American Journal of Medical Science and Innovation (AJMSI)

ISSN: 2836-8509 (ONLINE)

VOLUME 4 ISSUE 2 (2025)

PUBLISHED BY
E-PALLI PUBLISHERS, DELAWARE, USA

American Journal of Medical Science and Innovation (AJMSI)

Volume 4 Issue 2, Year 2025
ISSN: 2836-8509 (Online)
DOI: https://doi.org/10.54536/ajmsi.v4i2.6400
https://journals.e-palli.com/home/index.php/ajmsi

Molecular Mechanisms Linking Tobacco Smoke to DNA Damage and Carcinogenesis

Pritylata Mandal[1*], Md Rawfur Rahim[2], Effat Ara[3], Md. Saiful Islam[4], Md Mehedi Hasan[5]

Article Information

Received: October 22, 2025
Accepted: November 25, 2025
Published: December 26, 2025

Keywords

Carcinogenesis, DNA Damage, MicroRNA, Oxidative Stress, Tobacco Smoke

ABSTRACT

Although tobacco smoke is a powerful environmental carcinogen, the molecular pathways through which its components cause DNA damage and cancer have not been completely defined. To elucidate mechanisms of tobacco smoke extract (TSE) induced genotoxicity, human bronchial epithelial and lung carcinoma cells were treated with TSE. Exposure to TSE resulted in dose-dependent elevation of reactive oxygen species and lipid peroxidation, which were followed by the attenuation of antioxidant defense. Comet assays and γ-H2AX foci formation showed a marked DNA strand break, and the key genes and proteins of DNA repair (OGG1, XRCC1, XPA, ERCC1) were under expressed compared to normal levels indicating lowering repair capability. Epigenetic analyses revealed an increase in overall DNA methylation and induction of tumor inhibitor genes by promoter hypermethylation (p16^INK4a, RASSF1A, MGMT) and oncomiR activation (miR-21 overexpression), coupled with a loss of expression of other anti-oncogenic miRs including miR-34a/miR-200c. The apoptotic pathway was also initiated as revealed by upregulation of p53 and an increased ratio of Bax to Bcl-2. Overall, these results expose a mechanistic cascade whereby chronic oxidative stress DNA damage, repair inhibition and epigenetic disruption cooperate to drive cancer development. The research highlights the importance of preventive measures, early biomarker detection and tailored antismoking intervention to reduce cancer risk from tobacco use.

INTRODUCTION

Cigarette smoking persists among the most imperative preventable causes of sickness and premature death global (with more than 8 million deaths each year) (Gallucci *et al.,* 2020). However, despite extensive efforts to notify individuals and costly tobacco control measures, smoking still remains a major worldwide public health issue (West, 2017). Cigarette smoke is a very intricate assortment with over 7,000 chemical mixtures, including at least 70 known carcinogens and heavy metals (Setshedi *et al.,* 2025). These deleterious factors cause different types of cellular and molecular changes, oxidative stress or DNA alteration, genome instability that finally lead to cancer (Bouyahya *et al.,* 2024). Elucidation of the complex molecular mechanisms by which tobacco smoke causes DNA damage and initiates tumor genesis are essential for the expansion of cancer prevention or intervention strategies in order to reduce diseases associated with tobacco use (Halimuzzaman *et al.,* 2024; Korde *et al.,* 2025).

The atomic foundation of the cancer-inducing activity of tobacco smoke is largely attributed to presences within procarcinogens that need metabolic activation to electrophilic cancer-initiating agents (Sohel *et al.,* 2022; Xue *et al.,* 2014). Cytochrome P450 (CYP) enzymes including CYP1A1 and CYP2E1 catalyze this bioactivation following which the resulting metabolites can bind to DNA or proteins (Zanger & Schwab, 2013). The electrophilic metabolites can then react with covalent adducts associated with DNA bases , mainly at the guanine, and in this context create benzopyrene diol epoxide (BPDE)-DNA adducts. If not correctly repaired, these adducts lead to the misincorporation of nucleotides during DNA synthesis, giving rise to point mutations in crucial genes such as tumor suppressors (e.g., TP53) and proto-oncogenes (e.g., KRAS) (Jones *et al.,* 2025). These mutations disturb the signaling pathways of cells, thus allowing for uncontrolled cellular growth, resistance to apoptosis and ultimately carcinogenesis (Hashem *et al.,* 2022).

Aside from forming direct DNA adducts, tobacco smoke also induces severe oxidative stress in cells. Both ROS and RNS produced directly by the components of tobacco smoke or their metabolites react with critical cellular macromolecules such as DNA, lipids, and protein (Caliri *et al.,* 2021). Of the oxidative DNA lesions generated, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a well-accepted biomarker of oxidative DNA damage and is involved in tobacco smoke–induced carcinogenesis (Fenga *et al.,* 2017). Oxidative lesions overtime may accumulate, leading to the breaks of DNA strands, chromosomal aberrations and epigenetic changes that jeopardize the stability of genome. Additionally, tobacco smoke may induce oxidation stress which can lead to

[1] Department of Microbiology & Molecular Biology, Concord Diagnostics & Molecular Lab Limited, Dhaka, Bangladesh
[2] Department of Medicine and Health, University of Wollongong, New South Wales, Australia
[3] Department of Pharmacy, State University, Dhaka, Bangladesh
[4] Department of Public Health, Bangladesh Open University, Gazipur, Bangladesh
[5] Department of Narcotics Control, Ministry of Home Affairs, Dhaka, Bangladesh
[*] Corresponding author's e-mail: prity.dhaka@gmail.com

inhibition of DNA repair capacity such as the nucleotide excision repair or base excision repair further increasing the mutagenic potential of smoke (Schumacher *et al.,* 2021).

Epigenetic disruption is another key step connecting tobacco exposure with cancer initiation. Genes can be up or down-regulated by changes in methylation of promoters, alterations in histone alterations and non-coding RNA expression all caused by carcinogenic components found in tobacco smoke (Ullah *et al.,* 2022; Zong *et al.,* 2019). Both hypermethylation of promoters, in tumor inhibitor genes and hypomethylation, at oncogenic loci have been reported from smokers' tissues (Leng *et al.,* 2018). By inducing such epigenetic changes, smoking not only promotes tumorigenesis but also participates in the heritability of the smoking-induced genetic damage and places susceptibility among descendants (Vlachou *et al.,* 2025).

Inflammation induced by long-term exposure to tobacco compounds promotes additional DNA damage and tumor growth (Yamaguchi, 2019). The resulting persistent inflammation offers a milieu favoring the production of more ROS and RNS coupled with activation of pro-inflammatory transcription factors such as NF-κB and STAT3 as well as release of cytokines promoting cell proliferation and angiogenesis (Wang *et al.,* 2025). Oxidative stress, chronic inflammation and defective DNA repair are driving forces for the microenvironment to favor both initiation of cancer and its progression (Wang *et al.,* 2025).

LITERATURE REVIEW

Tobacco contains a lot of complex mixtures of chemicals, many of which are mutagenic and carcinogenic (Talhout *et al.,* 2011). In the last decades, several investigations have described biological mechanisms by which such toxicants exert DNA-damaging effects and emerge as chemicals promoting cancer (Huang & Zhou, 2021; Romanò *et al.,* 2025). The evidence in literature shows that the carcinogenic action of tobacco smoke is mediated by direct damage to the DNA as well as oxidative stress, defective DNA repair and alterations in the epigenetic profile leading to genome instability and tumorigenesis (Tang *et al.,* 2022).

