

REVIEW ARTICLE

The environmental impact on aging: Insights from buccal mucosa and molecular biomarkers

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Abstract

Buccal epithelial cells serve as a primary barrier against the inhalation and ingestion of harmful substances, working alongside immune system cells such as natural killer cells to protect the body from health-damaging factors. These epithelial cells can also be used as an alternative tissue source for monitoring the genotoxic effects of external factors such as chemical exposure. This assessment can be performed using molecular biomarkers of aging, which reflect biological age and indicate cellular aging acceleration due to internal and external damage factors, such as environmental hazards. In contrast to chronological age, which merely reflects the passage of time, biological age accounts for individual variation in aging processes. Molecular biomarkers are crucial for distinguishing between normal and pathological processes in the body and for identifying the effects of external factors such as chemical exposures. The identification of specific biomarkers enhances the ability to detect and monitor adverse biological responses and accelerated aging. This review aims to highlight the routes through which environmental hazards enter the body, the application of buccal epithelial cells in assessing genetic modifications, and the introduction of potential molecular biomarkers. However, further research is necessary to elucidate the roles of these biomarkers in determining aging rates and individual variability. Understanding their implications may also help identify new therapeutic targets for preventing premature aging, treating age-related diseases, and developing potential treatments.

Keywords: Aging; Buccal cell; Biomarkers; DNA damage; Exposure; Hazards

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1. Introduction

Aging is a complex and inevitable process characterized by the accumulation of damage caused by both internal and external factors. These factors may be endogenous, such as genetic predispositions, or exogenous, including environmental exposures and lifestyle influences.¹ Humans are continuously exposed to various health risks, including inherited, nutritional, and environmental hazards, which may lead to contact with toxic substances. These hazards, encountered in workplaces or industrial settings, have the potential to cause harmful effects, such as mutations, cancer, and congenital defects,² as well as accelerate the aging process through genetic and epigenetic changes.³

Hazardous substances enter the body through various routes such as the oral cavity.⁴ The oral mucosal epithelium plays a crucial role in protecting the body from chemical,

microbial, and physical threats, serving as a barrier between external aggressors and underlying tissues.⁵ Due to its constant exposure to environmental hazards, cells in this region, including buccal epithelial cells^{6,7} and immune cells, such as natural killer (NK) cells,⁸ are particularly valuable for studying genetic and epigenetic changes. Research on oral epithelial cells and oral immunity offers significant potential for identifying biomarkers of genetic damage, assessing the impacts of aging, and understanding susceptibility to age-related diseases.⁵ By exploring genetic changes within these cells, researchers may gain insights into the mechanisms of aging and develop strategies for the early detection of age-related conditions.

2. Buccal mucosa

The oral mucosal epithelium acts as a barrier between the underlying tissues and the external environment,⁵ protecting the body from chemical, microbial, and physical threats.⁹ The oral epithelium consists of basal, prickle, intermediate, and superficial layers, which comprise structural, progenitor, and mature cells.^{5,10} Due to constant exposure to environmental forces, oral mucosal epithelial cells undergo continuous shedding¹¹ and renewal.^{12,13} Homeostasis in this tissue is maintained by a rich source of epithelial stem cells.¹³ Cell division in the basal stem cells of the stratified squamous layer continuously replaces shedding cells.¹¹ Newly generated cells by mitosis in the basal layer of the oral epithelium migrate to the surface to replace the sloughed-off epithelial cells.¹⁰ Potential risk factors for genetic damage in buccal cells include reactive oxygen species (ROS), viruses, seasonal changes, lifestyle factors, and chemical and physical conditions in residential and occupational settings.¹⁴ As buccal cells form the first barrier to inhaled and ingested substances,^{12,15,16} they can serve as an alternative tissue source for monitoring the genotoxic effects of chemical exposure^{11,17,18} in occupational and environmental settings.¹⁹⁻²¹

