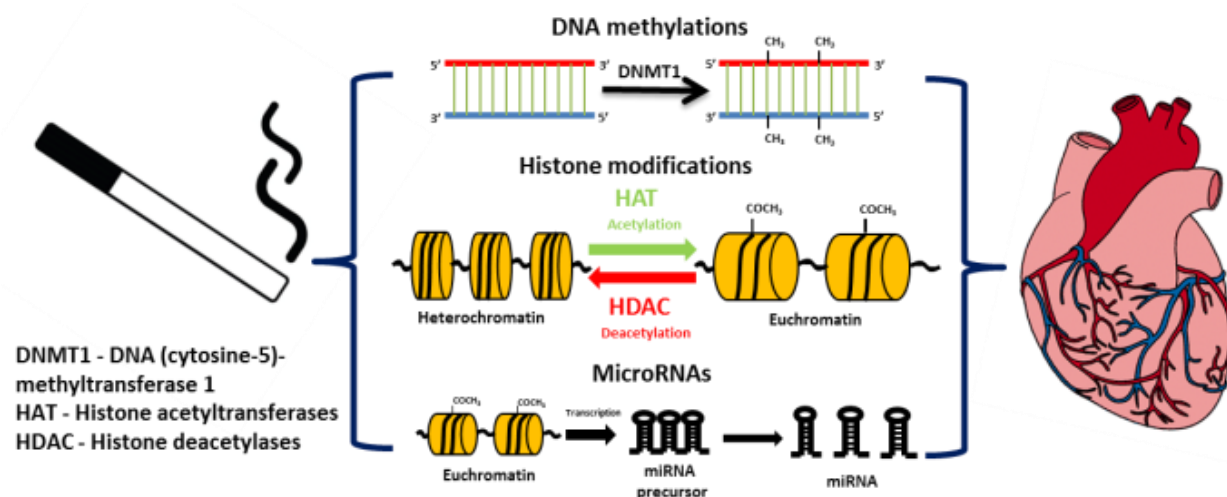
Great assortments of epigenetic molecules have been demonstrated to be involved in physiological and pathophysiological vascular settings, such as inflammatory process. For example, the significance of DNA methylation has been shown to be responsible for the regulation of inducible nitric oxide synthase (iNOS), whereas specific histone marks have been distinguished to be involved in the fixation of pro-inflammatory gene expression patterns in diabetic animals. Platelet function also seems to be crucially dependent on physiological epigenetic imprinting [8]. All these processes should be considered relatively important in CSE- and SHS-associated cardiovascular pathology. While most compounds that are present in CSE and SHS are non-mutagenic, some apparent non-mutagenic compounds can interfere

with gene expression by means of epigenetic signals. There is plentiful proof for smoking-induced epigenetic approaches in clinical research. For example, hypermethylated promoters have been recognized in active smokers. Despite the fact that promoter hypermethylation can persist for many years after smoking cessation, current active smokers exhibit a much higher level of DNA methylation than the former smokers, supporting the view that hypermethylation is reversible [17]. The CSE effect may be reflected in circulating cell-free DNA in the blood. It is perceived that any methylation of the tumor suppressor genes Kif1a, DCC, RARB, or NISCH may be served as promising plasma methylation markers for the detection of CSE-related lung cancer [18]. Hypermethylation in smoker tissues has been clarified by the abnormal levels of DNA methyltransferases (DNMTs) that are associated with smoking status in lung tumor samples. In vivo animal experiments have further demonstrated that nicotine-derived nitrosamine ketone (NNK) works through Akt signaling and DNMT1 protein in smoking groups. Subsequently, DNMT1 protein expression promotes elevated tumor suppressor gene hypermethylation [19].

Various studies have endeavored to examine epigenetic modifications observed in smokers' lung tissues. At this time, it is obscure whether the methylation is reversible and what would be the time period for demethylation events after CSE or SHS cessation [19]. Reversible smoking effects on DNA methylation have been documented in cultured lung cancer cells. CSE treatment-induced pro-metastatic oncogene synuclein-gamma (SNCG) demethylation was associated with gene overexpression in the face of just 3 days' treatment. The demethylation was accompanied by a twofold decrease of DNMT3B mRNA expression. Cessation of the exposure brought about the restoration of DNMT3B expression and accompanied recovery of SNCG CpG methylation [20]. A scheme is provided to summarize the epigenetic aspects of smoking exposure in the pathogenesis of CVD (**Figure 1**).