Chemical Composition and Carcinogenic Constituents of Tobacco Smoke

There are over 7,000 known chemicals in cigarette smoke, and these include nicotine, polycyclic aromatic hydrocarbons, tobacco-specific nitrosamines (TSNAs), aldehydes, aromatic amines and heavy metals (Setshedi *et al.,* 2025). Of these, PAHs like benzo[a]pyrene (BaP) and nitrosamines such as NNK (4(methylnitrosamino)-1-(3-pyridyl)-1-butanone) are known to be potent carcinogens (Yershova *et al.,* 2016). There has been research, such as that by Reed *et al.* (2018) have shown that both classes of monohalogenated benzene derivatives need to be metabolically triggered by cytochrome P450 enzymes to generate the corresponding reactive electrophilic intermediates, which react with DNA bases and create adducts. The DNA adducts formed can produce mutations if not effectively repaired, processes thought to be vital in the early stages of carcinogenesis.

DNA Adduct Formation and Mutagenesis

DNA adduct generation is one of the most direct molecular pathways between tobacco exposure and cancer (Ma *et al.,* 2019). Rodin & Rodin (2002) showed that BaP metabolites create stable adducts largely at guanine positions, resulting in G→T transversions commonly found at the TP53 gene in lung cancer patients with a smoking history. Likewise, investigations into NNK and NNN (N'-nitrosonornicotine) have demonstrated their capability to generate adducts of O6-methylguanine which result in mutations within critical oncogenes such as KRAS (Xue *et al.,* 2014). Repetitive cycles of these mutations disturb normal cell cycle maintenance and apoptosis, inducing cells to be prone to malignant transformation. The presence of these adducts in lung tissues and urine of smokers represents direct molecular evidence of the genotoxic action of tobacco smoke (Ma *et al.,* 2019).

Oxidative Stress and Reactive Species Generation

Oxidative damage is a key, facilitating partner of tobacco-promoted DNA damage. Tobacco smoke induces ROS and RNS, not only by external exposure but also by inflammatory and metabolic reactions (Caliri *et al.,* 2021). These reaction-active molecules impact DNA bases and induce oxidative lesions such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), a well-known biomarker of oxidative DNA damage (Valavanidis *et al.,* 2009). Increased 8-OHdG levels have been repeatedly found in the urine and tissue of smokers, which is related with a greater danger for cancer occurrence. In addition, downregulation of certain cellular antioxidants such as glutathione and the attenuation of other anti-oxidant enzymes (e.g., superoxide dysmutase – SOD) by tobacco smoke will exacerbate oxidative stress and facilitate more DNA damage and cell damage (Valavanidis *et al.,* 2009).

Impaired DNA Repair Mechanisms

DNA damaged repair capacity is vital for preserving genome solidity in cells (Chatterjee & Walker, 2017). Yet, tobacco smoke has been demonstrated to disturb several DNA repair schemes such as nucleotide excision repair, base excision repair, etc. (Tang *et al.,* 2022). Nasrallah *et al.* (2025) showed that cigarette smoke condensate (CSC) can decrease the expression levels of XRCC1 and OGG1, which are both critical DNA repair genes. Furthermore, polymorphisms of the repair genes were found linked with amplified lung cancer danger in smokers indicating that gene–environment interactions are important in tobacco carcinogenesis (Mei *et al.,* 2014). In addition, the inhibition of p53-mediated DNA damage response pathways by tobacco components compromises the

efficiency of cellular repair and enhances mutagenicity (Pitolli *et al.,* 2019).

Epigenetic Alterations Induced by Tobacco Smoke

Along with the induction of genetic mutations, tobacco smoke also stimulates extensive epigenetic changes that have more recently been appreciated as major drivers of carcinogenesis (Chen *et al.,* 2011). Cigarette smoke interferes with normal DNA methylation patterns, histone structures and the expression of non-coding RNAs (Breitling *et al.,* 2011). Particularly, promoter hypermethylation of key tumour suppressor genes including p16INK4a, RASSF1A and MGMT has been identified in the lung tissues of smokers, resulting in gene silencing and loss of tumour suppressive action (Wang *et al.,* 2004). In contrast, global DNA hypomethylation due to exposure of smoke may be involved in genomic instability and oncogenes activation. In addition, cigarette smoke deregulates microRNA (miRNA) levels by inhibiting tumor-suppressive miRNAs like the miR-34 and miR-200 families and activating oncogenic miRNAs like the potent oncomiR-21 contributing to promoting malignant transformation as well (Russ & Slack, 2012).

Inflammation and Microenvironmental Changes

Inflammatory microenvironment elicited by tobacco smoke favors the beginning and development of tumor (De La Iglesia *et al.,* 2020). Chronic exposure induces activation of transcriptional factors including NF-kB and STAT3, leading to creation of pro-inflammatory cytokines as IL-6 and TNF-a (Chung *et al.,* 2017). Not just ROS and RNS production, these mediators also increase VEGF-induced angiogenesis and cell proliferation. The crosstalk between inflammation and DNA damage signaling increased mutagenic pressure, fueling carcinogenic evolution (Kawanishi *et al.,* 2017).

Integration of Molecular Pathways in Carcinogenesis

The literature highlights that tobacco smoke–mediated carcinogenesis is based on multifactors of genotoxicity, antioxidant effect, and epigenetic factors (R.-J. Chen *et al.,* 2011). DNA adduct induction triggers mutagenesis, oxidative stress drives continued harm, defective repair leads to increased mutation load and epigenomic changes maintain distorted gene expression (Hwa Yun *et al.,* 2020). The net impact of these processes on the maintenance of genomic stability is disruption, resulting in activation of oncogenic pathways and concomitant silencing of tumor suppressors, which are typical hallmarks that drive cancer development (Dakal *et al.,* 2024).

Research Gap

Despite substantial research that has firmly established a link between tobacco smoke exposure and cancer, a number of important unknowns remain regarding the specific molecular pathways through which this occurs. Major studies to date have looked at these individual components and processes DNA adduct formation, oxidative stress, or epigenetic changes rather than combining them into a comprehensive molecular picture. the complicated web of cooperative interplays involving these pathways, and how they can together contribute to genomic instability and carcinogenesis remains far from clear. Furthermore, differences in individual genetic susceptibility, such as polymorphisms in genes involved in xenobiotic metabolism and DNA repair that often are ignored, are likely to preclude predicting differential cancer risks among smokers. Growing attention is paid to epigenetic disturbances as an important player, what its temporal relationship with mutations and DNA repair defects are currently poorly understood. Furthermore, most have been obtained in animal models or are from in vitro studies and there is scarce translational evidence to directly link molecular changes to distinct cancer phenotypes in humans. Hence, further comprehensive and integrative studies of the mechanisms by which tobacco smoke exposures elicit cumulative DNA damage through linked molecular, genetic and epigenetic routes are much needed if we are to better understand the mechanistic underpinnings of tobacco-associated carcinogenesis and make informed decisions about novel preventive measures as well as therapeutic interventions.