In addition, the fact that 92% of human cancers originate in epithelial cells highlights their significance for research^{20,22} and molecular studies. Furthermore, the direct exposure of buccal cells to environmental pollutants and their capability to metabolize carcinogens into reactive chemicals make them excellent sources for monitoring genotoxicity^{22,23} and for examining the relationship between occupational exposure and biomarkers.²⁴ Accordingly, buccal cells are an accessible source for investigating DNA damage.²⁵⁻²⁷ Interestingly, unlike blood lymphocytes, chromosomal damage in buccal cells continues to increase with age, potentially making them a valid biomarker for aging and a valuable resource for investigating genotoxicity and cytotoxicity parameters.²⁸

In addition, molecular modifications in the immune system, including oral immunity and related immune cells such as NK cells, can be used to identify the aging process. Since the majority of human pathogens are transmitted across mucosal surfaces, including the oral mucosae, studying oral immunity and the role of NK cells in defending against pathogens is essential.⁸ Buccal mucosal cells can be collected using cotton swabs, cytobrushes, the “swish and spit” method, or a modified version of the Guthrie card²⁹ for research related to identifying biomarkers and assessing risk factors such as exposure to environmental hazards.

3. Environmental exposure

Humans are exposed to inherited, nutritional, and environmental health risks.³⁰ Potentially harmful substances or situations, termed hazards,³¹ can cause toxic effects.^{32,33} These substances may be produced by agents in the workplace²³ and/or be prevalent in industrial areas, where they can induce mutations, cancer, and congenital defects.³⁰ Toxic materials typically enter the body by crossing barriers through dermal absorption, inhalation through the respiratory tract, or ingestion.^{31,34-36} In occupational settings, inhalation is the most important route of entry for toxic materials, followed by dermal contact and ingestion. Ingestion is generally considered the least important route of entry.⁴ Injection and ingestion are rare routes of chemical intake.³¹ It appears that ingestion may offer some protective role against increased sensitivity to certain materials, such as metals.^{37,38} However, chemical absorption more commonly occurs through other routes such as dermal adsorption of corrosive or irritants through the skin, respiratory tract, and eyes.³¹

Hazards can cause toxicological effects, including lethal or irreversible non-lethal outcomes, such as mutagenic effects after repeated or long-term exposure. The health effects of chemical exposures can be acute, chronic, local, systemic, reversible, or irreversible. The adverse biological effects of hazards are classified as very toxic, toxic, harmful, corrosive, and irritant.³¹ Figure 1 illustrates the entry routes and health effects of chemical exposures. The severity of damage caused by hazards depends on the toxicity level of the chemicals, the duration of exposure, the route of entry, and the individual's susceptibility to chemical effects (Table 1).

Since toxic exposure in occupational environments can cause numerous health problems,^{32,33} early identification of potential hazards is of utmost importance.³³ Workplace conditions and workers' behaviors are two major factors that increase the risk of toxic damage.³¹ For example, activities such as washing hands with petrol and contaminated

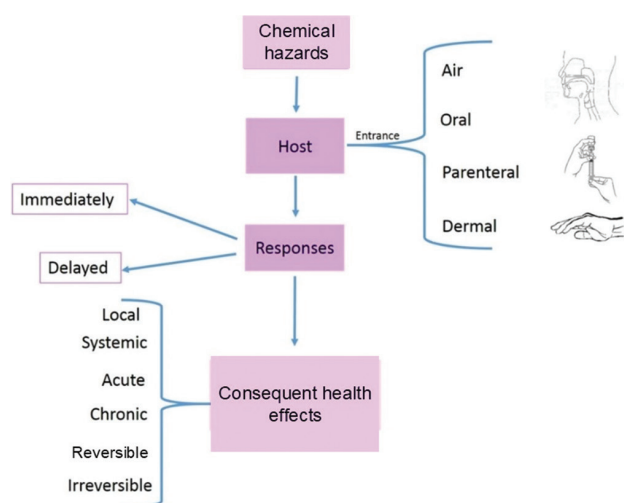


Figure 1. Entry routes of chemicals into the body and related reactions

Table 1. Factors affecting the toxicity level of chemicals in the body

Factors	Characteristics
Host	Age, heredity, gender, health status, immunology, nutrition, and hormones
Exposure dose	Concentration, rate of exposure, route of exposure, and duration
Chemicals properties	Composition (salt, free base, etc.), physical characters (particle size, liquid, solid, etc.), physical properties (instability, solubility, etc.), presence of impurities, breakdown product, and carrier
Others	Combination of chemicals, carrier