#### 5. DNA METHYLATION IN SMOKING-RELATED CARDIOVASCULAR DISEASES

Recent studies have indicated that DNA methylation is a critical contributor to the onset and development



**FIGURE 1. Epigenetics in smoking-associated cardiovascular diseases.** Epigenetic modifications are critical for cigarette smoking-related cardiovascular biological activity and occur mostly via DNA methylations, histone modifications, and microRNAs.

of cardiovascular pathology. DNA methylation is a heritable but also reversible covalent transfer of a methyl group to a nucleotide, and plays a crucial role in gene and ncRNAs transcriptional regulation. In mammals, most cytosines at CpG sites are methylated by DNMTs. An association between smoking and DNA methylation at different loci has been reported [21]. Most of these studies have examined individual CpG patterns at thousands of sites across the genome. Although a single methylated CpG may be linked to gene regulation and may affect disease risk, some of the loci reported in the research to be related to this regulation are genomic terrains within a length range of hundreds to thousands of bases [22]. Using the Human Methylation 450 K array, 8 of the 17 CpG sites related to exposure to tobacco smoking—F2RL3, GPR15, PSEN2, PRSS23, RARA, CPOX, AHRR, and RPS6KA2 are linked to genes showing expression in the whole blood. Moreover, gene interactions may be influenced by smoking-induced changes in DNA methylation [24]. In addition, a CpG differential methylation in LNX2 gene was identified in smokers and provided more information on THBS1 and MTSS1 and its relation to smoking [22]. The above study identified differential methylation at the genomic level in several genes and in genomic regions corresponding to four non-

coding RNAs (lncRNA01447, KIAA0087, miR-802, and Loc728554). Finally, there was a linear trend between current, former, and never smokers in the levels of methylation, suggesting that this is a reversible process [22].

Cigarette smoking is most clearly associated with DNA modifications to an aryl hydrocarbon receptor (AhR) pathway, and the AhR pathway was activated in atherosclerosis. Methylation of cg05575921 is significantly associated with smoking status. Significant functional associations between cg05575921 methylation in CD14<sup>+</sup> monocytes and carotid plaque scores were identified as CVD risk factors [23]. Moreover, ABCA1 (ATP-binding cassette A1) and GALNT2 (polypeptide *N*-acetylgalactosaminyltransferase 2) are two of these critical plasma lipoprotein genes variants that are associated with CHD. These two genes exert their effects on the pathogenesis of CHD through promoting transfer of intracellular cholesterol onto high-density lipoprotein (HDL) particles or onto catalyzing *O*-linked oligosaccharide biosynthesis of the lipid-poor form of the main HDL protein, apolipoprotein A1 (APOA1) [23]. One recent study indicates that promoter DNA hypermethylation of the ABCA1 and GALNT2 genes was linked with an increased risk of CHD, and both smoking and aging can contribute to the hypermeth-

ylation in an epigenetic manner [24]. Furthermore, another recent study showed a positive correlation between peripheral blood leukocytes' DNA methylation and prevalence of CVD, and reviewed global genomic DNA methylation within ALU and Satellite 2 repetitive elements in peripheral blood leukocytes as a more prominent clinical CVD risk factor in men than in women [25]. Analyses of peripheral blood leukocytes have shown that DNA from subjects with confirmed CHD had more substantial genomic methylation than that of healthy controls, indicating that DNA methylation may play a causative role during the development of CVD [21]. Moreover, a dynamic DNA methylation change of G-protein-coupled receptor 15 (GPR15) in response to smoking exposure was identified [17]. In summary, smoking is associated with significantly elevated DNA methylation across the genome.

However, another kind of DNA methylation challenge has been detected in platelets from smokers, in a study of monoamine oxidase-B (MAOB). These authors reported that "the methylation frequency of the MAOB gene promoter was markedly lower in smokers than in non-smokers, due to cigarette smoke-induced increase of nucleic acid demethylases activity" [22]. Meanwhile, in the research of smoking and factor II receptor-like 3 (F2RL3), data published thus far may have justified the interpretation that tobacco smoking leads to hypo-methylation in F2RL3. Plausible epigenetic relationship of such a risk of adverse outcomes in patients with CVD, however, is not convincing.