Research Questions

i. What are the mechanistic details of interaction between tobacco smoke chemical compounds in terms of mediating DNA damage to the target cells?

ii. What is the precise involvement of reactive oxygen and nitrogen in the induction of oxidative DNA damage, and ultimately genomic instability?

iii. What are the effects of tobacco smoke exposure on the effectiveness and control of vital repair pathways for DNA damage including nucleotide excision repair (NER) and base excision repair (BER)?

iv. How does tobacco smoke lead to epigenetic alterations, including those at the level of DNA methylation, histones and microRNAs, that are involved in carcinogenesis?

v. What is the interplay between genetic and epigenetic changes that conspire to promote the transition from tobacco-related DNA damage events to cancer?

Research Objectives

i. To isolate and characterize the principal carcinogens in tobacco smoke which produce molecular and genetic changes in human cells.

ii. To identify mechanisms by which tobacco smoke-generated reactive oxygen and nitrogen species induce oxidative DNA damage and genomic instability.

iii. To assess the effect of tobacco smoke exposure on DNA repair enzymes and pathways as they relate to maintenance of genomic integrity.

iv. To analyze the epigenetic alterations, i.e., DNA methylation, histone modifications, and microRNA expression, in tobacco smoke–induced carcinogenesis.

v. To consolidate the genetic, oxidative and epigenetic

information into an overarching molecular model of how tobacco smoke induced DNA damage leads to cancer.

MATERIALS AND METHODS

Tobacco smoke extract (TSE) was equipped according to the technique of Wetscher *et al.* (1995) with minor alterations. Standard commercial filter cigarettes having 12 mg tar and 0.9 mg nicotine were smoked on a single-port smoking machine (35 mL puff volume, 2 s duration, one puff per min). The smoke was passed through phosphate-buffered saline (PBS, pH 7.4) at 25°C to form TSE and then filtered (0.22 μm Viaflo; Millipore Sigma) to remove particles and stored at −80°C, Nicotine and tar concentrations were determined spectrophotometrically according to Côté *et al.* (2011). Human bronchial epithelial cells (BEAS-2B) and lung carcinoma cells, A549 were purchased from ATCC (U.S.A) and maintained in DMEM with 10% fetal bovine serum and 1% penicillin–streptomycin at 37°C in a humidified atmosphere of 5% CO_2. For treatments, cells were stimulated with various concentrations of TSE (0, 25, 50 and 100 μg/mL) according to Gao *et al.* (2020), whereas control groups were given PBS as the vehicle.

DNA damage assessment by alkaline comet assay DNA strand breaks were assessed by alkaline comet assay as described previously by Singh *et al.* (1988). Induced cells were mixed in 0.7% low melting point agarose, lysed by ice-cold lysis buffer followed by electrophoresis (25 V, 300 mA for 20 min). Slides were neutralized and stained with 20 μg/mL of ethidium bromide, and DNA damage was assessed as tail moment analyzed by CometScore software. γ-H 2 AX immunofluorescence Cells fixed with 4% paraformaldehyde were permeabilized with 0.2% Triton X-100 and treated with primary anti-γ-H 2 AX antibody, and then incubated with the Alexa Fluor 488–conjugated secondary antibody (Salvi *et al.,* 2020). Treated nuclei were counterstained with DAPI and foci examined with a Zeiss LSM 710 confocal microscope. Oxidative stress was detected as intracellular ROS determined by the DCFH-DA (LeBel *et al.,* 1992), lipid peroxidation through a TBARS assay of malondialdehyde (MDA) and antioxidant defense via reduced glutathione content (GSH) and superoxide dismutase activity using commercially available colorimetric kits (Cayman Chemicals, USA).

mRNA levels of selected DNA repair genes: a gene involved in base excision (OGG1 and XRCC1) and nucleotide excision repair (XPA, ERCC1), were determined by real-time PCR as previously described Vogel *et al.* (2006). Total RNA was isolated using the TRIzol reagent and then, reverse-transcribed into cDNA and amplified with SYBR Green Master Mix on an Applied Biosystems 7500 Fast Real-Time PCR System. GAPDH was taken as the internal reference, and the relative expression was analyzed by $2-\Delta\Delta Ct$. Global and promoter DNA methylation (p16^INK4a, RASSF1A, MGMT) was evaluated using the MethylFlash kit (Epigentek USA) and bisulfite conversion with MSP. The expression of specific microRNAs (miR-21, miR-34a, miR-200c) was detected by TaqMan assays with U6 as the internal control. The protein expression of DNA repair proteins (XRCC1 and OGG1), oxidative stress markers (Nrf2 and SOD1) as well as apoptosis regulators (p53, Bax, Bcl-2) were determined by Western blot. The equal amounts of protein (30 μg) were separated on 10% SDS-PAGE, transferred to PVDF membranes which were then blocked in 5% non-fat milk and incubated with respective primary (1:1000) and HRP-conjugated secondary antibodies. Bands were visualized with ECL detection reagent (Bio-Rad) and quantified by ImageJ. All experiments were done in triplicates, and data were expressed as mean ± SD and analyzed using one-way ANOVA followed by Tukey's post hoc comparison test (SPSS v26. 0 was assumed, with $p < 0.05$ considered significant.

RESULTS AND DISCUSSIONS

Results

The quantified concentrations for major cytotoxic and carcinogenic components of tobacco smoke extract (TSE) are presented in Table 1. The extract was abundant in total tar (560 ± 15 μg/mL) and nicotine (42.3 ± 2.1 μg/mL). Among the strong carcinogens, benzo[a]pyrene (a representative PAH), was registered at 18.6 ± 1.4ng/mL in this study; as for the tobacco-specific NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), it was determined to be 9.2 ± 0.9 ng/mL. Moreover, aldehyde compound was formaldehyde (6.8 ± 0.6 μg/mL). The occurrence of heavy metals, such as lead (0.85 ± 0.07 ng/mL).

Table 1: Chemical Composition of Prepared Tobacco Smoke Extract (TSE)

Parameters	Value
Nicotine (μg/mL)	42.3 ± 2.1
Total tar (μg/mL)	560 ± 15
Benzo[a]pyrene (ng/mL)	18.6 ± 1.4
NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) (ng/mL)	9.2 ± 0.9
Formaldehyde (μg/mL)	6.8 ± 0.6
Lead (ng/mL)	0.85 ± 0.07

At 100 µg/mL, the cell viability of BEAS-2B cells reduced to 49 ± 5% at 24 h and a rapidly decreasing to 32 ± 4% at 48h ($p < 0.05$) compared with control levels. A549 cells also reduced from 100 ± 2% in control to 61 ± 6% at 100 µg/mL at 24 h and decreased to (43 ± 5%) after another subsequent period of exposure after 48 h. Both cell lines were affected, but BEAS-2B cells were more sensitive to TSE-mediated cytotoxicity than A549 cells (Table 2).