water,³⁰ inhaling toxic vapors,³⁹ and exposure to brake lining products⁴⁰ and engine ignitions can elevate the risk of genotoxicity in mechanical workshops.^{30,41-45} Workers are primarily exposed to complex chemical mixtures of organic or inorganic compounds in the forms of gases, vapors, fumes, mist, and particles.⁴¹ Once these chemicals enter or come into contact with the body, they can induce cytotoxic and genotoxic effects,^{30,41-45} leading to health damage.³⁴⁻³⁶ For instance, exposure to high concentrations of diesel exhaust is associated with an increased risk of lung and gastric cancers.⁴⁶

One of the known health damages of environmental exposure is premature aging, which is associated with changes in metabolic rates and the body's ability to activate, detoxify, and excrete xenobiotic compounds. These changes can affect the extent of damage and toxicity, as well as the rate of aging.^{47,48} In addition, environmental exposure can result in modifications in the activation of immune system cells, including NK cells,^{49,50} whose maintenance is essential for healthy aging.⁴⁹

Accordingly, occupational health is a major societal concern. Further studies are needed to identify genotoxic agents at environmental exposures, particularly in occupational settings, to protect the body from health damage.^{41,51} Developing strategies to improve health and safety in workplaces, such as mechanical workshops, is essential.⁴⁶ In addition, actions should be taken to either reduce the levels of harmful chemicals or increase the protection of exposed individuals to minimize adverse health effects.³⁰ For instance, diluting chemicals or using less toxic substances can reduce the risk of toxicity⁵¹ and genotoxic effects from chemical hazards.^{37,52-55} These measures can help protect the body from molecular modifications caused by hazardous chemical exposure, which may accelerate biological aging.³

4. Aging process

Aging results from the cumulative effects of damage caused by endogenous and exogenous factors.^{48,56} It can be defined in terms of both chronological and biological aging. Chronological age is measured from birth and indicates the time a person has lived. While it is linked to declining health, morbidity, and mortality, it does not accurately reflect the internal biological processes or individual variation. Therefore, the focus has shifted toward using biological markers of aging to measure biophysiological aging processes and determine biological age, which reflects the aging of cells within the body. The concept of "aging biomarkers" was first introduced by Baker and Sprott⁵⁷ in 1988 to predict functional capability based on biological parameters.

However, aging transitions an organism from full maturity to death, diminishing reproductive capability and survival.⁵⁸ It occurs in both unicellular and multicellular organisms,⁵⁹ progressing differently across tissues due to specific intrinsic cellular mechanisms. There is ongoing debate regarding whether aging originates from a specific tissue, such as the brain,⁶⁰ or affects all tissues simultaneously.⁶¹ Despite this, aging is known to start at the cellular level due to DNA damage and reduced DNA repair capacity.^{62,63} Senescent cells remain metabolically active but, morphologically, exhibit an increased volume and a flattened cytoplasm, accompanied by changes in gene expression, nuclear structure, protein processing, and metabolism.⁶⁴ Various factors, especially ROS and nitrogen species such as hydroxyl radicals, peroxy radicals, ozone, and nitrogen oxides, induce oxidative stress, leading to DNA damage, accelerated aging, and age-related health issues.⁶⁵ The accumulation of ROS⁶⁶ and toxic metabolic byproducts in the body results in decreased physiological function, loss of homeostasis,⁵⁸ tissue atrophy, neoplasms, and reduced organ and tissue function.⁶⁷ ROS contribute to aging by causing DNA damage, mitochondrial

dysfunction, and altering protein and lipid content in the body (Figure 2). Aging has also been linked to changes in DNA methylation levels and epigenetic modifications, both of which are influenced by environmental factors such as occupational exposures. The effects of ROS on aging are thought to be mediated through histone modification and DNA methylation, processes that contribute to the regulation of gene expression.⁶⁸

