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REVIEW ARTICLE

A systematic review of smoking-related epigenetic alterations

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Abstract
The aim of this study is to provide a systematic review of the known epigenetic alterations caused by cigarette smoke; establish an evidence-based perspective of their clinical value for screening, diagnosis, and treatment of smoke-related disorders; and discuss the challenges and ethical concerns associated with epigenetic studies. A well-defined, reproducible search strategy was employed to identify relevant literature (clinical, cellular, and animal-based) between 2000 and 2019 based on AMSTAR guidelines. A total of 80 studies were identified that reported alterations in DNA methylation, histone modifications, and miRNA expression following exposure to cigarette smoke. Changes in DNA methylation were most extensively documented for genes including AHRR, F2RL3, DAPK, and p16 after exposure to cigarette smoke. Likewise, miR16, miR21, miR146, and miR222 were identified to be differentially expressed in smokers and exhibit potential as biomarkers for determining susceptibility to COPD. We also identified 22 studies highlighting the transgenerational effects of maternal and paternal smoking on offspring. This systematic review lists the epigenetic events/alterations known to occur in response to cigarette smoke exposure and identifies the major genes and miRNAs that are potential targets for translational research in associated pathologies. Importantly, the limitations and ethical concerns related to epigenetic studies are also highlighted, as are the effects on the ability to address specific questions associated with exposure to tobacco/cigarette smoke. In the future, improved interpretation of epigenetic signatures will lead to their increased use as biomarkers and/or in drug development.

Keywords Systematic review · Epigenetics · Tobacco/cigarette smoking · DNA methylation · Histone modifications · microRNA · Maternal/paternal smoking · Challenges · Ethics

Introduction

Cigarette smoking is the most prevalent preventable cause of death and disease in the world. According to a recent report by the CDC (Centres for Disease Control and Prevention), approximately 480,000 adults in the US die due to active or passive smoking every year (Jamal et al. 2018). While the effects of active smoking are well documented and include cardiovascular diseases, respiratory illnesses (pneumonia, influenza, bronchitis, emphysema, and chronic airway obstruction), and cancer (lungs, mouth, pharynx, larynx, esophagus, stomach, pancreas, uterine cervix, kidney, ureter, and bladder) (MacKenzie et al. 1994); smoking-related health problems and neurocognitive deficits are also widely associated with second-hand smoke exposure (Heffernan 2016; Lubick 2011). In fact, as of late special emphasis is being given to the effect of third-hand smoke exposure, which refers to the residual tobacco smoke pollutants that accumulate on surfaces and in dust after tobacco use in closed environments. It is believed that third-hand smoke could stay on surfaces for months and may yield secondary pollutants that are considered to be potential health hazards, especially to infants and children (Burton 2011; Protano and Vitali 2011).

The challenge with studying smoke-related diseases is that not all smokers develop smoke-related disorders, nor are all non-smokers safe from its effects (Saha et al. 2007; Terzikhan et al. 2016). Considering this, it is not far-fetched to consider that smoke-related changes in gene expression are regulated epigenetically. Epigenetics is the study of the covalent modifications of DNA, protein, or RNA without alteration of their primary sequences. These alterations function to regulate gene expression during development or in

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response to environmental stimulus. As the link between genes (nature) and environment (nurture), epigenetics is responsible for normal growth and development in mammals. During the last decade, epigenetics has emerged as an interdisciplinary field with enormous scientific and therapeutic potential. Epigenetic changes have been implicated in cancer, cardiovascular diseases, behavioral disorders, inflammation, aging, neurodegenerative diseases, and diabetes (Portela and Esteller 2010). Understanding the complexity of the epigenetic signatures associated with cigarette smoke exposure may provide information about key therapeutic targets.

The aim of this systematic review of literature is to: (a) conduct evidence-based analysis of the role of epigenetics in regulating cellular signaling in response to cigarette smoke exposure, and (b) determine the clinical value of targeting epigenetic markers for the screening, diagnosis, and treatment of smoke-related disease risks among humans. To achieve this, we performed a scoping search using PubMed and ScienceDirect to identify the available literature between 2000 and 2019 highlighting epigenetic alterations following smoke exposure in cellular, clinical, and animal-based models. We identified a total of 80 studies that met our inclusion/exclusion criteria, out of which 35 investigated alterations in DNA methylation; 11 identified changes in histone modifications and chromatin structure, and 12 compared the miRNA profile among smokers and non-smokers. It should be noted that there are no previously published systematic reviews that draw together all aspects of epigenetic changes following smoke exposure, as is the focus of the current review. Moreover, this review will also include discussion of the technical challenges and ethical concerns related to epigenetic studies in smoking-related research. A careful review and solution to these concerns are necessary to design improved research strategies to determine the epigenetic interplay in smoke-related inflammation/pathologies and identify better diagnostic biomarkers and therapeutic targets.

Methods

For this review, we cross checked our study results against the 'assessment tool of multiple systematic reviews' (AMSTAR) guidelines (Shea et al. 2007). The AMSTAR tool includes the following 11 items: (1) an a priori statement of research questions and inclusion/exclusion criteria, (2) duplicate literature searches by two or more co-authors, (3) use of at least two electronic search engines followed by a supplemental search of reviews, textbooks and secondary references with keywords and MESH statements reported in the "Methods" section, (4) specification of status of publication (e.g., grey literature, which we define as reports that are not published in peer-reviewed journals or scientific monographs) as an inclusion/exclusion criterion, (5) a list of studies excluded from the review, (6) a summary of study characteristics that meet the inclusion criteria, (7) a formal assessment of strength or limitations of individual studies, (8) consideration of strength of evidence in drawing conclusions, (9) pooling of study results in a quantitative meta-analysis accompanied by a test for heterogeneity, if possible, (10) assessment of publication bias, and (11) a statement of sources of support (Shea et al. 2007). Except for items 9 and 10, which are contingent on the feasibility of a formal meta-analysis, the AMSTAR checklist is applicable to this systematic review. The aim of this review was to identify and summarize the studies that examine the alterations in epigenetic markers (DNA methylation, histone modifications, and miRNA expression) in response to cigarette smoking.

Study selection

Electronic data sources PubMed and ScienceDirect were used to conduct the initial literature search. For both the sources, the following keywords were used as a search criterion: "epigenetics + smoking", "smoking + DNA methylation", "smoking + histone modifications", "smoking + chromatin remodelling", "smoking + miRNA", "epigenetic + maternal smoking", "epigenetic + paternal smoking", and "transgenerational epigenetics + smoking". Secondary references of retrieved articles were reviewed to identify publications not captured by the electronic search. The search and selection of relevant studies were conducted independently by three study authors (GK, RB, and ST) with all disagreements resolved by consensus.

Inclusion/exclusion criteria

To be included in this review, a study must report an epigenetic change caused due to smoke exposure in the form of alterations in: (i) DNA methylation, (ii) histone modifying marks or, (iii) miRNA profile. The EWAS studies included in this review generally comprise of clinical data; however, wherever needed, in vivo and in vitro data have also been referred to explain the mechanistic implications of observed epigenetic alterations. The study data included in this review only relate to pulmonary health outcomes (like asthma, COPD, emphysema, allergy, etc.), while other health impacts are excluded. However, for the purpose of pointing out the transgenerational effects of paternal smoking, references showing male infertility have been included.

Data extracted

The data from each study were tabulated, and the resulting summary tables were again cross checked with

disagreements resolved by consensus. Information extracted from each study for the purposes of this review included the following:

1. Type of study: clinical, cell-based or animal-based.
2. Description of the sample: type, size, composition, and source.
3. Smoke exposure categorization: current, former or never smokers for human samples or smoke exposure duration for in vitro and in vivo studies.
4. Endpoints of interest (see inclusion criteria).
5. Results for each study were mentioned in terms of the number of genes/CpG sites/miRNAs altered on smoking and its implications. When the result was reported in a qualitative fashion, the corresponding text was extracted from the original publication and reproduced verbatim.

Measure of strengths and weaknesses

Epigenome-wide studies were assessed based on the sample size, the method used for analysis of epigenetic alterations and the statistical method applied to deduce results. However, since no epigenome-wide data were available for the studies investigating alterations in the post-translational modifications at histones, we relied on the cell-based and animal-based studies in this area.

Results

A total of 1280 studies were retrieved after searching for the keywords in the two databases. After excluding the duplicates and screening the studies as per the inclusion/exclusion criteria, a total of 80 studies were included in this review (Fig. 1). Out of these, 35 studies investigated the DNA methylation alterations associated with smoking among human subjects; 11 studies identified the smoking-related changes in histone modifications and chromatin structure while 12 compared the miRNA profile among smokers and non-smokers (Table 1). Interestingly, cigarette smoking was found to have transgenerational effects on the offspring as well. To study this effect, the epigenetic changes associated with paternal and maternal smoking are listed separately. For the purpose of this review, we have included 7 and 15 studies associated with paternal and maternal smoking, respectively (Table 2). In the subsequent sections, the findings from each of these studies have been summarized in brief.