Table 2: Chemical Composition of Prepared Tobacco Smoke Extract (TSE)

Cell line	Treatment (µg/mL TSE)	24 h viability (%)	48 h viability (%)
BEAS-2B	0 (control)	100 ± 3a	100 ± 4a
	25	88 ± 4b	79 ± 5b
	50	71 ± 6c	56 ± 5c
	100	49 ± 5d	32 ± 4d
A549	0 (control)	100 ± 2a	100 ± 3a
	25	92 ± 3b	84 ± 4b
	50	78 ± 5c	65 ± 6c
	100	61 ± 6d	43 ± 5d

There was an evident dose-dependent enhancement of DNA strand breaks in both cell lines (Figure 1). In BEAS-2B cells, the average tail moment increased significantly from 0.78 ± 0.21 in control to 7.24 ± 0.92 at 100 µg/mL TSE ($p < 0.05$), while in A549 cells it was elevated from 0.62 ± 0.18 to 5.88 ± 0.80 across that concentration range.

A significant and dose-dependent increase of γ-H2AX foci was found in both tested cell lines (Figure 2). The average number of foci per BEAS-2B cells increased from 0.7 ± 0.4 in control to 11.3 ± 2.2 at a concentration of 100 µg/mL TSE ($p < 0.05$). In A549 cells, foci formation similarly increased from 0.5 ± 0.3 to 8.1 ± 1.7 within the same treatment range.

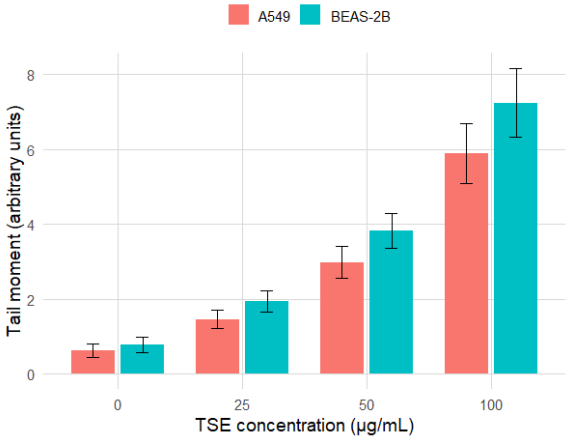

Figure 1: Comet Assay Mean Tail Moment (arbitrary units) after 24 h TSE Exposure

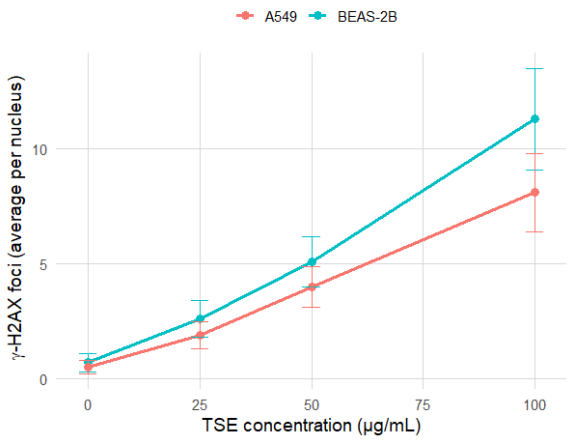

Figure 2: γ-H2AX foci (average foci per nucleus) after 24 h TSE Exposure

The data indicate concentration-dependent elevation of oxidative damage markers coupled with depletion of antioxidant defense (Table 3). The relative DCF fluorescence suddenly increased from 1.00 ± 0.08 in the control to 5.12 ± 0.36 with TSE at 100 µg/mL ($p < 0.05$). Also, lipid peroxidation estimated by the level of 1-malondialdehyde (MDA) increased from 0.46 ± 0.05 to 2.09 ± 0.18 nmol/mg. In contrast, the antioxidant signs of reduced glutathione (GSH) and superoxide dismutase (SOD) activity both decreased significantly from 8.6 ± 0.7 to 2.8±0.3 µmol/g for GSH and from 18.2±1.1 to 9.0±0.9 U/mg across the same concentration sequence used.

Both data demonstrate a relevant and dose dependent downregulation of all investigated genes (Table 4). Regarding components of base excision repair, the gene OGG1 was reduced from 1.00 ± 0.05 (control) to 0.39 ± 0.05 at 100 µg/mL and XRCC1 decreased from 1.00 ± 0.06 to 0.42 ± 0.04. Similarly, XPA and ERCC1 NER genes were also decreased from control levels of 1.00 ± 0.04 and 1.00 ± 0.07 to 0.54 ± 0.05 and 0.51 ± 0.06 at the highest dose ($p < 0.05$).

The amount of Nrf2 expression was found to rise gradually from 1.00 ± 0.06 (control) to 2.31 ± 0.18 at concentration of TSE 100 µg/mL ($p < 0.05$). Another protein, XRCC1 as well as OGG1 proteins responsible for DNA repair were also significantly downregulated (from 1.00 ± 0.05 to 0.41 ± 0.04 and from 1.00 ± 0.07 to 0.44 ± 0.05 respectively at the highest dose). At the same time,

Table 3: Oxidative Stress Markers after 24 h TSE Exposure

Marker (units)	0	25 µg/mL	50 µg/mL	100 µg/mL
DCF fluorescence (relative)	1.00 ± 0.08d	1.82 ± 0.12c	2.94 ± 0.20b	5.12 ± 0.36a
MDA (nmol/mg)	0.46 ± 0.05d	0.89 ± 0.08c	1.37 ± 0.12b	2.09 ± 0.18a
GSH (µmol/g)	8.6 ± 0.7a	6.1 ± 0.5b	4.3 ± 0.4c	2.8 ± 0.3d
SOD (U/mg)	18.2 ± 1.1a	15.4 ± 1.2b	12.1 ± 1.0c	9.0 ± 0.9d

Table 4: DNA Repair Gene Expression (qPCR; fold change vs control) after 24 h TSE Exposure

Gene	0	25 µg/mL	50 µg/mL	100 µg/mL
OGG1	1.00 ± 0.05a	0.89 ± 0.07a	0.63 ± 0.06b	0.39 ± 0.05c
XRCC1	1.00 ± 0.06a	0.91 ± 0.06a	0.67 ± 0.05b	0.42 ± 0.04c
XPA	1.00 ± 0.04a	0.94 ± 0.05a	0.76 ± 0.06b	0.54 ± 0.05c
ERCC1	1.00 ± 0.07a	0.95 ± 0.08a	0.72 ± 0.07b	0.51 ± 0.06c

Table 5: Protein Expression by Western Blot Densitometry (normalized to control = 1.00) after 24 h TSE Exposure

Protein	0	25 µg/mL	50 µg/mL	100 µg/mL
Nrf2	1.00 ± 0.06d	1.42 ± 0.09c	1.79 ± 0.13b	2.31 ± 0.18a
XRCC1	1.00 ± 0.05a	0.89 ± 0.06a	0.63 ± 0.05b	0.41 ± 0.04c
OGG1	1.00 ± 0.07a	0.92 ± 0.07a	0.68 ± 0.06b	0.44 ± 0.05c
p53 (total)	1.00 ± 0.09c	1.16 ± 0.08c	1.92 ± 0.12b	2.61 ± 0.20a
Bax/Bcl-2 ratio	1.00 ± 0.05c	1.12 ± 0.07c	1.67 ± 0.11b	2.45 ± 0.17a

the p53 total content and the pro-apoptotic Bax/Bcl-2 ratio also significantly increased up to 2.61 ± 0.20 and 2.45 ± 0.17, respectively at 100 µg/mL (Table 5).