## 6. HISTONE MODIFICATIONS IN SMOKING-RELATED CARDIOVASCULAR DISEASES

A nucleosome is a fundamental repeating unit of eukaryotic chromatin, which consists of 146 bp of DNA packed around an octameric histone core composed of two copies each of histones H2A, H2B, H3, and H4. Histones regulate the chromatin compaction degree. In this way they are able to manage the gene transcription and transcriptional silencing [26]. Within the nucleosomes or between nucleosomes, post-translational modifications of histones directly modulate chromatin compaction states by modifying acetylation of lysine residues between positively charged histones and negatively charged DNA. Histone post-translational modifications could therefore

influence phenotypic characteristics independent of the DNA sequence [27].

Given their involvement in fundamental cellular processes, particular dysfunction of histone post-translational modifications is found in vascular homeostasis and atherosclerosis biology. It has been shown that cigarette smoking causes local pulmonary inflammation and oxidative stress, leading to the release of pro-inflammatory mediators into the blood [28]. While some functions mediated by histone deacetylases (HDACs) are cytoplasmic, chromatin immunoprecipitation has shown that the fibroblast growth factor 2 (FGF2) promoter is bound by HDAC5. Transcriptional activity is thus assumed to be repressed by HDAC5 interacting with the FGF2 promoter [29]. On the other hand, endothelial nitric oxide synthase 4 (eNOS4), an important feature of vascular biology, has also been involved in post-translational histone modifications in endothelial cells. It has been proposed that histone modifications can maintain eNOS4 expression efficiently and that removing the histone mark is able to cause the hypoxic repression of the eNOS4 gene [30].

ROS imposes an effect on pro-inflammatory gene transcription in the signal pathways of several inflammatory lung disorders. From this aspect, NF- $\kappa$ B-dependent signal pathway is partially managed by the acetylation and methylation marks of specific histone H3 and H4 residues [31].

## 7. MICRORNAS IN SMOKING-RELATED CARDIOVASCULAR DISEASES

The third epigenetic mechanism is the down-regulation or silencing of specific genes expression mediated by small, non-coding RNAs. MicroRNAs (miRNAs) are a family of small, highly conserved non-coding RNAs of approximately 22 nucleotides. It has been reported that miRNAs play a critical role in many biological processes, such as signal transduction, cellular proliferation, differentiation, inflammation, and apoptosis, and their dysregulation has been related to many diseases. The biological role of miRNAs in the cardiovascular system has been demonstrated over the past decade, and miRNA has also been suggested to be an active component of the cellular response to smoking [32]. MiRNAs function by binding to the 3'-UTR of targeted mRNA, repressing translation or promoting mRNA decay. In



particular, miR-1, miR-206, miR-208a/b, and miR-499 were shown to play essential roles in heart diseases [33]. The potential of miRNAs as biomarkers for diagnosis, prognosis, and response to therapy of CVD has also been uncovered. MiR-133, a miRNA with experimentally verified roles in cardiac development and disease, is specifically expressed in cardiac and skeletal muscle. Overexpression of miR-133 protects cardiomyocytes from remodeling such as cardiac hypertrophy, myocardial infarction, and cardiac arrhythmia. One recent study revealed that nicotine decreased miR-133 level in atrial fibroblasts, and this resulted in atrial structural remodeling [34]. Serum response factor (SRF), an activator of miR-133, plays an important part in modulating myocardial function and development. SRF, as a downstream target protein of extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling cascade can be phosphorylated, resulting in up-regulation of miR-133 expression. Moreover, a recent study demonstrated that nicotine triggers myocardial apoptosis through inhibiting the MAPK/ERK–SRF–miR-133 pathway, which may reveal a novel strategy to protect the heart against smoking-induced injury [35].