Smoking-mediated alterations in DNA methylation

DNA methylation is the addition of a methyl group at the fifth carbon atom of cytosine (5mC) in the DNA strand (Fig. 2a) and is the most extensively studied epigenetic mechanism. Further, this modification plays a crucial role in regulating gene expression. Importantly, DNA methylation marks special sites called *CpG islands*, which exert important effects on gene transcription (Lim and Maher

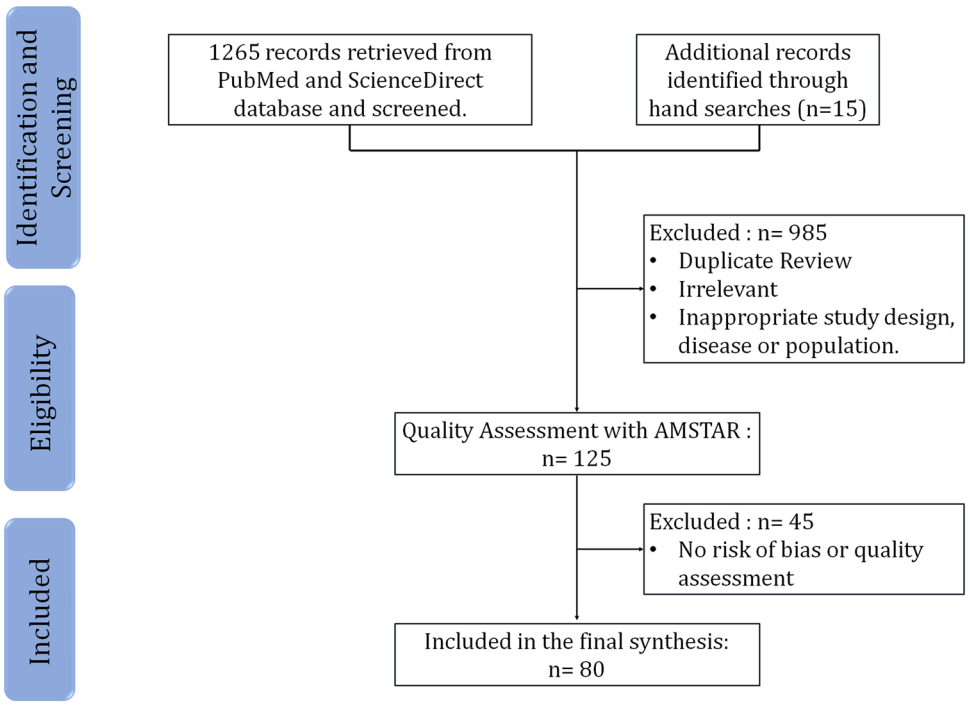

Fig. 1 AMSTAR flow diagram with reasons of exclusion. *AMSTAR* assessment tool of multiple systematic reviews

Table 1 List of reports demonstrating cigarette smoke-associated epigenetic alterations

Publication	Data source	Sample size	Sample type	Exposure characteristic	Platform	Outcome
DNA methylation						
Breitling et al. (2011)	General large-scale population wide cohort (ESTHER) study (Southwest Germany)	180 (50–60 years; both genders)	Peripheral blood	Current (60), Former (60) and Never smokers (60)	Infinium 27 K methylation assay; Sequenom MALDI-TOF mass spectrometry	Smoking-associated hypermethylation at coagulation factor II receptor-like 3 gene (*F2RL3*) locus
Prince et al. (2019)	Accessible resource for integrated epigenomics studies (ARIES); cohort of pregnant women (Avon, UK)	968 (14–16 years; both genders)	Blood	Current weekly (73) and non-weekly smokers (761)	HumanMethylation450 K BeadChip assay	Identified 11 differentially methylated CpG sites located on 7 gene regions associated with adolescent smoking
Costa et al. (2016)	Volunteers from Paraiba, Brazil	95 (30–67 years; both genders)	Oral epithelial cells	Current (29), former (36), and never (30) smokers	ELISA-based global DNA methylation and hydroxymethylation; MSP	Demonstrated that smoking habits are capable of inducing changes in global DNA methylation, but not hydroxymethylation
Sundar et al. (2017)	Lung tissue specimens collected by Department of Medicine and Pathology, Helsinki University Central Hospital	24 (45–71 years; both genders)	Peripheral lung tissues	Non-smokers (8), smokers with (8) and without (8) COPD	HumanMethylation450 K BeadChip assay	Suggested that DNA methylation of Nitric Oxide Synthase 1 adapter protein (*NOS1AP*), *BH3 interacting domain Death agonist* (*BID*), and Gamma Aminobutyric acid type A receptor Beta 1 subunit (*GABRB1*) may be used as epigenetic signatures in smokers and patients with COPD
de Vries et al. (2018a)	Participants from the observational population based (LifeLines) Cohort (Groningen, Netherlands)	658 (22–79 years; both genders)	Whole blood and lung tissues	Smokers with (381) and without (277) COPD	HumanMethylation450 K BeadChip assay and Pyrosequencing	Significantly associated 15 CpGs with pack years of which 10 were associated with lung function levels
de Vries et al. (2018b)	Participants from the observational population based (LifeLines) cohort (Groningen, Netherlands)	1561 (18–80 years; both genders)	Blood	Current (658) and Never (903) smokers	HumanMethylation450 K BeadChip assay	Concluded that there exists no significant association between DNA methylation levels and COPD, independent of smoking status
Qiu et al. (2012)	International COPD Genetics Network (ICGN) and the Boston Early Onset COPD Study (EOCOPD) participants	1454 participants (40-65 years; both genders)	White blood cells	With (801) and without (434) COPD	HumanMethylation 27K BeadChip assay	Observed 349 CpG sites significantly associated with the presence and severity of COPD, with majority relatively hypomethylated
Monick et al. (2012)	Participants of the Iowa adoptions studies (IAS)	165 participants (37–55 years; female)	Lymphoblasts and alveolar macrophages	Current (39), Former (46), and Never smokers (80)	HumanMethylation450 K BeadChip assay	Reported significant changes in *AHRR* methylation in DNA isolated from pulmonary alveolar macrophages and lymphoblasts

Table 1 (continued)

Publication	Data source	Sample size	Sample type	Exposure characteristic	Platform	Outcome
Su et al. (2016)	Healthy volunteers from general public in Durham and Chapel Hill, North Carolina	253 (19–63 years; both genders)	Blood	Smokers (172) and non-smokers (81)	HumanMethylation450 K BeadChip assay, RRBS	Identified new CpGs (616) associated with current smoking, pack-years, duration; and revealed unique profiles of smoking-associated DNA methylation and gene expression among immune cell types
Li et al. (2018)	Women from Australian mammographic density twins and sisters study	479 (40–70 years, female)	Blood	Never smokers (291), former (147), and current (41) smokers	HumanMethylation450 K BeadChip assay	Identified 39 smoking-associated CpGs located at 27 loci and demonstrated that smoking has a causal effect on peripheral blood DNA methylation, but not vice versa
Harlid et al. (2014)	Participants from National Institute of Environmental Health sciences (NIEHS) sister study (a nationwide prospective study amongst biological sister pairs with and without breast cancer)	200 females (45–56 years)	Blood	Current (12), former (70), and never smokers (118)	HumanMethylation450 K BeadChip assay	Confirmed smoking associations for 9 previously established CpGs and identified 2 potentially novel CpGs [G Protein Subunit gamma 12 (*GNG12*) and Protein tyrosine phosphatse non-receptor type 6 (*PTPN6*)]
Peluso et al. (2014)	Residents in Rayong province, Thailand	177 (26–49 years; both gender)	Peripheral blood leukocytes	Never (62), former (7), and current (108) smokers	Bisulfite PCR and Pyrosequencing	Associated hypermethylation of hypermethylated in Cancer 1(*HIC1*) gene with smoking
Philibert et al. (2013)	Participants from Adults in Making (AIM) project (a cohort of African-American men from six rural counties in Georgia)	107 (~22 years; men)	Blood serum	Smokers (58), non-smokers (49)	HumanMethylation450 K BeadChip assay	Identified hypomethylation of Aryl Hydrocarbon-receptor Repressor (*AHRR*) as a biomarker for smoking
Zhang et al. (2014)	General large-scale population-wide cohort (ESTHER) study (Southwest Germany)	3588 (50–75 years; both genders)	Blood	Never (1701), former (1136), and current smokers (654)	MALDI-TOF mass spectrometry	Associated hypomethylation at F2R like Thrombin or Trypsin receptor 3 (*F2RL3*) with smoking-related cardiovascular diseases, cancer, and other mortality
Beach et al. (2017)	Participants from two research projects: Strong African American Healthy Adult Project (SHAPE) and the Adults in the Making project (AIM).	616 (17–19 years; both genders)	Blood	Never, heavy or light smokers	HumanMethylation450 K BeadChip assay	Demonstrated for the first time that the impact of smoking on demethylation of cg05575921 is potentially qualified by an epigenetic regulatory motif: methylation of the first exon of methylene tetrahydrofolate reductase (*MTHFR*)
Reynolds et al. (2017)	Multi-Ethnic Study of atherosclerosis (MESA) participants from 6 field centres in the US	934 (59–79 years; both genders)	CD14 + monocyte	Never (420), former (458), and current (63) smokers	HumanMethylation450 K BeadChip assay	Suggested that alterations in methylation may not be a main mechanism regulating gene expression in monocytes in response to cigarette smoking

Table 1 (continued)

Publication	Data source	Sample size	Sample type	Exposure characteristic	Platform	Outcome
Ambatipudi et al. (2016)	Participants from European Prospective Investigation into Cancer and Nutrition (EPIC); a large prospective study conducted at 23 centres across 10 countries in Europe	910 (26.1–72.8 years; women)	White blood cells	Never (528), current (189), and former smokers (193)	HumanMethylation450 K BeadChip assay	Identified 748 differentially methylated CpG sites between smokers and non-smokers, of which 12 were novel
Wilson et al. (2017)	Participants from the Cooperative Health Research in the Augsberg region (KORA) study in Germany	1344 (40–70 years; both genders)	Blood	Current (280), former (449), and never (615) smokers	HumanMethylation450 K BeadChip assay	Indicated rapid reversion of altered methylation within the first 2 decades after smoking cessation; 52 differentially methylated CpGs between former and never smokers
McCartney et al. (2018)	Participants from Generation Scotland: Scottish family health study (GS: SFHS)	4905 (35–65 years; both genders)	Peripheral blood	Current (917), former (1466), and never (2522) smokers	Illumina HumanMethylationEPIC BeadChip	Proved smoking-based DNA methylation patterns are time and dose dependent which can be reversed on smoking cessation
Wan et al. (2012)	Participants from two cohorts: International COPD Genetics Network (ICGN) and Boston Early-onset COPD study (EOCOPD) cohort	1454 (45–65 years; both genders)	Blood	Former (887), current (499), and never (68) smoker	Infinum HumanMethylation27 K BeadChip assay	Identified 15 CpG sites significantly associated with current smoking; 2 sites associated with cumulative smoke exposure; and 3 sites associated with time since quitting cigarettes
Siedlinski et al. (2012)	Subjects from (Alpha 1 Antitrypsin (AAT) genetic modifiers study	316 (42–62 years; A1AT deficiency)	Blood sample	Current and former smokers	Illumina GoldenGate Methylation Cancer Panel I and Pyrosequencing	Demonstrated that smoking associates with global hypomethylation in subjects with alpha 1 antitrypsin (*AAT*) deficiency; methylation levels of 16 CpG sites were significantly associated with smoking
Gupta et al. (2019)	Participants from the Finnish Twin Cohort (FTC) study	310 (21–69 years; both genders)	Peripheral blood	Current smokers (cotinine levels > 4.85 ng/ml)	HumanMethylation450 K BeadChip assay	Observed that out of the 19 methylated Quantitative Trait Loci (meQTLs) associated with cotinine levels, 7 CpG sites showed molecular mediation by methylation suggesting that these sites may act as a causal mediator in regulating the effect of genetic variation on cotinine levels or smoking behavior
Dogan et al. (2017)	Participants from Framingham Heart Study (FHS)	2567 (52–77 years; both genders)	White blood cells	Smokers (121) and non-smokers (1476)	HumanMethylation450 K BeadChip assay and GeneChip HumanMapping 500 K Array	Reported that when genetic variations are consideredsmoking has a significant effect on DNA methylation signature of peripheral blood DNA at 4379 genes