5-Methylcytosine (5-mC) levels globally rose from 4.6 ± 0.3% in control to 6.8 ± 0.5% at TSE 100 µg/mL (Table 6). Promoter methylation of tumor suppressor genes was significantly increased with p16^INK4a increasing from 11 ± 3% to 48 ± 6%, RASSF1A from 8 ± 2% to 41 ±5% and MGMT from 6 ±2 % to33±4 % at the treatment range ($p < 0.05$).

Oncogenic miR-21 was also markedly increased, from 1.00 ± 0.05 to 5.36 ± 0.35 at 100 µg/mL TSE (Table 7). In comparison, tumor-suppressing miRNAs, miR-34a and miR-200c were downregulated in a dose-dependent manner (miR-34a from 1.00 ± 0.06 to 0.36 ± 0.03, miR-200c from 1.00 ± 0.07 to 0.48 ± 04).

Table 6: DNA Methylation Global and Promoter-Specific Changes after 48 h TSE Exposure

Measure	0	25 µg/mL	50 µg/mL	100 µg/mL
Global 5-mC (%)	4.6 ± 0.3c	5.1 ± 0.3c	5.9 ± 0.4b	6.8 ± 0.5a
p16^INK4a promoter (%)	11 ± 3d	22 ± 4c	34 ± 5b	48 ± 6a
RASSF1A promoter (%)	8 ± 2d	18 ± 3c	29 ± 4b	41 ± 5a
MGMT promoter (%)	6 ± 2d	14 ± 3c	23 ± 4b	33 ± 4a

Table 7: microRNA Expression (TaqMan assays; fold change vs control) after 24 h TSE Exposure

microRNA	0	25 µg/mL	50 µg/mL	100 µg/mL
miR-21	1.00 ± 0.05d	1.89 ± 0.12c	3.21 ± 0.20b	5.36 ± 0.35a
miR-34a	1.00 ± 0.06a	0.82 ± 0.05b	0.59 ± 0.04c	0.36 ± 0.03d
miR-200c	1.00 ± 0.07a	0.91 ± 0.06a	0.71 ± 0.05b	0.48 ± 0.04c

Discussion

The focus of the research was to elucidate the molecular mechanisms underlying the DNA damage and other carcinogenic effects caused by exposure to TSE. Our results portray a multifaceted view which combines the induction of oxidative stress, accumulation of DNA damage, inhibition of restoration mechanisms and an epigenetic as well as microRNA dysregulation to provide new aspects on tobacco smoke carcinogenicity. We found a strong dose-dependent rise in reactive oxygen species (ROS) and lipid peroxidation (MDA) together with a

decreased antioxidant defense, represented by GSHand SOD enzymatic activity after 24h of exposure to TSE. These results are consistent with meta-analysis data indicating that smokers have higher levels of oxidative DNA damage markers, for example urinary 8-oxo-dG which range from ~15 to 30% vs. non-smokers (Bellamri *et al.,* 2022; Ellegaard & Poulsen, 2016). Our findings extend and enhance that observation by showing defined concentration-dependent effect in vitro; pinpointing the ROS generation as a proximal event of genotoxicity caused by smoke. It has also been stressed in the literature that oxidative stress could function not only as a by-product of smoking, but rather it could also serve as an initiator and promoter of mutagenesis and carcinogenesis. For instance, 8-OHdG levels in airway were favorably connected with smoking indexes and tumor stage in patients with lung cancer (Cao *et al.,* 2016; Seo *et al.,* 2023). Therefore, our data lend support to the concept that TSE-induced oxidative stress is a central mechanistic nexus between smoke exposure and genomic instability.

Our comet assay (tail moment) and γ-H2AX foci analyses revealed notable and dose-dependent DNA single strand breaks and markers of double strand breaks following TSE exposure. These observations correlate with previous research on cigarette smoke condensate (CSC), which demonstrated enhanced DNA strand damage, sister-chromatid exchanges and production of micronuclei in the exposed cells (Bellamri *et al.,* 2022; DeMarini, 2004). Moreover, the observation of PAH-related DNA adducts including BPDE-N^2-dG in buccal cells in the present study offers a direct human correlate for genotoxic insults caused by tobacco smoke constituents (Chen *et al.,* 2022). We showed that the repair genes (OGG1, XRCC1, XPA and ERCC1) are downregulated and their repair-protein product is significantly inhibited which corroborates with previous studies has shown how tobacco smoke can hinder a conversion capacity (Kumar *et al.,* 2012). Such as the aldehydes in tobacco smoke directly modifying DNA-repair proteins, and down-regulating their repair activity have been postulated in literature (Tang *et al.,* 2022). Importantly, this "double-hit" of damage induction as well as the suppression of repair is crucial: DNA that cannot be repaired efficiently has an increased probability for mutagenic fixation.

Our results indicate that global 5-methylcytosine levels and promoter-specific hypermethylation of tumor inhibitor genes (p16^INK4a, RASSF1A, MGMT) are significantly enhanced. These findings are in alignment with previous reports demonstrating that tumorigenic agents in tobacco such as BaP induce genome-wide epigenetic changes, including promoter hypermethylation (of tumor suppressing loci) and hypomethylation (of regions favoring oncogenic transformation) (Wang *et al.,* 2023; Wang *et al.,* 2004). Epigenetic repression of toxicant resistant genes or cell-cycle regulator by smoking has been described elsewhere and may represent a contributory mechanism for carcinogenic transformation (Chen *et al.,* 2011). In terms of micro-RNAs, we found that miR-21 (an oncomiR) was upregulated and the expression levels of tumor-suppressive miR-34a and miR208 200c were decreased; these are findings consistent with the emerging evidence indicating that cigarette smoke exposure is able to modify the profiles of miRNA expression which in turn modulate apoptosis, proliferation and DNA damage response (Addissouky *et al.,* 2024). Collectively, both epigenetic and microRNA alterations appear to function downstream of oxidative and genotoxic stress in driving the establishment of tumor-type-permissive gene expression states.

Findings

i. Tobacco smoke extract (TSE) was responsible for a dose-dependent generation of oxidative stress, as exposed by ROS production and MDA levels, in combination with antioxidant defenses reduction (GSH, SOD).

ii. TSE exposure leads to more marked DNA damage, single-strand breaks and double-strand breaks as shown by the comet assay and γ-H2AX foci formation.

iii. Crucial DNA repair genes (OGG1, XRCC1, XPA, ERCC1) and proteins are inhibited after TSE treatment suggesting weakened potential of DNA repair.

iv. TSE induces epigenetic alterations, such as universal DNA hypermethylation and promoter-specific hypermethylation of the tumor inhibitor genes (p16^INK4a, RASSF1A, MGMT).

v. MicroRNA expression is changed, oncogenic miR-21: increased; tumor suppressing miR-34a and miR-200c: decreased) implying of post-transcriptional regulation of carcinogenesis.

vi. TSE triggers apoptotic pathways, manifested as enhanced p53 protein levels and increased Bax/Bcl-2.

Recommendations

i. Execute some preventive measures leading to a decrease in the exposure of tobacco smoke at individual and community levels.

ii. Explore antioxidant agents that can prevent ROS-induced DNA damage in high risk individuals.

iii. Investigate therapies based on inhibiting DNA repair pathways, such as pharmacological enhancers or gene therapy of repair factors.

iv. Create epigenetic and miRNA biomarkers for early detection and risk assessment of smoke-induced carcinogenesis.

v. Promote additional in vivo and longitudinal investigations to confirm the mechanistic connections between TSE and the long-term effects of TSE on genomic stability, carcinogenesis risk.

