Oxidative Stress and the Epigenome in Human Disease

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Abstract
Epigenetics refers to the study of the changes in gene expression that occur without changes in the DNA sequence. There is growing evidence that epigenetic modifications such as changes in the levels of DNA methylation or post-translational histone modifications are involved in the pathogenesis of many human diseases including cancer. Oxidative stress as a result of metabolic or environmental factors leads to excessive production of reactive oxygen species (ROS). ROS plays a role in many human diseases including cancer and pulmonary and cardiovascular diseases by promoting DNA damage and/or altering signaling pathways. This review article summarizes the most recent reports linking both oxidative stress and epigenetic mechanisms in the pathogenesis of chronic obstructive pulmonary disease (COPD), cardiovascular disease, lung, prostate and colorectal cancers. Here, we emphasize the importance that future studies should focus on epigenetic intervention strategies to treat diseases associated with oxidative stress.

Keywords
Oxidative stress; Reactive oxygen species; DNA methylation; Post-translational histone modifications; Chronic obstructive pulmonary disease; Atherosclerosis; Non-small cell lung cancer; Prostate cancer; Colorectal cancer

Introduction
The term “oxidative stress” refers to the state of a cell characterized by an imbalance between the production of reactive oxygen species (ROS) and the cell’s detoxification defense system, favoring a ROS-rich environment and/or reduced antioxidant reserves. Under normal circumstances, ROS are produced during physiological processes, such as cellular respiration, the activation of the arachidonic acid cascade and by enzymes, including cytochrome p450, nicotinamide adenine dinucleotide (NADH)/Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase and nitric oxide synthase. Oxidative stress is associated with numerous medical conditions, including pulmonary and cardiovascular diseases, as well as cancer.

At ground state, molecular oxygen (O2), required for aerobic metabolism, has two unpaired electrons, and can readily accept others. The mitochondria’s electron transport chain uses O2 as a terminal acceptor for the electrons from NADH and generates a proton motive force. However, if leakage of electrons occurs, O2 is rapidly reduced to the superoxide anion (O2−). Although O2− is not reactive itself, it can initiate the generation of ROS. Thus, it is necessary that cells have effective mechanisms for removing O2− and ROS. The generation of ROS may cause a wide range of DNA lesions including base modifications, deletions, strand breakage, and chromosomal rearrangements [1,2]. Oxidative stress can either drive genetic mutations or epigenetically regulate the expression of genes. The cellular antioxidants must respond to an overproduction of ROS before these highly reactive molecules adversely alter cellular structures, including DNA, proteins, and lipids. Severe oxidative stress may trigger apoptosis, necrosis, and cell death.

ROS are detoxified within the cell by several kinds of antioxidants [3]. Examples of endogenous defenses against ROS include the antioxidant enzymes glutathione-S-transferase P (GSTP1), glutathione peroxidase, catalase, superoxide dismutase (SOD), peroxiredoxin, and sulfiredoxin [3]. Examples of low molecular weight antioxidants include: glutathione, vitamin C, Vitamin A, and vitamin E [3]. Humans have three separate superoxide dismutases to reduce the O2− from the cell: the cytoplasmic Cu/Zn SOD1, the mitochondrial manganese SOD2, and the extracellular SOD3 enzyme.

Most of the chromatin in mammalian cells exists in a condensed, transcriptionally silent heterochromatic form. Euchromatin is less condensed, and contains most of the actively transcribed genes. Epigenetics refers to the study of a stably heritable phenotype that results from changes in the chromatin that alter gene expression without alterations in the DNA sequence [4]. DNA and histone proteins can be chemically modified with epigenetic marks that alter the electrostatic nature of the chromatin or alter the affinity of chromatin-binding proteins. The chromatin structure, or the “epigenome”, is regulated by a large number non-coding RNAs and histone-modifying and DNA methylation enzymes [5]. The three major mechanisms of epigenetic regulation include DNA methylation, post-translational histone modifications, and non-coding RNAs including micro RNAs.

DNA methylation plays an important role in embryonic development, genomic imprinting, X-chromosome inactivation, and the preservation of chromosome stability. DNA methylation at the promoter region of genes is associated with repression of gene transcription by maintaining the chromatin in a closed state [6]. During DNA methylation a methyl group is added to the carbon-5 position of the cytosine pyrimidine ring by DNA methyltransferases...
to form 5-methylcytosine (5-MeC) [6]. The chromatin is maintained in a closed state by recruitment of the methyl-CpG-binding-domain protein complexes that also contain HDACs that remove acetyl groups from the histone’s N-terminal domains and keep the chromatin in a closed configuration making the chromatin inaccessible to transcription factors and co-activators [7,8]. A family of DNA methyltransferase enzymes (DNMTs) is involved in de novo DNA methylation and methylation maintenance. DNMT1 is predominantly responsible for maintaining cellular levels of CpG methylation whereas DNMT3A and DNMT3B are critical for de novo methylation during embryogenesis [9]. The absence of 5-MeC in DNA promoters allows acetylation of histones permitting a number of transcription factor complexes to access the chromatin and promote transcription of a specific genomic region [8].