Molecular modifications in immune cells, such as NK cells, can play a critical role in the initiation and progression of senescence and age-related diseases,⁶⁹ including infection, malignancies, and inflammatory disorders. These changes are consequences of decreased immune system function and a process known as inflammaging, which refers to the chronic, low-grade inflammation associated with aging.⁷⁰ Therefore, NK cells in the buccal mucosa provide an interesting resource for assessing aging in the body.⁸

Exposure to environmental hazards can transform normal cells into abnormal ones⁷¹ by interacting with genetic material and changing DNA and/or RNA in cells.⁷² These genetic alterations can affect cellular functions, including those of immune system cells such as NK cells.⁵⁰ This transformation may occur within a latency period of 4 – 40 years following initial exposure.⁷¹ As aging is a time-related process resulting from life-long exposure to low and natural levels of environmental agents, regular exposure to chemicals in daily occupational activities poses a higher risk of premature aging, health damage, and decreased productivity.⁶⁷ Biomarkers of aging and biological aging can assess these risks, reflecting individual-specific age-related vulnerabilities, physiological function, and overall health.³

5. Related molecular biological markers of aging

Aging is a heterogeneous process, with significant variability in health outcomes among individuals of the

same chronological age. At the individual level, different cells, tissues, and organs age at different trajectories. Biological aging can be affected by exposure to chemical hazards, particularly through DNA damage in cells such as buccal cells, which serve as the first line of defense against foreign particles entering the body.⁹

DNA damage resulting from both internal and external factors poses a significant threat to living cells.⁷³ Genotoxic agents derived from exogenous or endogenous sources – including UV light, ionizing radiation, chemicals, and the intrinsic biochemical instability of DNA – can disrupt cellular homeostasis by causing DNA damage^{74,75} and altering its chemical structure.⁷⁶ DNA damage, along with chromosomal changes caused by mutations, DNA adducts, DNA breaks, and alkali-labile sites,⁷⁷ can trigger⁷⁸ and accelerate the aging process.⁷⁷ The effects of chemical exposure on genotoxic materials can be monitored³⁰ using biomarkers, which assess molecular and cellular events in biological systems.^{79,80} These biomarkers, as listed in Table 2, are categorized into three groups: exposure, susceptibility, and early effects^{24,81} (Figure 3).

Exposure biomarkers measure substances, metabolites, or products resulting from the interaction of target molecules with tissue, reflecting external and/or internal

Table 2. Types of biomarkers

Biomarkers	Description
Exposure	Biomarkers of internal dose, biomarkers of biologically effective dose (e.g., DNA adducts)
Effect	DNA breaks, chromosome aberrations, micronuclei, sister chromatic exchange, biochemical effects, oxidative damage, DNA repair enzymes, metal-binding proteins
Susceptibility	Single nucleotide polymorphisms, telomere length, epigenetics, metabolism enzyme induction, varying DNA repair capacity