Limitations

i. Cell line models (BEAS-2B and A549) could not recapitulate in vivo exposure or tissue microenvironment completely.

ii. TSE exposure was acute (24–48 h), and therefore limited any inferences on chronic and cumulative impacts on long-term smoking.

iii. Functional implications of epigenetic modifications and microRNA changes were implied; evidence was not direct for mutation fixing or evolution.

iv. The current investigation covered specific genes, proteins and microRNAs; thus other relevant pathways might not have been identified.

CONCLUSION

Our study shows that tobacco smoke extract (TSE) promotes a complex molecular hierarchy that drives cellular transformation and DNA damage potentially leading to cancer. TSEs exposure induces oxidative stress, DNA strand breaks, downregulates key processes of DNA repair and induces epigenetic and miRNA modifications that result in impaired genomic integrity and prone to malignant transformation. The included mechanistic understanding demonstrates that oxidative injury, repair failure and gene-expression reprogramming intersect to promote tobacco smoke–induced carcinogenesis. This should be confirmed in vivo using chronic exposure models, by studying long-term effects of the cumulative DNA damage, and also investigate the genomic or epigenomic variations between individuals that may affect their susceptibility. Furthermore, studies targeted at specific interventions such as antioxidant use, DNA repair enhancement or modulation of epigenetic changes might provide new preventive and therapeutic drug regimens to counteract the carcinogenic potential of tobacco smoke.

REFERENCES

Addissouky, T. A., El Sayed, I. E. T., Ali, M. M. A., Wang, Y., El Baz, A., Elarabany, N., & Khalil, A. A. (2024). Oxidative stress and inflammation: Elucidating mechanisms of smoking-attributable pathology for therapeutic targeting. *Bulletin of the National Research Centre, 48*(1), 16. https://doi.org/10.1186/s42269-024-01174-6

Bellamri, M., Walmsley, S. J., Brown, C., Brandt, K., Konorev, D., Day, A., Wu, C.-F., Wu, M. T., & Turesky, R. J. (2022). DNA Damage and Oxidative Stress of Tobacco Smoke Condensate in Human Bladder Epithelial Cells. *Chemical Research in Toxicology, 35*(10), 1863–1880. https://doi.org/10.1021/acs.chemrestox.2c00153

Bouyahya, A., Bakrim, S., Aboulaghras, S., El Kadri, K., Aanniz, T., Khalid, A., Abdalla, A. N., Abdallah, A. A., Ardianto, C., Ming, L. C., & El Omari, N. (2024). Bioactive compounds from nature: Antioxidants targeting cellular transformation in response to epigenetic perturbations induced by oxidative stress. *Biomedicine & Pharmacotherapy, 174*, 116432. https://doi.org/10.1016/j.biopha.2024.116432

Breitling, L. P., Yang, R., Korn, B., Burwinkel, B., & Brenner, H. (2011). Tobacco-Smoking-Related Differential DNA Methylation: 27K Discovery and Replication. *The American Journal of Human Genetics, 88*(4), 450–457. https://doi.org/10.1016/j.ajhg.2011.03.003

Caliri, A. W., Tommasi, S., & Besaratinia, A. (2021). Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer. *Mutation Research/Reviews in Mutation Research, 787*, 108365. https://doi.org/10.1016/j.mrrev.2021.108365

Cao, C., Lai, T., Li, M., Zhou, H., Lv, D., Deng, Z., Ying, S., Chen, Z., Li, W., & Shen, H. (2016). Smoking-promoted oxidative DNA damage response is highly correlated to lung carcinogenesis. *Oncotarget, 7*(14), 18919–18926. https://doi.org/10.18632/oncotarget.7810

Chatterjee, N., & Walker, G. C. (2017). Mechanisms of DNA damage, repair, and mutagenesis. *Environmental and Molecular Mutagenesis, 58*(5), 235–263. https://doi.org/10.1002/em.22087

Chen, K.-M., Sun, Y.-W., Krebs, N. M., Sun, D., Krzeminski, J., Reinhart, L., Gowda, K., Amin, S., Mallery, S., Richie, J. P., & El-Bayoumy, K. (2022). Detection of DNA adducts derived from the tobacco carcinogens, benzo[a]pyrene and dibenzo[def,p]chrysene in human oral buccal cells. *Carcinogenesis, 43*(8), 746–753. https://doi.org/10.1093/carcin/bgac058

Chen, R.-J., Chang, L. W., Lin, P., & Wang, Y.-J. (2011). Epigenetic Effects and Molecular Mechanisms of Tumorigenesis Induced by Cigarette Smoke: An Overview. *Journal of Oncology, 2011*, 1–14. https://doi.org/10.1155/2011/654931

Chung, S. S., Wu, Y., Okobi, Q., Adekoya, D., Atefi, M., Clarke, O., Dutta, P., & Vadgama, J. V. (2017). Proinflammatory Cytokines IL-6 and TNF- α Increased Telomerase Activity through NF- ϰ B/STAT1/STAT3 Activation, and Withaferin A Inhibited the Signaling in Colorectal Cancer Cells. *Mediators of Inflammation, 2017*, 1–11. https://doi.org/10.1155/2017/5958429

Côté, F., Létourneau, C., Mullard, G., & Voisine, R. (2011). Estimation of nicotine and tar yields from human-smoked cigarettes before and after the implementation of the cigarette ignition propensity regulations in Canada. *Regulatory Toxicology and Pharmacology, 61*(3), S51–S59. https://doi.org/10.1016/j.yrtph.2010.03.004

Dakal, T. C., Dhabhai, B., Pant, A., Moar, K., Chaudhary, K., Yadav, V., Ranga, V., Sharma, N. K., Kumar, A., Maurya, P. K., Maciaczyk, J., Schmidt-Wolf, I. G. H., & Sharma, A. (2024). Oncogenes and tumor suppressor genes: Functions and roles in cancers. *MedComm, 5*(6), e582. https://doi.org/10.1002/mco2.582

De La Iglesia, J. V., Slebos, R. J. C., Martin-Gomez, L., Wang, X., Teer, J. K., Tan, A. C., Gerke, T. A., Aden-Buie, G., Van Veen, T., Masannat, J., Chaudhary, R., Song, F., Fournier, M., Siegel, E. M., Schabath, M. B., Wadsworth, J. T., Caudell, J., Harrison, L., Wenig,

B. M., … Chung, C. H. (2020). Effects of Tobacco Smoking on the Tumor Immune Microenvironment in Head and Neck Squamous Cell Carcinoma. *Clinical Cancer Research, 26*(6), 1474–1485. https://doi.org/10.1158/1078-0432.CCR-19-1769

DeMarini, D. M. (2004). Genotoxicity of tobacco smoke and tobacco smoke condensate: A review. *Mutation Research/Reviews in Mutation Research, 567*(2–3), 447–474. https://doi.org/10.1016/j.mrrev.2004.02.001

Ellegaard, P. K., & Poulsen, H. E. (2016). Tobacco smoking and oxidative stress to DNA: A meta-analysis of studies using chromatographic and immunological methods. *Scandinavian Journal of Clinical and Laboratory Investigation, 76*(2), 151–158. https://doi.org/10.3109/00365513.2015.1127407