- Post-translational histone modifications include lysine acetylation, arginine and lysine methylation, serine phosphorylation, and lysine ubiquitination, and sumoylation. Lysine acetylation is usually associated with transcriptional activation but the functional consequences of lysine and arginine methylation depend on the specific site of the residue within the histone tail [10-13]. For example, methylation of histone H3 at lysine 4 is linked to transcriptional activation, whereas methylation of histone H3 at lysine 9 or lysine 27 is associated with transcriptional repression [11-13]. The post-translational histone modifications allow the chromatin to have a dynamic structure and constitute the docking site for distinct chromatin-binding proteins; for example, the histone acetyltransferases (HATs) and their counterpart, the histone deacetylases (HDACs), or the histone methyltransferases (HMTases), and their opposite, the histone demethylases, direct between a transcriptionally active or transcriptionally silent chromatin [14]. The “histone code” is now also widely accepted and states that specific histone modifications on the same or different histone tails act sequentially or in combination regulate the expression of a specific region within the chromatin [15]. Dysregulated histone post-translational modifications have been shown to be important in both predictive and prognostic value in various diseases such as cancer [16-21].

- Non-coding RNAs (ncRNAs) have changed the view of the “central dogma” in that these play fundamental roles in regulating protein levels by modulating transcription and translation to either ultimately increase or decrease protein levels. Small ncRNAs include PIWI-interacting RNAs (piRNAs), transition initiation RNAs (tiRNAs), and microRNAs (miRNAs). Mid-size ncRNAs include small nucleolar RNAs (snoRNAs), promoter upstream transcripts (PROMPTS), and transcription start sites (TSS)-associated RNAs (TIARs). Long ncRNAs include the ultra-conserved regions (U-CRUs) and long intergenic ncRNAs (linncRNAs)[22]. miRNAs can regulate downstream gene expression by binding to the 3’ untranslated region (UTR) of a mRNA resulting in mRNA degradation and translational repression [22,23]. Long-ncRNAs are mostly known to modulate the chromatin structure, and thus change DNA condensation, resulting in less transcription [22]. Small and mid-size ncRNA regulate transcription and translation. Some long ncRNAs even regulate the expression of other microRNAs.

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Cardiovascular Disease

Cardiovascular diseases are the leading cause of death in industrialized nations [32]. There is a growing body of evidence suggesting that both epigenetic modifications and oxidative stress may play a role in the pathogenesis of many cardiovascular diseases.

- Nitric oxide synthases (NOSs) play an important role in cardiovascular diseases and are known to be epigenetically modulated. Nitric Oxide (NO) plays an important cardioprotective role against cardiovascular diseases by regulating blood pressure, vascular tone, and inhibiting platelet aggregation and leukocyte adhesion. NO is produced by three isoforms of NOS encoded by separate genes on different chromosomes: neuronal NOS (NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3). eNOS is constitutively expressed and responsible for the majority of NOS produced by the vascular endothelium and, therefore, represents the major source of bioactive NO. Methylation plays a role in the expression of the various forms of NOS. For example, iNOS is expressed in atherosclerotic plaque, but repressed by methylation in most tissues. The reaction of NO with superoxide forms peroxynitrite and decreases NO bioavailability, which enhances cellular oxidative stress. Peroxynitrite increases endothelial dysfunction and stimulates prothrombotic effects such as increased platelet reactivity and lipid peroxidation. Inactivation of NO by ROS is recognized as a key mechanism underlying the reduced NO availability and the development of endothelial dysfunction, which may be an important contributor to disease pathophysiology.

Cancer

The “epigenetic progenitor” model of human cancer proposed by Feinberg, Ohlsson, and Henikoff states that epigenetic changes in gene expression impact carcinogenesis through aberrant silencing of tumor suppressors genes and the improper activation of oncogenes [33]. Further epigenetic derangements and genetic mutations are acquired as this epigenetically altered progenitor population expands, ultimately leading to carcinogenesis.

- Oxidative stress has been clearly linked to the development of various cancers. Oncogenic-driven cancer cells generate increased ROS as byproducts of their augmented metabolism to promote and maintain tumorigenicity [34-36]. Since high levels of ROS can induce cell death, cancer cells adapt to ROS stress by upregulating intracellular antioxidant proteins in order to maintain ROS levels that is characterized as a group of disorders with similar respiratory symptoms, including cough, sputum production, systemic inflammation, obstruction of lung airflow, and decreases in respiratory function [25]. COPD patients also have an increased risk of developing lung cancer.