Table 1 (continued)

Publication	Data source	Sample size	Sample type	Exposure characteristic	Platform	Outcome
Leng et al. (2015)	Participants from two longitudinal cohorts: Lovelace smokers cohort and veterans smokers cohort	1675 (40–74 years; mostly men)	Sputum samples	Smokers	MSP and HumanOmni2.5–4v1-H BeadChip	Provides proof-of-concept substantiating that two trans acting loci with an enhancer SNP predisposes O-6-methylguanine-DNA methylatransferase (*MGMT*) methylation leading to its reduced transcription in the lungs of smokers
Soria et al. (2002)	Participants from the ongoing chemoprevention trial at MD Anderson Cancer Center	100 former smokers with prior history of cancer (35–78 years; both genders)	Bronchial brush samples	100 former smokers with at least a 20-pack-year smoking history; at least 12 months of smoking cessation; prior cancer history (≥ 6 months after treatment)	MSP	Promoter hypermethylation at cyclin-dependent kinase inhibitor 2A (*p16*), Death associated Protein Kinase (*DAPK*), and Glutathione-S-transferase pi 1 (*GSTP1*) genes in former smokers with non small cell lung cancer (NSCLC)
Belinsky et al. (2002)	Participants from multispecialty chest clinic at New Mexico Veterans Health Care System (NMVHCS)	141 (44–81 years; both genders)	Bronchial epithelial cells (clinical samples)	Current (60) and former (81) smokers	MSP	Strong association between *p16* methylation in the bronchial epithelium with smokers and incidence of lung cancer
Fasanelli et al. (2015)	Participants from the Norwegian women and cancer study (*NOWAC*) longitudinal cohort	132 (47–64 years; both genders)	Blood	Former (41), current (72), and never (11) smokers	HumanMethylation450 K BeadChip assay	Hypomethylation of CpG sites at *AHRR* and *F2RL3* genes may mediate the effect of tobacco in lung cancer
Ostrow et al. (2013)	Volunteers for specialized program of research excellence in lung cancer	20 (34–78 years, both gender)	Blood	Former (6), current (2), and non-smokers (12)	Quantitative MSP	Showed that tobacco smoke induces hypermethylation in the Nischarin (*NISCH*) gene promoter before any detectable cancer
Zaghlool et al. (2015)	Middle eastern population	123 (22–56 years; both genders)	Blood	Smokers (13) and non-smokers (108)	HumanMethylation450 K BeadChip assay	Smoking-related variations in four genes were observed [*AHRR, F2RL3, G protein-coupled receptor (GPR1)* and *GF11*)
Shenker et al. (2013)	Participants from the italian component of the European Prospective Investigation into Cancer and Nutrition cohort	180 (both genders)	Blood	Current (33), former (45), and never smokers (102)	HumanMethylation450 K BeadChip assay	Validated the previously identified associations with methylation and smoking for the *F2RL3* and *AHRR* probes and further identified *AHRR* probes that were significantly associated with smoking

Table 1 (continued)

Publication	Data source	Sample size	Sample type	Exposure characteristic	Platform	Outcome
Sun et al. (2013)	Participants of the african-american descent from the Genetic Epidemiology Network of Arteriopathy (GENOA) study	972 (56–76 years; both genders)	Peripheral leukocytes	Current (122), former (283) and never (567) smokers	Illumina Infinum Human Methylation 27K BeadChip	Established association of DNA methylation sites in *F2RL3* and *GPR15* with current smokers in African American (AA) population and identified that the smoking-associated methylation changes in AA population are not different than those observed in Caucasians
Zhu et al. (2016)	Chinese participants from coke oven cohort, acute coronary syndrome (ACS) patients, and the Wuhan-Zhuhai (WHZH) cohort	596 (30–70 years; both genders)	Blood	Current (273), former (64), and never (259) smoker	HumanMethylation450 K BeadChip assay	Identified 318 smoking-associated CpGs in Chinese population, among which 161 CpGs annotated to 123 genes that were not associated with smoking in recent studies of Europeans and African Americans
Xu et al. (2010)	Subjects from Mid-South Tobacco Family (MSTF) study	300 (African American; both genders; 34–51 years)	Blood	150 smokers and 150 non-smokers	MSP	Proved association of nicotine dependence with methylation of the Catechol-O-methyltransferase (*COMT*) promoter, implying that methylation plays a role in smoking dependence
Lee et al. (2016)	Participants in a Korean COPD cohort	100 participants (53–84 years; both genders)	Blood	Never smokers (39); former smokers (30), and current (31) smokers	HumanMethylation450 K BeadChip assay	Identified 87 significant differentially methylated regions in current smokers compared to never smokers of which 66 were novel loci
Elliot et al. (2014)	Participants from Southhall and Brent Revisited (SABRE) cohort	189 (40–50 years; both genders; South Asian and European decent)	Blood	Never smokers (129); former smokers (24), and current (36) smokers	HumanMethylation450 K BeadChip assay	Identified association between smoking status and ethnic group at the *AHRR* locus; also the differences in DNA methylation status at both single CpG sites and combined smoking score with respect to ethnicity
Histone modification						
Kaur et al. (2018)	C57Bl/6 mice and human airway type II epithelial cells (A549)	5–6 mice	Lung tissue and A549 cells	4 h/day, 5 days/week for a period of 6 consecutive weeks in vivo; 24-h CSE exposure in vitro	ChIP assay	Histone modifications (H3K4me3, H3K9ac, H3K9me3, H4K20me3) at the *Nlrp10* promoter region regulate its expression in response to smoke exposure both in vivo and in vitro
Marwick et al. (2004)	Sprague–Dawley rats	24 Rats (male)	Lung tissue	Cigarette smoke exposure (4 cigarettes per day)	Electrophoretic shift assay and HDAC activity	Cigarette smoke exposure results in an influx of inflammatory cells and chromatin modifications regulated by HDAC2 in rat lungs
Ito et al. (2001)	Human volunteers	29 (27–30 years; both genders)	Human bronchial biopsy and BAL macrophages	Smokers (13) and non-smokers (16)	ChIP Assay and HDAC activity	Associated cigarette smoking with reduced HDAC2 expression which correlated with the increase in H4 acetylation

Table 1 (continued)

Publication	Data source	Sample size	Sample type	Exposure characteristic	Platform	Outcome
Ito et al. (2005)	Patient registry and COPD tissue Bank maintained since 1972	56 (58–69 years; both genders)	BAL fluid and peripheral lung tissue	Non-smokers (11) and COPD patients (35)	ChIP Assay and HAT/HDAC Activity	Showed a positive correlation between HDAC activity and disease severity in COPD; demonstrated that interleukin-8 mRNA and histone-4 acetylation at the NF-κB binding site of the interleukin-8 promoter were induced in peripheral lung tissue from COPD patients
Szulakowski et al. (2006)	Human volunteers	56 (58–70 years; both genders)	Peripheral lung tissue	Current smokers with COPD and without COPD; ex-smokers with COPD and a non-smoker	EMSA	Identified increased expression of acetylated histone H4 in smokers with and without COPD; while increase in acetylation of histone H3 was observed only in ex-smokers with COPD
Sundar et al. (2012)	Human bronchial epithelial cells (H292)	Repeated three times	In vitro	1% CSE	ChIP assay and immunoblotting	Demonstrated that CSE-mediated activation of Mitogen Stress-activated Protein kinase 1 (*MSK1*) resulted in phospho-acetylation of histone H3 (Ser10/Lys9) and acetylation of histone H4 (Lys12)
Sundar et al. (2014)	C57BL/6 J mice and human bronchial epithelial cells (H292)	Four per group	Lung tissues for mice	1 puff/min of 2 s duration and 35 mL volume (mice); 1% CSE	Bottom-up mass spectrometry	Identified histone marks on histones H3 and H4 associated with smoke exposure
Sundar et al. (2016)	C57BL6/J mice and human samples (COPD patients)	4–6 mice per group	Lung tissue	Two 1-h exposures (1 h apart) daily for 3 consecutive days (acute); 6 mo exposure (chronic)	Immunoblotting and qPCR	Identified significant downregulation of Dnmt1, Dnmt3a, Dnmt3b, Hdac2, Hdac4, Hat1, protein arginine methyltransferase (Prmt1), and Aurora kinase B (Aurkb) along with induction of chromatin histone marks (H3K56ac and H4K12ac) by cigarette smoke
Yang et al. (2006)	Human monocytic (MonoMac6) cells	Repeated 3–4 times	In vitro	CSE (1, 2.5, 5%) treatment	HDAC activity and immunoprecipitation	Demonstrated that CSE reduced histone deacetylase (HDAC 1-3) activity and was associated with post-translational modification of HDAC1, HDAC2, and HDAC3 by nitrotyrosine and aldehyde-adduct formation
Yao et al. (2010)	129/SVJ mice and MonoMac6 cells	Repeated three times	In vitro	0.25–1% CSE; 21 hr exposure of mice for 3 days	ChIP assays	Determined that Preotein Kinase C (PKCζ) plays an important role in CSE/aldehyde- and LPS-induced lung inflammation through acetylation of RelA/p65 and histone modifications via chromatin binding protein (CBP)

Table 1 (continued)