Fenga, C., Gangemi, S., Teodoro, M., Rapisarda, V., Golokhvast, K., Docea, A. O., Tsatsakis, A. M., & Costa, C. (2017). 8-Hydroxydeoxyguanosine as a biomarker of oxidative DNA damage in workers exposed to low-dose benzene. *Toxicology Reports, 4*, 291–295. https://doi.org/10.1016/j.toxrep.2017.05.008

Gallucci, G., Tartarone, A., Lerose, R., Lalinga, A. V., & Capobianco, A. M. (2020). Cardiovascular risk of smoking and benefits of smoking cessation. *Journal of Thoracic Disease, 12*(7), 3866–3876. https://doi.org/10.21037/jtd.2020.02.47

Gao, X., Zhang, S., Wang, L., Yu, L., Zhao, X., Ni, H., Wang, Y., Wang, J., Shan, C., & Fu, Y. (2020). Anti-Inflammatory Effects of Neochlorogenic Acid Extract from Mulberry Leaf (Morus alba L.) Against LPS-Stimulated Inflammatory Response through Mediating the AMPK/Nrf2 Signaling Pathway in A549 Cells. *Molecules, 25*(6), 1385. https://doi.org/10.3390/molecules25061385

Halimuzzaman, Md., Sharma, J., Hossain, M. I., Akand, F., & Khan, N. N. (2024). Healthcare Service Quality Digitization with Enterprise Resource Planning. *Journal of Angiotherapy, 8*(5). https://doi.org/10.25163/angiotherapy.859716

Hashem, S., Ali, T. A., Akhtar, S., Nisar, S., Sageena, G., Ali, S., Al-Mannai, S., Therachiyil, L., Mir, R., Elfaki, I., Mir, M. M., Jamal, F., Masoodi, T., Uddin, S., Singh, M., Haris, M., Macha, M., & Bhat, A. A. (2022). Targeting cancer signaling pathways by natural products: Exploring promising anti-cancer agents. *Biomedicine & Pharmacotherapy, 150*, 113054. https://doi.org/10.1016/j.biopha.2022.113054

Huang, R., & Zhou, P.-K. (2021). DNA damage repair: Historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. *Signal Transduction and Targeted Therapy, 6*(1), 254. https://doi.org/10.1038/s41392-021-00648-7

Hwa Yun, B., Guo, J., Bellamri, M., & Turesky, R. J. (2020). DNA adducts: Formation, biological effects, and new biospecimens for mass spectrometric measurements in humans. *Mass Spectrometry Reviews, 39*(1–2), 55–82. https://doi.org/10.1002/mas.21570

Jones, K., Dye, E., Gilkes, N., Xia, D., Jiang, S., & Li, W. (2025). Mechanisms of DNA repair and mutagenesis induced by acetaldehyde, acrolein, aristolochic acids, and vinyl chloride. *Journal of Environmental Science and Health, Part C,* 1–38. https://doi.org/10.1080/26896583.2025.2545086

Kawanishi, S., Ohnishi, S., Ma, N., Hiraku, Y., & Murata, M. (2017). Crosstalk between DNA Damage and Inflammation in the Multiple Steps of Carcinogenesis. *International Journal of Molecular Sciences, 18*(8), 1808. https://doi.org/10.3390/ijms18081808

Korde, A., Ramaswamy, A., Anderson, S., Jin, L., Zhang, J., Hu, B., Velasco, W. V., Diao, L., Wang, J., Pisani, M. A., Sauler, M., Boffa, D. J., Puchalski, J. T., Yan, X., Moghaddam, S. J., & Takyar, S. S. (2025). Cigarette smoke induces angiogenic activation in the cancer field through dysregulation of an endothelial microRNA. *Communications Biology, 8*(1), 511. https://doi.org/10.1038/s42003-025-07710-y

Kumar, A., Pant, M. C., Singh, H. S., & Khandelwal, S. (2012). Reduced expression of DNA repair genes (XRCC1, XPD, and OGG1) in squamous cell carcinoma of head and neck in North India. *Tumor Biology, 33*(1), 111–119. https://doi.org/10.1007/s13277-011-0253-7

LeBel, C. P., Ischiropoulos, H., & Bondy, S. C. (1992). Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. *Chemical Research in Toxicology, 5*(2), 227–231. https://doi.org/10.1021/tx00026a012

Leng, S., Diergaarde, B., Picchi, M. A., Wilson, D. O., Gilliland, F. D., Yuan, J.-M., Siegfried, J. M., & Belinsky, S. A. (2018). Gene Promoter Hypermethylation Detected in Sputum Predicts FEV1 Decline and All-Cause Mortality in Smokers. *American Journal of Respiratory and Critical Care Medicine, 198*(2), 187–196. https://doi.org/10.1164/rccm.201708-1659OC

Ma, B., Stepanov, I., & Hecht, S. S. (2019). Recent Studies on DNA Adducts Resulting from Human Exposure to Tobacco Smoke. *Toxics, 7*(1), 16. https://doi.org/10.3390/toxics7010016

Mei, C., Hou, M., Guo, S., Hua, F., Zheng, D., Xu, F., Jiang, Y., Li, L., Qiao, Y., Fan, Y., & Zhou, Q. (2014). Polymorphisms in DNA repair genes of XRCC1 , XPA , XPC , XPD and associations with lung cancer risk in C hinese people. *Thoracic Cancer, 5*(3), 232–242. https://doi.org/10.1111/1759-7714.12073

Nasrallah, N. A., Lee, B., Wiese, B. M., Karam, M. N., Mickler, E. A., Zhou, H., Paolelli, N., Stearman, R. S., Geraci, M. W., & Sears, C. R. (2025). Cigarette smoke and decreased DNA repair by Xeroderma Pigmentosum Group C use a double hit mechanism for epithelial cell lung carcinogenesis. *Oncotarget, 16*(1), 396–409. https://doi.org/10.18632/oncotarget.28724

Pitolli, C., Wang, Y., Candi, E., Shi, Y., Melino, G., & Amelio, I. (2019). p53-Mediated Tumor Suppression: DNA-Damage Response and Alternative Mechanisms. *Cancers, 11*(12), 1983. https://doi.org/10.3390/cancers11121983

Reed, L., Arlt, V. M., & Phillips, D. H. (2018). The role of cytochrome P450 enzymes in carcinogen activation and detoxication: An in vivo–in vitro paradox. *Carcinogenesis, 39*(7), 851–859. https://doi.org/10.1093/carcin/bgy058

Rodin, S. N., & Rodin, A. S. (2002). On the origin of p53 G:C → T:A transversions in lung cancers. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 508*(1–2), 1–19. https://doi.org/10.1016/S0027-5107(02)00106-9

Romanò, A., Pagiatakis, C., Gornati, R., Bernardini, G., & Papait, R. (2025). Epigenetics: A link between toxicants and diseases. *iScience, 28*(6), 112613. https://doi.org/10.1016/j.isci.2025.112613

Russ, R., & Slack, F. J. (2012). Cigarette-Smoke-Induced Dysregulation of MicroRNA Expression and Its Role in Lung Carcinogenesis. *Pulmonary Medicine, 2012,* 1–9. https://doi.org/10.1155/2012/791234