Cigarette smoke contains a number of free radicals and chemical compounds, representing the major source of inhaled ROS leading to the deregulated expression of pro-inflammatory genes [26,27]. Recently it was found that cigarette smoke post-translationally modifies histone deacetylase 2 (HDAC2), a class I histone deacetylase, resulting in a reduction of its enzymatic activity [26]. A smoke-dependent HDAC2 inactivation by post-translational phosphorylation via casein protein kinase 2 (CK2) was also reported in macrophages, human bronchial and primary small airway lung epithelial cells and, in vivo, in the mouse lung [28]. The inactivation by phosphorylation of HDAC2 results in its ubiquitination and proteosomal degradation. In COPD patients, inflammation and cellular senescence are exacerbated by tobacco smoke [25]. A decreased HDAC2 activity has been associated with inflammation and senescence in COPD patients resulting in an increase in H3 and H4 acetylation, the activation of nuclear factor of kappa light polypeptide gene enhancer in B-cells (NF-κB) transcription factor, and deregulated expression of proinflammatory genes [28-30]. Levels of the NAD (+) dependent histone deacetylase sirtuin 1 (SIRT1) have also been shown to be reduced in patients with COPD, further demonstrating that HDAC expression and oxidative stress is associated with COPD [31].

Chronic Obstructive Pulmonary Disease (COPD)

The lungs are exposed to numerous sources of endogenous and exogenous sources of oxidants derived from mitochondrial respiration, phagocyte activation, air pollutants, noxious gases, and cigarette smoking [24]. Chronic obstructive pulmonary disease (COPD) represents the fourth cause of mortality worldwide, and
allow protumorigenic signaling without resulting in cell death [37–42]. In fact, studies have shown that disabling antioxidant mechanisms triggers ROS-mediated cell death in many forms of human cancers [43–46]. Increasing evidence also has linked the regulation of many pathways associated the homeostasis of oxidative stress to epigenetic mechanisms [47].

Lung cancer is the leading cause of cancer deaths worldwide and only 13% of lung cancer patients survive more than 5 years [47]. Non-small cell lung cancers (NSCLCs) represent 80% of all lung cancers and are often diagnosed at an advanced stage with poor prognosis. SOD1 has been shown to be over expressed at higher molecular levels in lung adenocarcinomas, a subtype of NSCLC [48]. SOD1 converts superoxide to hydrogen peroxide (H2O2) and molecular oxygen, the cytosol, the nucleus, and the intermembrane space of the mitochondria. SOD1 protects the cell from oxidative stress and subsequent cell death by maintaining low levels of superoxide in the cytosol. A more recent study reported that inhibition of SOD1 by the small molecule ATN-224 reduced tumor burden in a mouse model of NSCLC suggesting a potential clinical application for the treatment of patients with various forms of NSCLC [49]. ATN-224-dependent SOD1 inhibition in various NSCLC cells increased superoxide, diminished the enzyme activity of the antioxidant glutathione peroxidase, and increased intracellular levels of H2O2.

Elevated levels of HDAC1 mRNA have been reported in more advanced stages of this disease (Stage III or IV) [31,50]. Murine switch-independent 3-associated (mSin3A), a critical scaffold on which the multi-component HDAC co-repressor complex assembles, has also been reported to have decreased expression in NSCLC [31,50]. Additionally, the ATP-dependent SWI/SNF chromatin remodeling complexes members have been reported to be dysregulated in NSCLC [31,50]. In NSCLC, mutations are also found within the lysine acetyltransferase KAT3A in a subset of patients and polymorphisms have been identified which are associated with an increased risk for lung cancer including the lysine methyltransferases KMT1B and KMT8 [31]. Polymorphisms in the methyl-CpG binding domain 1 (MBD-1) have been associated with an increased risk of developing lung cancer [31].