Induction of altered miRNA expression by chemical agents in target cells is an established mechanism. Mechanisms activated by the let-7 and miR-34 miRNAs families can protect against the p53-related activation of DNA-repair processes, cell-cycle arrest, and apoptosis [36]. In addition, numerous physiological and pathological events in cardiovascular system are partially controlled by multiple miRNAs. Due to their easy accessibility, miRNAs in circulating blood have been widely accepted as diagnostic biomarkers of CVD.

## 8. PERSPECTIVES OF EPIGENETICS OF SMOKING-RELATED CARDIOVASCULAR DISEASES

At this point, we only begin to understand the potential of modern epigenetic patterns in CVD in CSE and SHS affected individuals. For smoking, some promising loci discovered using the cluster-based technology have replicated across numerous studies and have shown promises for the clinical settings. For example, the smoking-related differential F2RL3 methylation observed in experimental studies was

scrutinized for methylated modifications indicative of smoking exposure or habit [37]. The following action will be trying to translate these findings into clinical tools that can then be studied in interventions aimed at distinguishing smoking behavior, monitoring smoking cessation during and after treatment, and assessing risk for related health hazard [38].

The great hope of epigenetic signals is to recognize novel genetic codes manipulating the cardiovascular responses to CSE or SHS, which lies on or involves the contribution of associating tobacco smoking exposure to cardiovascular clinical events. In spite of the fact that this appears to be principally possible, it remains to be seen whether epigenetics will actually exist in this regard and whether the epigenetics in CVD vary markedly between smoking and nonsmoking individuals [39]. Such observational studies depended on systemic epigenome-wide methylation assessment in chromosomes obtained from peripheral blood samples, which tend to be more accessible in expansive epidemiological cohorts. But given the potentially pronounced tissue-specificity of DNA methylation, peripheral blood may not be the most relevant sample matrix if epigenetic adjustments pertinent to cardiovascular sickness are so sought. Pure research work, which could experimentally confirm the results of methylation changes in cardiovascular candidate genes that could be caused by CSE in cultured cells, seems to be lacking [40]. These outcomes should motivate an ever-growing number of epigenome-wide data sets of CVD or CHD, which usually will feature at least crude smoking behavior data that have neither been assembled nor been evaluated with CSE- or SHS-related diseases. Diverse data on sequence modification regulatory mechanisms and gene expression can also be predicted to offer a prominent feature between epigenetic patterns and disease phenotypes.

## REFERENCES

1. Board JBS. Joint British Societies' consensus recommendations for the prevention of cardiovascular disease (JBS3). *Heart* 2014; 100 Suppl 2:ii1–ii67. doi: 10.1136/heartjnl-2014-305693.
2. Kim S, Karin M. Role of TLR2-dependent inflammation in metastatic progression. *Ann N Y Acad Sci* 2011; 1217:191–206. doi:

- 10.1111/j.1749-6632.2010.05882.x.
3. Sevenoaks MJ, Stockley RA. Chronic obstructive pulmonary disease, inflammation and co-morbidity: a common inflammatory phenotype? *Respir Res* 2006; 7:70. doi: 10.1186/1465-9921-7-70.
  4. Morris PB, Ference BA, Jahangir E, Feldman DN, Ryan JJ, Bahrami H, et al. Cardiovascular effects of exposure to cigarette smoke and electronic cigarettes: clinical perspectives from the Prevention of Cardiovascular Disease Section Leadership Council and Early Career Councils of the American College of Cardiology. *J Am Coll Cardiol* 2015; 66(12):1378–91. doi: 10.1016/j.jacc.2015.07.037.
  5. Zhang Y, Ren J. Epigenetics and obesity cardiomyopathy: from pathophysiology to prevention and management. *Pharmacol Ther* 2016; 161:52–66. doi: 10.1016/j.pharmthera.2016.03.005.
  6. Fagnocchi L, Mazzoleni S, Zippo A. Integration of signaling pathways with the epigenetic machinery in the maintenance of stem cells. *Stem Cells Int* 2016; 2016:8652748. doi: 10.1155/2016/8652748.
  7. Vaiserman A. Epidemiologic evidence for association between adverse environmental exposures in early life and epigenetic variation: a potential link to disease susceptibility? *Clin Epigenetics* 2015; 7(1):96. doi: 10.1186/s13148-015-0130-0.
  8. Breitling LP. Current genetics and epigenetics of smoking/tobacco-related cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2013; 33(7):1468–72. doi: 10.1161/ATVBAHA.112.300157.
  9. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 2008; 9(2):102–14. doi: 10.1038/nrg2290.
  10. Ober C, Vercelli D. Gene-environment interactions in human disease: nuisance or opportunity? *Trends Genet* 2011; 27(3):107–15. doi: 10.1016/j.tig.2010.12.004.
  11. Ong TP, Ozanne SE. Developmental programming of type 2 diabetes: early nutrition and epigenetic mechanisms. *Curr Opin Clin Nutr Metab Care* 2015; 18(4):354–60. doi: 10.1097/MCO.0000000000000177.
  12. Lane RH. Fetal programming, epigenetics, and adult onset disease. *Clin Perinatol* 2014; 41(4):815–31. doi: 10.1016/j.clp.2014.08.006.
  13. Patterson AJ, Chen M, Xue Q, Xiao D, Zhang L. Chronic prenatal hypoxia induces epigenetic programming of PKC $\epsilon$  gene repression in rat hearts. *Circ Res* 2010; 107(3):365–73. doi: 10.1161/CIRCRESAHA.110.221259.
  14. Hochberg Z, Feil R, Constancia M, Fraga M, Junien C, Carel JC, et al. Child health, developmental plasticity, and epigenetic programming. *Endocr Rev* 2011; 32(2):159–224. doi: 10.1210/er.2009-0039.
  15. Castaldelli-Maia JM, Ventriglio A, Bhugra D. Tobacco smoking: from 'glamour' to 'stigma'. A comprehensive review. *Psychiatry Clin Neurosci* 2016; 70(1):24–33. doi: 10.1111/pcn.12365.
  16. Rahman MM, Laher I. Structural and functional alteration of blood vessels caused by cigarette smoking: an overview of molecular mechanisms. *Curr Vasc Pharmacol* 2007; 5(4):276–92.
  17. Wan ES, Qiu W, Baccarelli A, Carey VJ, Bacherman H, Rennard SI, et al. Cigarette smoking behaviors and time since quitting are associated with differential DNA methylation across the human genome. *Hum Mol Genet* 2012; 21(13):3073–82. doi: 10.1093/hmg/dds135.
  18. Ostrow KL, Hoque MO, Loyo M, Brait M, Greenberg A, Siegfried JM, et al. Molecular analysis of plasma DNA for the early detection of lung cancer by quantitative methylation-specific PCR. *Clin Cancer Res* 2010; 16(13):3463–72. doi: 10.1158/1078-0432.CCR-09-3304.
  19. Lin RK, Hsieh YS, Lin P, Hsu HS, Chen CY, Tang YA, et al. The tobacco-specific carcinogen NNK induces DNA methyltransferase 1 accumulation and tumor suppressor gene hypermethylation in mice and lung cancer patients. *J Clin Invest* 2010; 120(2):521–32. doi: 10.1172/JCI40706.
  20. Liu H, Zhou Y, Boggs SE, Belinsky SA, Liu J. Cigarette smoke induces demethylation of prometastatic oncogene synuclein-gamma in lung cancer cells by downregulation of DNMT3B. *Oncogene* 2007; 26(40):5900–10. doi: 10.1038/sj.onc.1210400.
  21. Wojciechowski M, Czapinska H, Bochtler M. CpG underrepresentation and the bacterial CpG-specific DNA methyltransferase M.MpeI. *Proc Natl Acad Sci USA* 2013; 110(1):105–10. doi: 10.1073/pnas.1207986110.
  22. Sayols-Baixeras S, Lluís-Ganella C, Subirana I, Salas LA, Vilahur N, Corella D, et al. Identification of a new locus and validation of previously reported loci showing differential methylation associated with smoking. The REGICOR study. *Epigenetics* 2015; 10(12):1156–65. doi: 10.1080/15592294.2015.1115175.
  23. Tall AR, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. *Nat Rev*