Salvi, A., Amrine, C. S. M., Austin, J. R., Kilpatrick, K., Russo, A., Lantvit, D., Calderon-Gierszal, E., Mattes, Z., Pearce, C. J., Grinstaff, M. W., Colby, A. H., Oberlies, N. H., & Burdette, J. E. (2020). Verticillin A Causes Apoptosis and Reduces Tumor Burden in High-Grade Serous Ovarian Cancer by Inducing DNA Damage. *Molecular Cancer Therapeutics, 19*(1), 89–100. https://doi.org/10.1158/1535-7163.MCT-19-0205

Schumacher, B., Pothof, J., Vijg, J., & Hoeijmakers, J. H. J. (2021). The central role of DNA damage in the ageing process. *Nature, 592*(7856), 695–703. https://doi.org/10.1038/s41586-021-03307-7

Seo, Y.-S., Park, J.-M., Kim, J.-H., & Lee, M.-Y. (2023). Cigarette Smoke-Induced Reactive Oxygen Species Formation: A Concise Review. *Antioxidants, 12*(9), 1732. https://doi.org/10.3390/antiox12091732

Setshedi, K. Z., Maity, A., Nyakale, A., Ramahlare, S., Chauke, V. P., Nkomzwayo, T., Mandiwana, V., Ray, S. S., & Hlekelele, L. (2025). Removal of Tobacco Specific Carcinogenic Nitrosamines in Mainstream Cigarette Smoke and Aqueous Solution─A Review. *ACS Omega, 10*(21), 20949–20967. https://doi.org/10.1021/acsomega.4c08529

Singh, N. P., McCoy, M. T., Tice, R. R., & Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research, 175*(1), 184–191. https://doi.org/10.1016/0014-4827(88)90265-0

Sohel, Md. S., Shi, G., Zaman, N. T., Hossain, B., Halimuzzaman, Md., Akintunde, T. Y., & Liu, H. (2022). Understanding the Food Insecurity and Coping Strategies of Indigenous Households during COVID-19 Crisis in Chittagong Hill Tracts, Bangladesh: A Qualitative Study. *Foods, 11*(19), 3103. https://doi.org/10.3390/foods11193103

Talhout, R., Schulz, T., Florek, E., Van Benthem, J., Wester, P., & Opperhuizen, A. (2011). Hazardous Compounds in Tobacco Smoke. *International Journal of Environmental Research and Public Health, 8*(2), 613–628. https://doi.org/10.3390/ijerph8020613

Tang, M., Lee, H.-W., Weng, M., Wang, H.-T., Hu, Y., Chen, L.-C., Park, S.-H., Chan, H., Xu, J., Wu, X.-R., Wang, H., Yang, R., Galdane, K., Jackson, K., Chu, A., & Halzack, E. (2022). DNA damage, DNA repair and carcinogenicity: Tobacco smoke versus electronic cigarette aerosol. *Mutation Research/Reviews in Mutation Research, 789*, 108409. https://doi.org/10.1016/j.mrrev.2021.108409

Ullah, Md. R., Rahman, Md. A., Haque, Md. N., Sharker, Md. R., Islam, M. M., & Alam, Md. A. (2022). Nutritional profiling of some selected commercially important freshwater and marine water fishes of Bangladesh. *Heliyon, 8*(10), e10825. https://doi.org/10.1016/j.heliyon.2022.e10825

Valavanidis, A., Vlachogianni, T., & Fiotakis, C. (2009). 8-hydroxy-2' -deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis. *Journal of Environmental Science and Health, Part C, 27*(2), 120–139. https://doi.org/10.1080/10590500902885684

Vlachou, M., Kyrkou, G., Georgakopoulou, V., Kapetanaki, A., Vivilaki, V., Spandidos, D., & Diamanti, A. (2025). Smoke signals in the genome: Epigenetic consequences of parental tobacco exposure (Review). *Biomedical Reports, 23*(3), 1–9. https://doi.org/10.3892/br.2025.2024

Vogel, U., Nexø, B. A., Tjønneland, A., Wallin, H., Hertel, O., & Raaschou-Nielsen, O. (2006). ERCC1, XPD and RAI mRNA levels in lymphocytes are not associated with lung cancer risk in a prospective study of Danes. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 593*(1–2), 88–96. https://doi.org/10.1016/j.mrfmmm.2005.06.021

Wang, H., Liu, B., Chen, H., Xu, P., Xue, H., & Yuan, J. (2023). Dynamic changes of DNA methylation induced by benzo(a)pyrene in cancer. *Genes and Environment, 45*(1), 21. https://doi.org/10.1186/s41021-023-00278-1

Wang, J., Lee, J. J., Wang, L., Liu, D. D., Lu, C., Fan, Y.-H., Hong, W. K., & Mao, L. (2004). Value of p16 INK4a and RASSF1A Promoter Hypermethylation in Prognosis of Patients with Resectable Non–Small Cell Lung Cancer. *Clinical Cancer Research, 10*(18), 6119–6125. https://doi.org/10.1158/1078-0432.CCR-04-0652

Wang, M., Xiao, Y., Miao, J., Zhang, X., Liu, M., Zhu, L., Liu, H., Shen, X., Wang, J., Xie, B., & Wang, D. (2025). Oxidative Stress and Inflammation: Drivers of Tumorigenesis and Therapeutic Opportunities. *Antioxidants, 14*(6), 735. https://doi.org/10.3390/antiox14060735

West, R. (2017). Tobacco smoking: Health impact, prevalence, correlates and interventions. *Psychology & Health, 32*(8), 1018–1036. https://doi.org/10.1080/08870446.2017.1325890

Wetscher, G. J., Bagchi, M., Bagchi, D., Perdikis, G., Hinder, P. R., Glaser, K., & Hinder, R. A. (1995). Free radical production in nicotine treated pancreatic

tissue. *Free Radical Biology and Medicine, 18*(5), 877–882. https://doi.org/10.1016/0891-5849(94)00221-5

Xue, J., Yang, S., & Seng, S. (2014). Mechanisms of Cancer Induction by Tobacco-Specific NNK and NNN. *Cancers, 6*(2), 1138–1156. https://doi.org/10.3390/cancers6021138

Yamaguchi, N. H. (2019). Smoking, immunity, and DNA damage. *Translational Lung Cancer Research, 8*(S1), S3–S6. https://doi.org/10.21037/tlcr.2019.03.02

Yershova, K., Yuan, J., Wang, R., Valentin, L., Watson, C., Gao, Y., Hecht, S. S., & Stepanov, I. (2016). Tobacco-specific N-nitrosamines and polycyclic aromatic hydrocarbons in cigarettes smoked by the participants of the Shanghai Cohort Study. *International Journal of Cancer, 139*(6), 1261–1269. https://doi.org/10.1002/ijc.30178

Zanger, U. M., & Schwab, M. (2013). Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacology & Therapeutics, 138*(1), 103–141. https://doi.org/10.1016/j.pharmthera.2012.12.007

Zong, D., Liu, X., Li, J., Ouyang, R., & Chen, P. (2019). The role of cigarette smoke-induced epigenetic alterations in inflammation. *Epigenetics & Chromatin, 12*(1), 65. https://doi.org/10.1186/s13072-019-0311-8