Publication	Data source	Sample size	Sample type	Exposure characteristic	Platform	Outcome
Moodie et al. (2004)	Human alveolar epithelial cells (A549)	n.s.	In vitro	CSE (1, 2.5, 5, 10%) treatment	HDAC activity	Identified that exposure of alveolar (A549) epithelial cells to CSE results in altered chromatin deacetylation due to dysregulated HDAC activity
MicroRNA						
Gross et al. (2014)	Subjects recruited through Iowa Institute for Clinical and Translational Science Clinical Core	Eight (four smokers; four non-smokers)	Alveolar macrophages from clinical samples	5% CSE	RT-PCR	Cigarette smoke alters alveolar macrophage miRNA expression, in part by SUMOylation of DICER which modifies its enzyme activity
Huang et al. (2014)	Samples from the First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China	249 (45–65 years; Male)	Serum	Non-smokers (65), smokers (100), and lung cancer (84) patients	miRCURY LNA microRNA array version 16.0	Observed 105 differentially expressed miRNAs in the sera of smokers, of which the expression of 73 miRNAs was downregulated
Willinger et al. (2017)	Participants enrolled for the Framingham heart study (FHS)	5023 (40–73 years; both genders)	Blood	Current (524), former (2079), and never (2420) smokers	Targeted TaqMan miRNA assays	Identified six miRNAs that were characteristically dysregulated in the context of smoking, five of which were downregulated in smokers vs. non-smokers
Advani et al. (2017)	Human bronchial epithelial cells (H292)	Not specified	In vitro	0.1% CSE for 12 months	Affymetrix GeneChip miRNA Arrays	Identified 112 upregulated and 147 downregulated miRNAs (by twofold) in cigarette smoke-treated H292 cells
Wang et al. (2015)	Samples from Weill Cornell NIH Clinical and Translational Science Center	19 (34–57 years; both genders)	Small airway epithelial cells	Non-smokers (9) and smokers (10)	Affymetrix miRNA 2.0 arrays	Reported that 12 out of the 34 miRNAs did not revert to normal levels with Wnt/β-catenin signaling pathway being the enriched pathway after smoking cessation for 3 months
Izzotti et al. (2009)	Sprague–Dawley rats	16 rats	Lung tissues for rats	5 cigarettes at one time, 6 h/day divided into 2 rounds of 3 h with a 3-h interval, 5 days/wk	qPCR	Demonstrated twofold decrease in 126 miRNAs and induction of 7 miRNAs in cigarette smoke-exposed rats
Gu et al. (2018)	Human tissues, C57BL/6 J mice and human airway bronchial epithelial cell (BEAS-2B)	28 (53–73 years; both genders); 12 mice	Lung tissues	Never smokers (6), smokers with (16) and without (6) COPD	qPCR	Observed positive correlation between miR-195 and phosphorylation of Akt in lung tissues of COPD patients
Shen et al. (2017)	Patients at The Second Affiliated Hospital of Kunming Medical University	80	Blood	Non-smoker non-COPD (20); smoker non-COPD (20); smoker with stable COPD (20); smoker with acute exacerbation COPD (20)	miRCURY LNA™ microRNA Array	Observed that expression of 56 miRNAs was regulated in the study groups; decreased miR-149-3p may induce inflammatory responses in COPD patients through TLR-4/NF-κB dependent pathway

Table 1 (continued)

Publication	Data source	Sample size	Sample type	Exposure characteristic	Platform	Outcome
Banerjee et al. (2015)	Subjects enrolled in Current Controlled Trials	70 (23–55 years; both genders)	Blood	Never (20), former (20), and current (30) smokers	qPCR	Identified differential expression of miR-124 and let-7a between the smoking and control groups
Van Pottelberge et al. (2011)	Human subjects	32 (47–72 years; both genders)	Sputum	Current smokers with (12) and without (10) COPD and never smokers (10)	Stem-loop reverse transcription-qPCR	Identified 34 differentially expressed miRNAs between never and current smokers without COPD; 8 miRNAs were significantly downregulated in current smokers with COPD
Anderson et al. (2018)	Human subjects	140 (23–71 years; both genders)	Blood	Current (39) and never (101) smokers	qPCR	Identified elevated levels of total WBCs, neutrophils, monocytes, lymphocytes, C-reactive protein (CRP), MCP-1, IFNγ, and reduced levels of miR-21 in smokers
Shi et al. (2016)	Human subjects	100 (21–30 years; Male)	Blood	Never (40) and current (60) smokers	Agilent human microRNA array	Determined that 35 miRNAs were differentially expressed—while 24 miRNAs were up-regulated, 11 miRNAs were down-regulated in smokers

MALDI-TOF matrix-assisted laser desorption ionization time-of-flight, *MSP* methylation-specific PCR, *RRBS* reduced representation bisulfite sequencing, *ChIP* chromatin immunoprecipitation, *BAL* bronchoalveolar lavage, *EMSA* electrophoretic mobility shift assay, *CSE* cigarette smoke extract, *n.s.* not specified

2011; Edwards et al. 2017). Generally, but not always, the hypermethylation of CpG islands on gene promoters leads to gene silencing (Yang and Schwartz 2011). This may be explained by the need for accessibility of the gene promoter to transcription factors and other regulatory units for induction of gene transcription, which can be restricted by the presence of DNA methylation (Lim and Maher 2011).

Functionally, DNA methylation is catalyzed by a protein family known as DNA methyltransferases (DNMTs) which transfer methyl groups from *S*-adenosyl-L-methionine (SAM) to the 5-carbon position of cytosine residues in DNA (Jin and Robertson 2013). On the contrary, DNA demethylation is mediated by the ten-eleven translocation (TET) enzymes, which add a hydroxyl group onto the methyl group of 5mC to form 5hmC (5-hydroxymethyl cytosine) (Moore et al. 2013). A brief description of the roles of methyltransferases and demethylases is provided in Table 3.

Several epigenome-wide association studies (EWAS) have shown an association between the modifications in DNA methylation in the blood of smokers and their smoking status (Breitling et al. 2011; de Vries et al. 2018a; Harlid et al. 2014; Li et al. 2018; Philibert et al. 2013; Prince et al. 2019; Su et al. 2016). These studies have identified several CpGs mapping to specific genes that are altered on smoke exposure. Some of the common hits identified from such studies include coagulation factor II (thrombin) receptor-like 3 (*F2RL3*) (Sun et al. 2013; Zaghlool et al. 2015), aryl hydrocarbon receptor repressor (AHRR) (Elliott et al. 2014; Fasanelli et al. 2015; Monick et al. 2012; Philibert et al. 2013; Zaghlool et al. 2015), cyclin-dependent kinase inhibitor (*p16*) (Belinsky et al. 2002; Soria et al. 2002), and death-associated protein kinase (DAPK) (Soria et al. 2002).

F2RL3 encodes a protein involved in platelet activation, intimal hyperplasia, and inflammation (Breitling et al. 2011; Fasanelli et al. 2015). It has been suggested to be a strong predictor of mortality during smoking-induced cardiovascular diseases and cancers (Breitling et al. 2011; Zhang et al. 2014). Likewise, hypomethylation at specific CpG loci of *AHRR* (the repressor of the aryl hydrocarbon receptor) has been associated with tobacco smoking and is linked to increased risk of lung cancer among smokers (Fasanelli et al. 2015; Prince et al. 2019; Shenker et al. 2013). Also, methylation at *p16* and *DAPK* promoter has been reported in former patients with non-small cell lung carcinoma (NSCLC) (Soria et al. 2002). Such reports suggest that the smoking-associated DNA methylation changes could act as biomarkers to predict the susceptibilities toward major pulmonary diseases among smokers.

However, contradictory evidences exist with regard to DNA methylation. For instance, Qiu et al. (2012) studied DNA methylation changes in the WBCs from 1454 smokers with and without COPD. They identified 349 CpG sites that were associated with the presence and severity of COPD.

Table 2 List of studies demonstrating the effect of maternal/paternal cigarette smoking on the offspring

Publication	Data source	Sample size	Sample type	Exposure characteristic	Platform	Outcome
Paternal smoking						
Laqqan et al. (2017)	Fertile Male volunteers	108 (30–46 years, male)	Sperm cells	Current (51) and never (57) smokers	Infinium HumanMethylation450 BeadChip	Identified 11 differentially methylated CpGs associated with smoking in the sperm DNA; strong positive correlation between the sperm DNA methylation at CpG in mitogen activated protein kinase 8 interacting protein (MAPK8IP) and tyrosine kinase receptor (TKR) gene-related amplicon, sperm count and sperm motility
Alkhaled et al. (2018)	Human subjects	28 (25–50 years; male)	Spermatozoa	Current (14) and never (14) smokers	Infinium HumanMethylation450 BeadChip	Observed significant difference in DNA methylation of 11 CpGs and normal sperm parameters between the study cases and the control groups
Hamad et al. (2018)	Human subjects who attended Laboratory of Assisted Reproduction and Andrology at the Department of Obstetrics and Gynaecology, Saarland University	109 (30–50 years; male)	Sperm cells	Non-smokers (54) and smokers (55)	ELISA-based global DNA methylation assay	Demonstrated that DNA methylation significantly correlates with sperm parameters and sperm non-condensed chromatin which is altered with smoking
Jenkins et al. (2017)	Samples collected from general population	156 (30–35 years; male)	Semen samples	Non-smokers (78) and smokers (78)	Infinium HumanMethylation450 BeadChip	Identified 141 differentially methylated CpGs (132 unique genes) associated with smoking
Marczylo et al. (2012)	Volunteers undergoing fertility assessment at the Leicester Royal Infirmary, UK	13 (24–44 years; male)	Semen samples	Smokers (6) and non-smokers (7)	In-house customized miRNA microarray	Identified 28 significant differentially expressed miRNAs in smokers (out of 102 miRNAs) compared with non-smokers; 4 of these were associated with healthy sperm and normal embryo development
Xu et al. (2013)	C57BL/6 J mice	60 (5–6 week-old male)	Mice testis	Smoke exposed (30) and controls (30)—2 cigarettes daily for 2-wk	MSP	Showed that four CpGs near the *Pebp1* (spermatogenesis-associated protein) transcriptional start site were hypermethylated during CSE challenge
Hillemacher et al. (2008)	German individuals with Caucasian history	298 (25–73 years; both genders)	Genomic DNA	Smokers and non-smokers among parents and offspring	Global DNA methylation assessed as per (Bleich et al. 2006)	Observed no effect of smoking on global DNA methylation; however, reported an association of offspring's DNA methylation with paternal DNA methylation if both were non-smokers

Table 2 (continued)