- Immunol* 2015; 15(2):104–16. doi: 10.1038/nri3793.
24. Peng P, Wang L, Yang X, Huang X, Ba Y, Chen X, et al. A preliminary study of the relationship between promoter methylation of the ABCG1, GALNT2 and HMGCR genes and coronary heart disease. *PLoS One* 2014; 9(8):e102265. doi: 10.1371/journal.pone.0102265.
  25. Kim M, Long TI, Arakawa K, Wang R, Yu MC, Laird PW. DNA methylation as a biomarker for cardiovascular disease risk. *PLoS One* 2010; 5(3):e9692. doi: 10.1371/journal.pone.0009692.
  26. Ramachandran S, Henikoff S. Nucleosome dynamics during chromatin remodeling in vivo. *Nucleus* 2016; 7(1):20–6. doi: 10.1080/19491034.2016.1149666.
  27. Bowman GD, Poirier MG. Post-translational modifications of histones that influence nucleosome dynamics. *Chem Rev* 2015; 115(6):2274–95. doi: 10.1021/cr500350x.
  28. Hwang JW, Yao H, Caito S, Sundar IK, Rahman I. Redox regulation of SIRT1 in inflammation and cellular senescence. *Free Radic Biol Med* 2013; 61:95–110. doi: 10.1016/j.freeradbiomed.2013.03.015.
  29. Wang Z, Zang C, Cui K, Schones DE, Barski A, Peng W, et al. Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* 2009; 138(5):1019–31. doi: 10.1016/j.cell.2009.06.049.
  30. Qian J, Fulton D. Post-translational regulation of endothelial nitric oxide synthase in vascular endothelium. *Front Physiol* 2013; 4:347. doi: 10.3389/fphys.2013.00347.
  31. Chung S, Sundar IK, Hwang JW, Yull FE, Blackwell TS, Kinnula VL, et al. NF-kappaB inducing kinase, NIK mediates cigarette smoke/TNFalpha-induced histone acetylation and inflammation through differential activation of IKKs. *PLoS One* 2011; 6(8):e23488. doi: 10.1371/journal.pone.0023488.
  32. Jamaluddin MS, Weakley SM, Zhang L, Kougias P, Lin PH, Yao Q, et al. miRNAs: roles and clinical applications in vascular disease. *Expert Rev Mol Diagn* 2011; 11(1):79–89. doi: 10.1586/erm.10.103.
  33. Wong LL, Wang J, Liew OW, Richards AM, Chen YT. MicroRNA and heart failure. *Int J Mol Sci* 2016; 17(4). doi: 10.3390/ijms17040502.
  34. Pan ZW, Lu YJ, Yang BF. MicroRNAs: a novel class of potential therapeutic targets for cardiovascular diseases. *Acta Pharmacol Sin* 2010; 31(1):1–9. doi: 10.1038/aps.2009.175.
  35. Leung A, Natarajan R. Noncoding RNAs in vascular disease. *Curr Opin Cardiol* 2014; 29(3):199–206. doi: 10.1097/HCO.0000000000000054.
  36. Landau DA, Slack FJ. MicroRNAs in mutagenesis, genomic instability, and DNA repair. *Semin Oncol* 2011; 38(6):743–51. doi: 10.1053/j.seminoncol.2011.08.003.
  37. Fasanelli F, Baglietto L, Ponzi E, Guida F, Campanella G, Johansson M, et al. Hypomethylation of smoking-related genes is associated with future lung cancer in four prospective cohorts. *Nat Commun* 2015; 6:10192. doi: 10.1038/ncomms10192.
  38. Andersen AM, Dogan MV, Beach SR, Philibert RA. Current and future prospects for epigenetic biomarkers of substance use disorders. *Genes (Basel)* 2015; 6(4):991–1022. doi: 10.3390/genes6040991.
  39. Ho SM, Johnson A, Tarapore P, Janakiram V, Zhang X, Leung YK. Environmental epigenetics and its implication on disease risk and health outcomes. *ILAR J* 2012; 53(3–4):289–305. doi: 10.1093/ilar.53.3-4.289.
  40. Cortessis VK, Thomas DC, Levine AJ, Breton CV, Mack TM, Siegmund KD, et al. Environmental epigenetics: prospects for studying epigenetic mediation of exposure-response relationships. *Hum Genet* 2012; 131(10):1565–89. doi: 10.1007/s00439-012-1189-8.