In the United States, prostate cancer (CaP) is the most commonly diagnosed non-skin cancer and the second-leading cause of cancer deaths [51]. Several studies have reported decreased levels of Erythroid 2p45 (NF-E2)-related factor 2 (NRF2) and members of the glutathione-S-transferase (GST) mu family in human CaP [52]. NRF2 is a basic-region leucine zipper (bZIP) transcription factor that regulates the expression of phase II detoxifying/antioxidant enzymes, including glutathione-S-transferase (GST), UDP-glucuronosyltransferase (UGT), hemeoxygenase-1 (HO-1), NADPH: quinone oxidoreductase (NQO), glutamate cysteine ligase (CGL) and gamma glutamylcysteine synthase (yGCS), by binding in combination with small Maf proteins to antioxidant response elements (AREs) in promoter regions [53]. The expression of NRF2 in prostate tumors from TRAMP mice has also been shown to be suppressed epigenetically by promoter CpG methylation and histone modifications [54]. The treatment of TRAMP cells with the epigenetic methylation inhibitor, 5-aza-2-deoxycytidine (5-aza-dC), and the histone deacetylase inhibitor trichostatin A (TSA) restored NRF2 expression and increased the expression of NRF2 and its downstream antioxidant and detoxification enzymes [54,55]. Three specific CpG sites in the NRF2 promoter were found to be hypermethylated in clinical CaP samples [56]. CpG sites showed methylation that inhibited the transcriptional activity of NRF2 in LNCap cells but LNCap cells treated with 5-aza/TSA restored the expression of NRF2 and its downstream target genes, decreased expression levels of DNMT1 and HDAC proteins, increased RNA Pol II and H3Ac, and decreased H3K9me3, MBD2, and MeCP2 at CpG sites of the human NRF2 promoter [56]. Moreover, the expression and activity of SOD, catalase, and GPx have also been found to be decreased in plasma, erythrocytes, and CaP tissues confirming the role of NRF2 and its target genes in controlling oxidative stress in CaP and confirming the existence of an epigenetic mechanism involved in its regulation [57,58].

A recent study reported that ROS silenced the tumor suppressor, RUNX3, by epigenetic regulation and may be associated with the progression of colorectal cancer [59]. The runt-domain transcription factor 3 (RUNX3) is known to be a tumor suppressor involved in various cancers, including gastric cancers [60–63]. Approximately 45–60% of human gastric cancers have been reported to display loss of RUNX3 expression [64]. The Kang et al. [59] study reported that RUNX3 mRNA and protein expressions were down-regulated in response to H2O2 in the SNU-407 human colorectal cancer cell line. H2O2 treatment increased RUNX3 promoter methylation and the ROS scavenger, N-acetylcysteine (NAC) and 5-aza-dC, decreased it. The downregulation in methylation may open the door to targeting epigenetic mechanisms of NAC. 5-aza-dC treatment prevented the decrease in RUNX3 mRNA and protein levels by H2O2 treatment. Additionally, this same study also reported that H2O2 treatment resulted in DNMT1 and HDAC1 up-regulation with increased expression and activity, increased binding of DNMT1 to HDAC1, and increased DNMT1 binding to the RUNX3 promoter. H2O2 treatment also inhibited the nuclear localization of RUNX3, which was also abolished by NAC treatment. When RUNX3 is translocated to the nucleus it acts as a tumor suppressor; however, cytoplasmic RUNX3 does not elicit tumor suppressor activity [65].

DNA methylation and down-regulation of CDX1 has been observed in a number of colorectal carcinoma derived cell lines and in patient samples. The Zhang et. al. [66] study examined whether oxidative stress regulated the expression of the cecal type homeobox-1 (CDX1) tumor suppressor gene in colorectal cancer cells [66]. The results of the study suggested that silencing of CDX1 expression by oxidative stress in colorectal cancer cells may be mediated by epigenetic mechanisms. Additionally, treatment with H2O2 down regulated CDX1 mRNA level and protein expression in the T-84 human colorectal cancer cell line. The down regulation of CDX1 at the mRNA and protein level induced by H2O2 was further abolished by separate treatment of either NAC or 5-aza-dC. Treatment with H2O2 also increased CDX1 promoter methylation and 5-aza-dC reversed this effect. In this same study, H2O2 also induced the up regulation of DNMT1 and HDAC1 expression and activity.

ROS induced by DNA hypomethylation is an important factor for the progression of genomic instability and is, in turn, a source of ROS accumulation. One of the main causes of genomic instability is thought to be a result of alterations in oxygen metabolism which can give rise to increased levels of ROS. Genomic instability arises in a few cells capable of sustaining the ROS production. These cells accumulate further changes possibly due to epigenetic factors and to gene mutations induced by the high ROS levels, acquire selective advantage and can proliferate, even with their genomic instability. Pregeny of these cells may exhibit memory of genome changes that can lead to a transformed phenotype [67,68].

Conclusions

Oxidative stress, as a consequence of ROS accumulation, increases exponentially with age, in parallel with a decline in the cell repair machinery, resulting in many diseases associated with aging including cancer and respiratory and cardiovascular diseases [69]. It is possible that targeting epigenetic regulators may be an important new therapeutic avenue for suppressing oxidative stress in cancer and other human diseases. A fundamental question now in the field of epigenetics is to understand the biochemical mechanisms underlying ROS-dependent regulation of epigenetic modification, which may open the door to identifying new therapeutic modalities. For example, in oncology, further studies of the epigenetic mark profiles from primary tumor samples will provide important information on the role of methylation of the CpG islands or other epigenetic marks in the promoter regions of tumor suppressor genes. Deciphering the methylation status of tumor suppressor genes may contribute to the regulation of the transcriptional activity of tumor suppressor genes,
which could be used in cancer preventive and therapeutic treatment.

References