Publication	Data source	Sample size	Sample type	Exposure characteristic	Platform	Outcome
Maternal smoking						
Fa et al. (2016)	Women seeking a legal abortion in a regional hospital within Region Midtjylland, Denmark	40 (both genders)	First trimester fetal tissues and placentas	Pregnant women [smokers (17) and non-smokers (23)]	Bisulfite PCR and pyrosequencing	Identified gender-specific differences in global DNA methylation levels; however, no significant DNA methylation changes were reported in response to the first trimester maternal cigarette smoking
Joubert et al. (2012)	Participants enrolled in Norwegian mother and child cohort study and Newborn Epigenetics Study (NEST)	1098 (new born; both genders)	Cord blood	Mothers (smokers (555) and non-smokers (543))	Infinium HumanMethylation450 BeadChip	Identified statistically significant associations between maternal smoking in pregnancy and methylation in cord blood at 26 CpGs mapping to 10 genes
Joubert et al. (2014)	Participants from Norwegian mother and child cohort study	1042 infants	Cord blood	Mothers [smokers (136), non-smokers (520) and quite during pregnancy (386)]	Infinium HumanMethylation450 BeadChip	Demonstrated that differential methylation in the offspring occurs only when the embryo is exposed to smoke due to maternal smoking during pregnancy while paternal, grandmaternal or maternal smoking prior to pregnancy have no effect
Richmond et al. (2018)	Participants from the Avon Longitudinal study of Parents and Children (ALSPAC) cohort	1640 (20–60 years; both genders)	Mother–offspring blood	Mothers [smokers (216) and non-smokers (538)]; offspring [smokers (252) and non-smokers (634)]	Infinium HumanMethylation450 BeadChip	Observed associations at 15 CpG sites in 11 gene regions: myosin (*MYO1G*), *FERM domain containing 4A (FRMD4A), cytochrome P450 (CYP1A1), contactin associated protein type 2 (CNTNAP2), ADP ribosylation like factor 4C (ARL4C), AHRR, TIFA inhibitor (TIFAB), MDM4 regulator of p53 (MDM4), AX748264, dopamine receptor D1 (DRD1), FTO alpha ketoglutarate dependent dioxygenase (FTO) with prenatal smoking*
Breton et al. (2009)	Participants from the Children's health study	348 kids in kindergarten and first grade	Buccal cells	Mothers [Smokers (102) and non-smokers (239)]	Pyrosequencing	Showed that maternal smoking leads to significantly reduced methylation of *AluYb8* and differential methylation of CpG loci in eight genes in the offspring
Suter et al. (2010)	Pregnant women admitted to either Ben Taub General Hospital or St. Luke's Episcopal Hospital (Houston, TX)	34 placentas	Placental tissue	Non-smokers (19) and smokers (15)	Bisulfite sequencing	Determined that in utero tobacco exposure significantly increases placental *CYP1A1* expression in association with differential methylation at a critical Xenobiotic response element (XRE)

Table 2 (continued)

Publication	Data source	Sample size	Sample type	Exposure characteristic	Platform	Outcome
Suter et al. (2011)	Human subjects	36 placentas	Placental tissue	Non-smokers (18) and smokers (18)	Human HG-12 expression and HumanMethylation27 BeadChip array	Observed significant correlation between placental transcriptional regulation and differential CpG methylation in only 25 genes among non-smokers but in 438 genes among smokers
Herberth et al. (2014)	Participants from the mother-child study from Lifestyle and Environmental Factors and their influence on Newborn Allergy risk (LINA)	441	Maternal and cord blood	Mothers [smokers (55) and non-smokers (385)]	qPCR	Determined that maternal tobacco smoke exposure during pregnancy correlates with the level of miRNA-223 expression in blood which affects children's cord blood Treg cell numbers and subsequent allergy risk
Richmond et al. (2015)	Offspring in the Avon Longitudinal Study of Parent and children (ALSPAC) cohort	790	Cord blood	Mothers [smokers (91) and non-smokers (699)]	Infinium HumanMethylation450 BeadChip	Demonstrated that methylation at 15 CpG sites in 7 gene regions [*AHRR, MYO1G, GFI1, CYP1A1, CNTNAP2,* Krupple like factor 13 (*KLF13)* and ATPase phospholipid transporting 9A (*ATP9A*)] in cord blood was associated with maternal smoking
Maccani et al. (2010)	Human subjects	25 placentas	Placental tissue	Mothers [smokers (8) and non-smokers (17)]	qPCR	Reported significant downregulation of miR-16, miR-21, and miR-146a in cigarette smoke-exposed placentas compared to the controls
Murphy et al. (2012)	Participants from the New-born Epigenetics STudy (NEST)	428	Cord blood	Mothers [smokers (75), non-smokers (222) and quit during pregnancy (110)]	Bisulfite pyrosequencing	Observed that smoking-related hypermethylation was most pronounced in male offsprings for whom approximately 20% of smoking-related low birth weight was mediated by DNA methylation at the Insulin-like growth factor 2 (*IGF2*) *differentially methylated region* (*DMR*)
Morales et al. (2016)	Subjects enrolled for INfancia y medio ambiente (INMA) project	450 mother–child pairs	Placental tissue	Mothers [smokers (68) and non-smokers (359)]	Infinium HumanMethylation450 BeadChip	Reported 50 CpGs, representing 46 loci, differentially methylated in smokers compared with non-smokers
Markunas et al. (2014)	Infants from the Norway Facial Cleft (NCL) study	898 mother–child pairs	New-born blood	Mothers [smokers (287) and non-smokers (602)]	Infinium HumanMethylation450 BeadChip	Identified 10 genes with newly established links to maternal smoking; amongst which *FRMD4A, ATP9A, acetylgalatosaminyltransferase 2 (GALNT2),* and maternally expressed 3 (*MEG3)* were implicated in processes related to nicotine dependence, smoking cessation, and placental and embryonic development

Table 2 (continued)

Publication	Data source	Sample size	Sample type	Exposure characteristic	Platform	Outcome
Patil et al. (2013)	Participants from the Isle of Wight birth cohort	245 females (currently ~ 18 years of age) exposed to maternal smoking	Peripheral blood leukocytes	Mothers [smokers (n.s.) and non-smokers (n.s.)]	Infinium HumanMethylation450 BeadChip	Concluded that both genetic variants of IL13 and DNA methylation changes due to maternal smoking are responsible for asthma-related lung function in the offsprings
Tehranifar et al. (2018)	Participants from the New York women's birth cohort	89 females with self-reported history of smoking status of their mothers	Blood (collected twice at birth through age 7 and then in midlife between 38 and 46 years age)	Mothers [smokers (32) and non-smokers (57)]	Infinium HumanMethylation450 BeadChip	Reported 17 differentially methylated CpGs associated with maternal smoking; several of these extended to mid-life independent of the subjects' own active smoking status

MSP methylation-specific PCR, *CSE* cigarette smoke extract; *n.s.* not specified

Most of the associated CpG sites were hypomethylated and were found on genes associated with immune and inflammatory system pathways, responses to stress and external stimuli, wound healing, and coagulation cascades. These findings led the group to conclude that epigenetic changes might cause COPD (Qiu et al. 2012). On the contrary, de Vries et al. conducted similar investigations on the whole blood of 1561 individuals from a Dutch cohort comprising current and never smokers but found no association between the DNA methylation and the occurrence of COPD in the study groups. Few of the reasons described by the authors for such contradiction were the fact that the study group associated with the work of Qiu et al. had severe COPD with an FEV1/FVC ratio less than 0.7. Additionally, the platform used to study DNA methylation by both the groups varied and the former did not include confounding factors like age and sex in their analyses (de Vries et al. 2018b). Such contradictions are not uncommon when reviewing epigenetic studies and just highlight the highly dynamic nature of DNA methylation.

Evidence has suggested that various genetic variants mediate the smoking-associated DNA methylation changes (Dogan et al. 2017; Gupta et al. 2019; Leng et al. 2015; Siedlinski et al. 2012). This proves that gene and environment work at tandem to decide the disease fate in individuals. This is the reason behind variations observed in smoking-related methylation changes across populations (Elliott et al. 2014; Shenker et al. 2013; Dogan et al. 2014; Sun et al. 2013; Lee et al. 2016; Xu et al. 2010; Zaghlool et al. 2015; Zhu et al. 2016).

Interestingly smoking-related DNA methylation changes are strongly associated with the smoking habits and time since smoking cessation (Ambatipudi et al. 2016; Breitling et al. 2011; Li et al. 2018). Studies have found that there exists a marked reversibility of methylation changes after smoking cessation at certain gene loci (Ambatipudi et al. 2016; McCartney et al. 2018; Wan et al. 2012; Wilson et al. 2017), whereas differential DNA methylation for certain other genomic locations remain unaffected even years (up to 22 years) after smoking cessation (Ambatipudi et al. 2016). In one of the most extensive studies suggesting the reversion of DNA methylation marks on smoking cessation, a team of investigators led by Ambatipudi observed that CpGs that were hypermethylated in current smokers showed decreased methylation with increasing time since smoking cessation and vice versa. In contrast, four CpG sites [cg01940273 (*ALPPL2*), cg05951221 (*ALPPL2*), cg11554391 (*AHRR*), and cg21566642 (*ALPPL2*)], were recognized during this study that did not show any decrease in the methylation levels even 14.1–22 years after smoking cessation (Ambatipudi et al. 2016). Wan et al. classified the CpG methylations into two categories—'rapidly reversible' and 'slowly reversible'—based on their differential methylation following

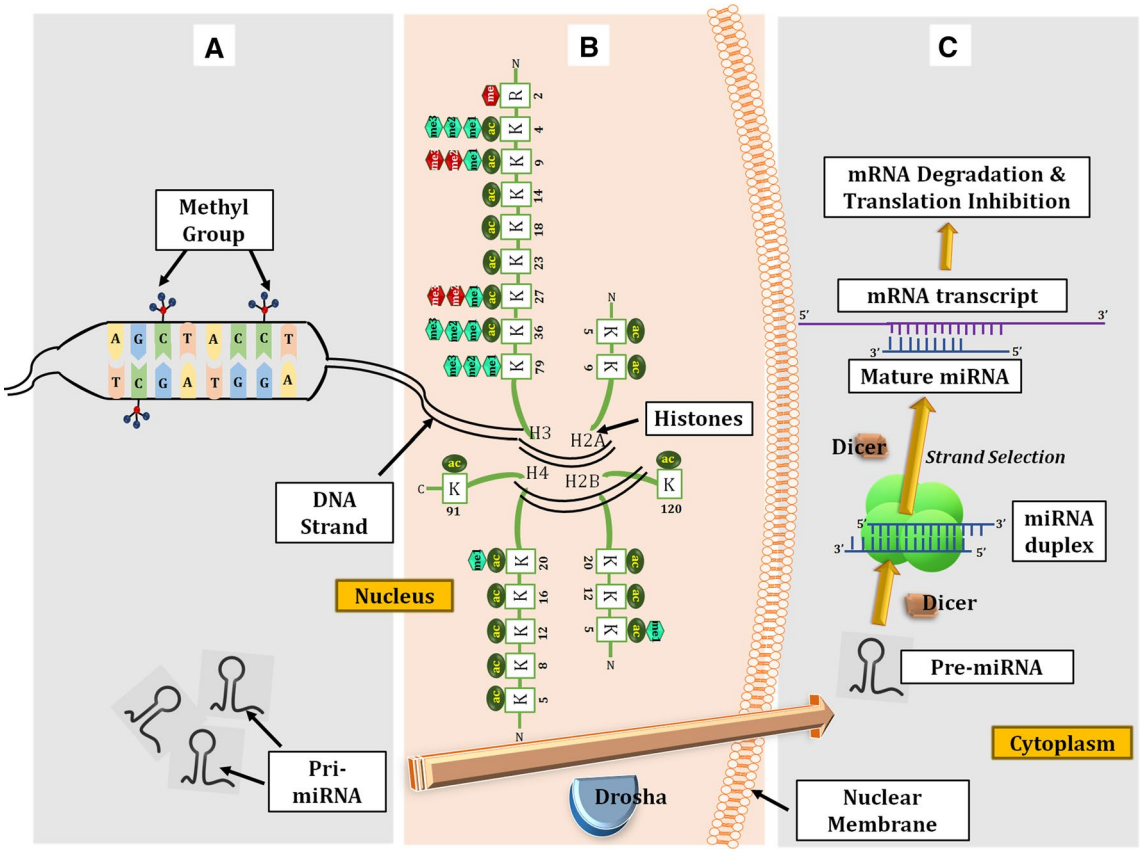

Fig. 2 Mechanisms of epigenetic regulation. The addition of a methyl ($–CH_3$) group to the cytosine residue on the DNA strand is termed as 'DNA methylation' which is the most widely studied epigenetic regulation. In general, it causes suppression of gene expression by hindering the binding of regulatory proteins onto the DNA strand (**a**). The second level of epigenetic regulation occurs at histone level by addition of chemical groups such as acetyl (ac), methyl (me), phosphoryl, ubiquitinyl, etc., to the lysine (K), arginine (R), serine or threonine residues. The figure depicts some of the major histone marks on histone 2A (H2A), 2B (H2B), 3 (H3), and 4 (H4) listing the position (numbers denoted at the bottom) and type of amino acid residue (K or R) and the nature of the modification [ac, me, me1 (mono-methylation), me2 (dimethylation), and me3 (trimethylation)]. The inducible histone modifications are depicted in green; while the repressive histone signatures are shown in red (**b**). MicroRNAs (miRNA) are the third level of epigenetic regulation within the cell. Like messenger RNAs (mRNA), miRNAs are also transcribed by RNA polymerase II. However, unlike mRNAs, the pri-miRNAs are translocated to the cytoplasm by the nuclear enzyme Drosha; where it is converted to miRNA by the action of Dicer enzyme as shown in the figure. The miRNA shows complementarity to the sequence of its target mRNA. On binding of the miRNA to the mRNA, a duplex is formed which prevents the binding of the translational machinery to the mature mRNA, and thus halts gene expression (**c**) (color figure online)

smoking cessation in the peripheral blood from current, former, and never smokers. The genes myosin light chain kinase (*MYLK*) and leucine-rich repeat neuronal 3 (*LRRN3*) were identified as 'rapidly-reversible' while *G-Protein-coupled receptor protein 15* (GPR15) and Fas ligand (FASLG) as 'slowly-reversible' CpG sites in this study (Wan et al. 2012). A slight contradiction to this view was provided in reports by Philibert et al. (2016) and Wilson et al. (2017), which showed that the magnitude of this reversion on smoking cessation is larger than normal individuals who have never smoked. This suggests overcorrection of DNA methylation marks on smoking cessation and adds another layer of complexity to the regulation of DNA methylation and gene expression in current and former smokers (Philibert et al. 2016; Wilson et al. 2017).

Despite our knowledge about the differential DNA methylation on smoke exposure, not many studies have been conducted to correlate the gene expression with CpG methylation changes. Most of the current investigations have been conducted using peripheral blood from smokers due to ease of sampling. However, DNA methylation changes on smoking may differ in multiple tissues (Hammons et al. 1999; Monick et al. 2012; Peters et al. 2007; Satta et al. 2008; Suzuki et al. 2007), which has not been examined in detail so far. Most of the current studies test genome-wide changes in DNA methylation signatures on smoking. The platforms and approaches used for each of these studies differ and so do the assumptions and statistics used to deduce outcomes. It, thus, becomes difficult to compare such genome-wide studies and find specific targets to serve as biomarkers or

Table 3 Enzymes involved in epigenetic regulation

Enzyme family	Name	Function	Effect on gene expression*
DNA methyltransferase (Jin and Robertson 2013; Kaneda et al. 2004; Milutinovic et al. 2004; Moore et al. 2013; UniProt 2019a)	DNMT1	Responsible for maintenance of DNA methylation Involved in DNA repair	Repression
	DNMT3A	Responsible for de novo DNA methylation Essential for genetic imprinting	Repression
	DNMT3B	Responsible for de novo DNA methylation	Repression
	DNMT3L	Functions in association with DNMT3A and 3B as it lacks the catalytic domain Required for genomic imprinting, retrotransposon methylation, compaction of the X chromosome	Repression
DNA demethylase (Moore et al. 2013; UniProt 2019b)	TET1, TET2, TET3	Mediates 5-methylcytosine (5mC) conversion into 5-hydroxymethylcytosine (5hmC)	Induction
Histone methyltransferase (Hyun et al. 2017)	SET1A, SET1B, SET7/9, MLL	Responsible for H3K4 methylation	Induction
	SUV39H1, SUV39H2, G9a	Responsible for H3K9 methylation	Repression
	EZH1, EZH2	Responsible for H3K27 methylation	Repression
	NSD1, NSD2, NSD3	Responsible for H3K36 methylation	Induction
	SUV4-20H1, SUV4-20H2	Responsible for H4K20 methylation	Repression
Histone demethylases (Hyun et al. 2017)	LSD1, LSD2	Responsible for H3K4 demethylation	Repression
	JHDM2, JHDM3	Responsible for H3K9 demethylation	Induction
	UTX, UTY	Responsible for H3K27 demethylation	Induction
	JHDM1, JHDM3	Responsible for H3K36 demethylation	Repression
	PHF8, PHF2	Responsible for H4K20 demethylation	Induction
Histone acetylation (HATs) (Carrozza et al. 2003)	GNATs MYST-related HATs p300/CBP HAT General transcription factor HATs Nuclear hormone-related HATs	Responsible to catalyze the transfer of an acetyl group from acetyl-CoA to the lysine 1-amino groups on the N-terminal tails of histones	Induction
Histone deacetylation (HDACs) (de Ruijter et al. 2003)	Class I (HDAC 1, 2, 3, and 8) Class II (HDAC 4, 5, 6, 7, 9, and 10) Class III (Sirutins) Class IV (HDAC 11)	Responsible for removal of acetyl groups from the histones	Repression

*This table shows the generally observed effect of the mentioned enzymes on gene expression

therapy. Finally, several of the EWAS investigations focus on the phenotypic outcome of cigarette smoking such as COPD, lung cancer, etc. (Carvalho et al. 2012; Fasanelli et al. 2015; Qiu et al. 2012), but they do not examine the effects of smoking per se. Such explorations are crucial to understand the molecular mechanisms affected on smoking and identifying potential biomarkers for early detection of various smoking-related disease outcomes (Lee and Pausova 2013).

Cigarette smoke-induced chromatin remodeling and changes in histone modifications

Histone modifications are chemical modifications in the form of methylation, acetylation, ubiquitination, and/or phosphorylation of specific amino acids [particularly lysine (Lys), serine (Ser), threonine (Thr), and tyrosine (Tyr)] on their N-terminal tail, which influence chromatin packaging and, in turn, transcriptional activity. *Inducible* histone modifications loosen the DNA association with histones, thus providing a permissive environment for transcription, whereas *repressive* histone modifications tighten the chromatin packaging, thus repressing gene expression. The inducible or repressive nature of a histone modification is determined based on the: (a) type of histone modification, (b) modified amino acid base, and (c) position of modification (Bannister and Kouzarides 2011; Moore et al. 2013), as depicted in Fig. 2b.

Of note, histone modifications not only regulate chromatin structure, but also recruit remodeling enzymes to reposition nucleosomes. Like DNA methylation, the addition/removal of chemical groups to histone tails is also catalyzed by a group of enzymes collectively termed

as histone-modifying enzymes (HMEs) (Bannister and Kouzarides 2011). The names and functions of some of the common HMEs are listed in Table 3.

The in vitro and in vivo studies have shown downregulation of histone deacetylase 2 (HDAC2) expression and activity in smoking-induced lung inflammation (Ito et al. 2001; Marwick et al. 2004; Moodie et al. 2004; Sundar and Rahman 2016). In fact, reduction in HDAC2 has been reported in the lung tissues from COPD and severe/smoking asthma patients as well, thus stating its role in the disease pathophysiology (Barnes 2009; Ito et al. 2002; 2005). Furthermore, HDAC1 expression is often found to be reduced in smoke-challenged cells and bronchial biopsies from asthmatics; which is not surprising as it is associated with HDAC2 in the nucleus (Adenuga et al. 2009; Ito et al. 2002). Function of other HDACs in smoke-related inflammation is uncertain (Barnes 2009). Additionally, the expression of histone acetyltransferases (HATs)—CREB-binding protein (CBP) and p300-CBP-associated factor (PCAF)—has been found to be unaltered in response to cigarette smoke challenge in vitro (Ito et al. 2001).

Reports suggest that inactivation of HDAC2 leads to increased acetylation at histone 3 (H3) and 4 (H4) in the lungs of smokers, COPD patients, cigarette smoke-exposed animals (rat), and cellular (A549) models (Ito et al. 2005; Marwick et al. 2004; Moodie et al. 2004; Szulakowski et al. 2006). However, most of these studies investigate global changes in the histone marks in association with smoking and do not provide much information about the site-specific regulation of gene transcription (Marwick et al. 2004).

Nevertheless, cigarette smoke exposure does not just alter the histone acetylation marks, but also affects other histone modifications. This was reported by Sundar et al. in smoke-exposed mouse lungs and human bronchial epithelial (H292) cells. Using a bottom-up mass spectrometry approach, this group identified acetylation at H3K56, H4K8, H4K12, and H4K16 in smoke-exposed mouse lungs and H292 cells as compared to the controls. In the same study, distinct site-specific histone post-translational modifications at H3K27me1, H3K27me2, H4K31me2, and H4R35me2 were found to be associated with smoke exposure. These findings suggest a strong link between chromatin compaction, replication, and transcriptional control in response to cigarette smoke challenge (Sundar et al. 2014).

Contrary to the abovementioned studies, our group undertook a targeted approach to study the changes in the histone signatures at the promoter site of NLRP10 (family of NOD-like proteins) gene in response to smoke challenge (Kaur et al. 2018). We demonstrated NLRP10-mediated caspase-1 activation, cytokine/chemokine production (IL-1β, IL-18, MCP-1, and IL-17A), and NF-κB and MAPKs expression in the lungs of second-hand smoke-exposed C57Bl/6 mice and cigarette smoke extract-challenged human lung epithelial cells with type II characteristics. To identify upstream mediators of NLRP10 regulation, we investigated changes in the epigenetic signatures on the *Nlrp10* promoter region following smoke exposure and observed significant changes in active (H3K4me3 and H3K9ac) as well as repressive (H3K9me3 and H4K20me3) gene markers on histone 3 and histone 4 both in vivo and in vitro. Furthermore, alterations in the respective histone acetyl- and methyltransferases (PCAF, SET1, ESET, SUV20H1) correlated well with the alterations observed in the histone markers (Kaur et al. 2018). Such targeted studies are important to understand the mechanism of regulation of inflammatory responses on smoke exposure at molecular level.

Despite all these evidences, establishing a direct link between altered expression of HATs/HDACs leading to chromatin remodeling and gene transcription at specific gene sites has many challenges (Marwick et al. 2004). The histone PTMs vary based on the duration of smoke exposure, type of cell/tissue exposed to smoke, and the experimental models used for the study; making it difficult to ascertain their exact role under physiological and disease state (Marwick et al. 2004; Sundar et al. 2014). Furthermore, there are contrasting evidences with regard to the expression and activity of various HDACs in smoke-related disease conditions (Ito et al. 2005; Sohal et al. 2013). The histone modifications are dynamic in nature and gene transcription is affected by a concerted effect of the histone modifications which adds to the complexity of studying such a phenomenon (Bannister and Kouzarides 2011; Sundar et al. 2014).

Smoke-associated alterations in miRNA expression

MicroRNA (miRNA) are a class of small endogenous RNAs approximately 22 nucleotides in length that play an important regulatory role by targeting specific mRNAs for degradation and/or translational repression in both animal and plant cells. These are single-stranded RNA molecules produced from hairpin-shaped precursors, also known as pri-miRNAs, by the action of two RNase III-type proteins: *Drosha* (in nucleus) and *Dicer* (in cytoplasm) (Wahid et al. 2010) (Fig. 2c).

There are currently a total of 1917 known mature human miRNAs in miR-Base, the central online repository for miRNA sequences and annotation, which accounts for 1–5% of all predicted human genes (Banerjee and Luettich 2012; Wang et al. 2015). Functionally, miRNAs regulate developmental processes, cellular homoeostasis, and responses to various stimuli by binding to a target mRNA and altering its protein expression. (Banerjee and Luettich 2012; Wahid et al. 2010).

Reports indicate distinct miRNA profiles among smokers and non-smokers (Advani et al. 2017; Andersson et al. 2018; Banerjee et al. 2015; Gross et al. 2014; Huang et al. 2014;

Shen et al. 2017; Wang et al. 2015; Willinger et al. 2017). In general, these studies have found differences in the expression of several miRNAs following exposure to smoke. Most of the dysregulated miRNAs have been found to be associated with lung development, airway epithelium differentiation, inflammation, and cancer (Banerjee and Luettich 2012; Wang et al. 2015; Willinger et al. 2017).

However, just like DNA methylation, changes in the miRNA profile appear to be reversible in nature (Ambatipudi et al. 2016; Wang et al. 2015). Wang et al. observed that 3 months after quitting smoking, 22 of the 34 differentially expressed miRNAs returned to their normal levels, including the miRNAs related to cancer/inflammation (miR-181a), airway epithelium differentiation (miR-449b), and lung development (miR-214 and miR-127). Interestingly, the remaining 12 differentially expressed miRNAs associated with smoking did not revert to their normal levels even after smoking cessation. Further, the miRNAs with sustained dysregulated expression are associated with carcinogenesis and chronic airway diseases (miR-218, miR-133a, miR-133b, miR-487b, and miR-1246) (Wang et al. 2015). Importantly, the Wnt/β-catenin signaling pathway was found to be significantly enriched in the target genes linked with persistently dysregulated miRNAs, which is in accordance with the previous knowledge that Wnt pathway dysregulation is associated with diseases, including lung cancer and COPD (Heijink et al. 2013; Konigshoff and Eickelberg 2010; Wang et al. 2015).

Currently, miRNAs are being targeted as candidates for biomarkers and drug discovery for various disease conditions (Banerjee and Luettich 2012; Janssen et al. 2013). However, certain considerations must be made before making advances in this respect. Smoking-related dysregulation of miRNAs is dependent on multiple factors including the dose of exposure, duration of exposure, and cell types exposed. Confounding factors such as age, race, and other epigenetic changes could also affect miRNA expression (Banerjee and Luettich 2012; Izzotti et al. 2009, 2011; Wang et al. 2015). To date, miRNA-based research has been exploratory in nature. The main challenge ahead is the need for a targeted approach to narrow down and identify specific candidates for biomarker or drug development in smoke-related diseases. Of note, several of the miRNAs identified during such studies are regulated by DNA methylation, which signifies the cross talk between the epigenetic signatures and their regulatory mechanisms (Lyn-Cook et al. 2014; Wang et al. 2015). In the future, a detailed investigation of the cross talk between different epigenetic mechanisms will be necessary to fully appreciate their therapeutic potential.

Effects of maternal smoking

Altered DNA methylation and dysregulated miRNA expression have also been assessed to identify transgenerational effects of maternal smoking (Breton et al. 2009; Herberth et al. 2014; Jenkins et al. 2017; Knopik et al. 2012; Suter et al. 2011). In this regard, studies by Suter et al. 2010 and 2011 showed significant hypomethylation in the placentas of babies born to mothers who smoked during pregnancy compared to that of non-smoking mothers. This hypomethylation was found to correlate with increased placental CYP1A1 expression, which may have implications for xenobiotic metabolism in the offspring (Suter et al. 2010; 2011). Likewise, hypermethylation of the brain-derived neurotrophic factor (*BDNF*) might be responsible for its lowered expression with subsequent behavioral consequences in infants, children, and adolescents exposed in utero to maternal cigarette smoking (Knopik et al. 2012).

While assessing the gender-specific methylation differences in offspring in relation to maternal smoking, Murphy et al. (2012) tested the methylation profiles of two imprinted genes, H19 and IGF2, in cord blood. The results from this study suggest that there is a more pronounced epigenetic effect of maternal smoking on male offspring than on females (Murphy et al. 2012). This is not surprising as sex differences in DNA methylation patterns have been previously reported among Dutch famine victims in response to caloric restriction (Heijmans et al. 2007). Other population-wide studies of human disasters have further revealed heightened phenotypic responses and risk among males (Catalano et al. 2005; Khashan et al. 2011). One of the criticisms of this paper, however, was that it only tested the methylation status at two different regions of the imprinted genes. Nevertheless, methylation still holds significance as it plays an essential role in regulating growth and its deregulation may lead to disease and disorder in the growing fetus (Murphy et al. 2012). Another group of researchers studied the DNA methylation status at six CpG sites on the IL-13 gene in peripheral blood leukocytes of offspring to study the correlation between maternal smoking and asthma-related lung function. While a strong correlation between differential methylation at the cg13566430 site and maternal smoking during pregnancy was established, disease outcome in the offspring remained unclear due to lack of data relating to asthmatic traits (Patil et al. 2013). However, Herberth et al. were able to establish an association between maternal tobacco smoke exposure and cord blood miR223 expression, which was responsible for reduced Treg numbers, suggesting increased allergy risk in offsprings later in life (Herberth et al. 2014). Additionally, a recent study by Richmond et al. proved that maternal smoking during pregnancy is associated with persistent alterations in DNA methylation in the exposed offspring. These changes in the DNA methylation pattern were observed to exist even 18 years after

prenatal exposure (Richmond et al. 2018). Overall, it can be concluded that changes in DNA methylation signatures and the miRNA profile caused by maternal smoking may not only increase disease susceptibilities in exposed offspring but are also transmitted to the next generation.

Effects of paternal smoking

Interestingly, the intergenerational effects of smoking are not limited to the smoking habits of mothers. Jenkins et al. identified 141 differentially methylated CpGs in the DNA of sperm from men who smoke compared with non-smokers. The differential methylation occurred more frequently at regions reported to display H3K4 and H3K27 methylation in mature spermatozoa (Jenkins et al. 2017). Functionally, H3K4 methylation is associated with gene activation while methylation at H3K27 is a gene repression signature. But despite opposite functions both these modifications are responsible for development, lineage commitment, and differentiation (Eissenberg and Shilatifard 2010; Nichol et al. 2016). These changes could not only account for reduced sperm count and motility in men who smoke, but also affect the fetal development of their offspring at later stages (Gunes et al. 2018). Another study tested the sperm quality of smoking and non-smoking males and reported differential miRNA expression in the spermatozoa from smokers vs. non-smokers. In fact, four (hsa-miR-146b-5p, hsa-miR-509-5p, hsa-miR-519d, and hsa-miR-652) of the differentially methylated miRNAs identified in this study are known to be altered in infertile men, thus suggesting that smoking might be associated with male sterility. Moreover, the major pathways affected by these differentially expressed miRNAs are known to be involved in cell differentiation, proliferation, and death. Thus, these pathways likely play a vital role during sperm and early embryo development (Marczylo et al. 2012). In a similar type of study, Hamad and his group used a whole-genome DNA methylation assay to study the differences in global DNA methylation among smokers and non-smokers. The results of this study revealed a significant increase in the levels of global DNA methylation in the sperm of smokers. Previous work showed that the production of low quality and apoptotic spermatozoa could be linked to altered spermatogenesis that resulted in global DNA hypermethylation (Hamad et al. 2018). These findings further suggest that global DNA methylation might affect normal spermatogenesis and thereby affect male fertility as well as the future progeny of men who smoke.

Challenges and ethical concerns related to epigenetic research

Despite the great potential of epigenetic research about the future of medicine, the research community is currently unable to tap its full potential. Hence, it is important to discuss the challenges and ethical concerns hindering advances in this area. While some of the challenges and ethical concerns may be directly encountered during research pertaining to smoke-related epigenetic alterations, most of these challenges are more generic in nature and are not restricted to any one disease condition.

Challenges

Study of the human epigenome is still in its infancy, as we are just beginning to understand the complexities of epigenetic signatures in disease and development (Zheleznyakova et al. 2017). Several challenges lie ahead on this venture of exploring the full potential of the epigenome. First, while there is only one genome in all individuals of a species, the epigenome exhibits tissue-specific variations (Costa et al. 2016; de Vries et al. 2018a; Leng et al. 2015). In this regard, it should be noted that most previous epigenetic studies were conducted using whole blood, which itself is comprised of multiple cell types. Therefore, it is likely that the epigenetic alterations detected in mixed tissues/whole blood arise from differences in cell composition between tissues/samples from diseased and control subjects (Weinhold 2006; Zheleznyakova et al. 2017). Thus, future experiments will be required to further assess tissue-specific differences in the epigenome.

Additionally, an individual's epigenetic make-up could be affected by multiple confounding factors such as age, genetic background, environmental exposure, clinical conditions, etc. Such confounders cause discordance and discrepancies while comparing similar epigenetic cohort studies, thus affecting the end deductions (Knopik et al. 2012; Zheleznyakova et al. 2017). During our study, we found several instances where inclusion of confounding factors altered the study results completely. A good example to this is the contradictions in the findings by Qiu et al. (2012) and de Vries et al. (2018b) that have been explained earlier in this review.

Additional technical difficulties are known to arise when conducting epigenetic research. For example, bisulfite sequencing is the most extensively used technique to study DNA methylation. In fact, most of the studies listed in this review used this technique to study DNA methylation alterations with respect to smoke (Breitling et al. 2011; de Vries et al. 2018a; Prince et al. 2019; Sundar et al. 2017). However, bisulfite sequencing cannot differentiate between 5mC and 5hmC. This raises the possibility that the observed DNA methylation changes could be an overrepresentation due to the lack of assay specificity (Zheleznyakova et al. 2017).

In addition, few biological/chemical tools, such as antibodies and selective inhibitors or activators, exist to study epigenetic changes in vitro and in vivo. Thus, this limits the scope of understanding of this phenomenon. Currently

scientists primarily use genetic association combined with molecular tools, including gene silencing, protein overexpression, and catalytic-inactive mutants, to determine relevant disease targets. However, each of these approaches has certain caveats which make them less than ideal (Campbell and Tummino 2014). We ourselves ran into this challenge when attempting to identify the HDACs/HATs responsible for histone modifications in vitro and in vivo (Kaur et al. 2018). We attempted to target the trimethylation on histone 3 and 4 during this study; however, finding an antibody that specifically binds to trimethyl and not mono- or di-methyl on histones remains a struggle.

Finally, improvements to overcome batch variability and to nullify the effects of confounding factors are needed in high-throughput technologies. These include computational capability, analytical techniques, mechanistic studies, and bioinformatic strategies (Campbell and Tummino 2014; Weinhold 2006).

Ethical concerns

Epigenetic research has largely been kept from the forefront of the drug development process due to ethical issues associated with the gathering of patient information. For example, it is well known that environmental factors including diet and exposure to chemical toxicants such as pesticides, diesel exhaust, and tobacco smoke increase disease risks; however, such exposures are frequently linked to poverty, standard of living, and working conditions of the exposed individuals, which places the onus on law and policymakers. Evidences related to epigenetic effects, including transgenerational effects, suggest that some individuals are predisposed to be more affected by adverse environmental conditions than other individuals. Thus, the focus shifts from populations with greater susceptibility to those receiving disproportionate exposure, thus resulting in calls for environmental justice advocates to address these injustices (Rothstein et al. 2009). Something that is important to mention here is the fact that during this literature review we found that most population-based studies were conducted on Caucasians (Ambatipudi et al. 2016; McCartney et al. 2018; Wan et al. 2012; Wilson et al. 2017). However, it is well known that some population is clearly predisposed to pulmonary health problems like COPD caused by smoking (El-Zein et al. 2012). Study of only specific population groups should thus be avoided both in research and during drug development, but it is something that is not practiced.

Another important aspect of extensive, ongoing epigenetic studies is the generation of a wealth of sensitive information regarding future health issues in patients and the possibility of transmitting those risks to offspring. Our investigations of maternal/paternal smoking clearly showed that offspring inherit not just genes, but also epigenes (Breton et al. 2009; Jenkins et al. 2017; Joubert et al. 2014; Marczylo et al. 2012). However, epigenes are not often considered, and thus, neither are the privacy and confidentiality issues surrounding such information. Another factor to consider is that unlike our genetic information, epigenetic effects are environmentally induced and might also be reversed, as seen in the case of smoke-induced DNA methylation and miRNA alterations (Ambatipudi et al. 2016; Wang et al. 2015). Though relevant, such questions have not yet been addressed (Rothstein et al. 2009; Shabani et al. 2018).

Related to the abovementioned challenges is the issue of equitable access to health care. Regarding genetics, both public and private providers are reluctant to approve various clinical genetic services on the grounds of these being experimental and not medically essential. Considering this, the success and popularity of epigenetic testing seems uncertain. Particularly, the issue of access to healthcare will be critical for individuals likely to work and live in hazardous environments (Rothstein et al. 2009). Moreover, the current medical system is treatment oriented and does not encourage means of disease prevention, which is where the true potential of epigenetic research lies.

Advancements in epigenetic studies have raised awareness of intergenerational equity and thereby broadened the scope of our duties to future generations. Ardent supporters of scientific advancements might believe that measures to prevent the transmission of epigenetically harmful signatures to future generations must be encouraged; however, critics may argue that this would interfere with the natural order of things (Rothstein et al. 2009). Thus, future policies must consider these concerns so that epigenetic information can be utilized as a diagnostic tool or treatment method for life-debilitating disease.

Discussion

Systematic review of the literature to identify epigenetic alterations on cigarette smoke exposure revealed heterogeneous results. Our search criteria identified 80 studies focusing mainly on 3 of the widely known epigenetic mechanisms, DNA methylation (35 studies), histone modifications/chromatin remodeling (11 studies), and miRNA expression (12 studies). Among these, most of the studies were conducted using human and/or clinical samples (69 studies); while 9 were employed in vitro and 8 used in vivo study models to identify epigenetic changes. Considerable evidence is available in the literature suggesting that cigarette smoking regulates DNA methylation signatures. In fact, several genes which have been commonly cited in multiple studies (e.g., *AHRR, p16, F2RL3, and DAPK*) could be developed as biomarkers to assess disease progression. These studies have several merits including large sample size, correlation

with confounding factors such as smoking status; smoking history, smoking cessation, robust approach, and efficient summary statistics. Likewise, miRNA expression studies also have great potential to identify probable biomarkers and drug targets. However, unlike DNA methylation, other epigenetic markers have not been extensively investigated.

The major criticism of epigenetic studies related to smoke-associated diseases is the heterogeneity of sample size, sample type, and assessment techniques used to determine epigenetic alterations. Further, non-inclusion of comorbidities and factors such as demographics, genetic variations, BMI, and other health determinants for statistical corrections, and the lack of evidence regarding the consequences of the observed changes are the major drawbacks of these studies. In this context, three studies included in this review compared the DNA methylation alterations among smokers based on their demographics and provided contrasting outcomes. The study conducted by Zhu et al. measured methylation marks at > 485,000 CpGs in current, former, and never smokers from a Chinese cohort and compared their results to previous studies conducted on Europeans and African Americans. This group identified 161 CpGs annotated to 123 genes that were not associated with smoking in Europeans or African Americans and concluded that these sites were specific to the Chinese population (Zhu et al. 2016). Sun et al. conducted a methylome-wide study using 972 African Americans to identify DNA methylation sites associated with smoking in this population and compared their results with previous work done in Caucasians. They concluded that the two ethnic groups share common associations with cigarette smoking despite their distinct genetic backgrounds (Sun et al. 2013). While both of these studies compared their observation with data from a previously published study to draw conclusions, Elliot et al. performed a methylome-wide study in a population-based cohort including 1711 first-generation South Asian migrants and 1762 people of European origin aged 40–69 living in West London, UK. In this study, they found distinct smoking-associated methylation changes at both single CpG sites and overall smoking score (constructed based on methylation profile) based on ethnicity (Elliott et al. 2014). While most of the DNA methylation studies have been conducted in European populations, the studies conducted in other populations have not considered demographic differences in their results. Thus, there exists a wide knowledge gap with regard to the effect of genes and epigenes on smoking-associated disease susceptibilities among various populations. The abovementioned examples highlight the importance of inclusion of confounding factors like ethnicity in the DNA methylation studies. Future studies, thus, need to address these gaps to draw more meaningful conclusions.

Since a wide variety of cells/tissue samples have been used in epigenetic studies to deduce the effects of smoking, it is challenging to identify common epigenetic signatures and associated molecular mechanisms as these changes are predominantly tissue specific (Gutierrez-Arcelus et al. 2015). The information about various types of cellular and tissue models used in individual epigenetic studies is included in Tables 1 and 2. Likewise, it is difficult to compare the information about epigenetic changes from different studies due to the measurement of different variables (DNA methylation, histone modification or miRNA) in terms of individual signatures. While the studies included in our systematic review identify the top candidate genes, chromatin modifications, and miRNAs altered during smoke exposure, none of them explored the interconnections between various epigenetic markers. In future, such associations must be explored to tap the full potential of epigenetics in deducing disease mechanisms and developing therapies.

Conclusions

The evidence presented in this systematic review is suggestive of a vital role of epigenetic changes in regulating smoking-associated alterations and disease development. Better study design to interconnect different epigenetic marks, inclusion of confounding factors, and association with disease outcomes could improve the quality of future research.

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Compliance with ethical standards

Conflict of interest The employment affiliation of the authors is shown on the cover page of the manuscript. The authors declare no conflict of interest. All the authors participated in the study design and interpretation of the findings. We declare that none of the authors have participated in any regulatory or legal proceedings related to the contents of this paper.

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