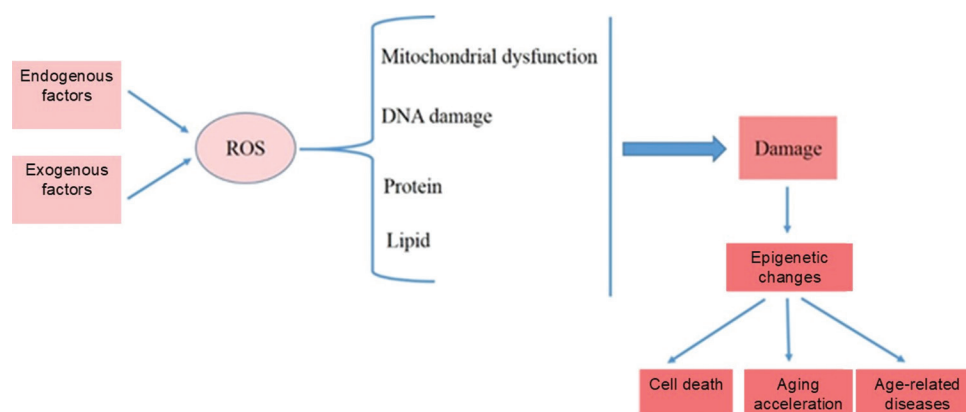


Figure 2. A schematic representation of reactive oxygen species effects on cellular senescence

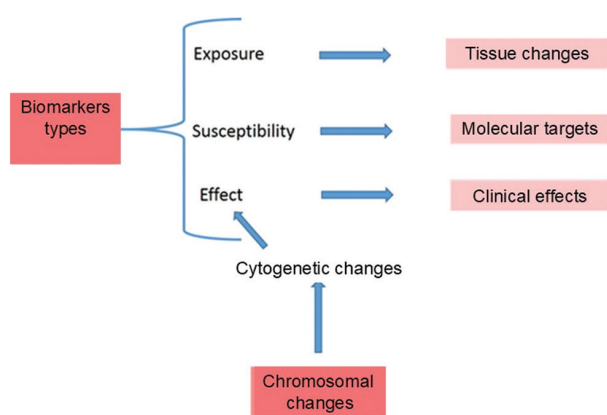


Figure 3. Biomarkers associated with environmental exposure

exposures.^{24,82,83} These biomarkers indicate short-term DNA or chromosomal damage in cytogenetic analyses³⁰ and serve as sensitive indicators of recent human exposures to hazards.⁸⁴ Consequently, the potential risk posed by hazards and their ability to induce long-term health problems can be determined.³⁰ Biomarkers of effect show early health effects, such as health impairment and critical and clinical effects.^{24,82,83} Biomonitoring the effects of exposure through various cytogenetic tests enables the evaluation of genotoxic effects by detecting DNA damage, such as sister chromatid exchange and micronuclei.⁴⁶ Micronuclei, an example of such biomarkers,⁸⁴ are formed due to chromosomal damage,⁸⁵ typically arising from chromosomal fragmentation or lagging.⁸⁶ This biomarker is evaluated in cells, such as buccal cells, and reflects genotoxic damage in the dividing basal cell layer of target organs 1 – 3 weeks earlier.⁴⁶ Micronucleus levels tend to increase with age.⁸⁶ The first effective biomarkers used to assess the aging process were micronuclei, sister chromatid exchange, and chromosome aberrations.⁷⁷

Biomarkers of susceptibility indicate an individual's increased sensitivity to specific target molecules or metabolic processes, leading to a higher dose of the target compound upon exposure.^{24,82,83} These biomarkers reveal the inherent or acquired capability of an organism to metabolize xenobiotic substances upon chemical exposure, resulting in variations in metabolic pathways and toxic responses among individuals. Examples of susceptibility biomarkers include cytochrome P-450 (CYPs) and glutathione transferases (GSTs).⁸⁷ Telomere shortening and epigenetic modifications are also included in this group.⁸⁸ Epigenetic biomarkers reflect stable changes to the genome that affect gene expression or silencing.^{3,89} Factors such as DNA methylation levels, cell metabolism, and signal transmission control through epigenetic mechanisms play significant roles in human longevity.⁶⁸

Moreover, changes in biomarkers related to NK cells, such as cytokines, including interferon (IFN)- γ , IFN- β , and interleukins (IL-2), as well as alterations in NK cell types, are associated with aging. NK cells may also exhibit telomere shortening, a marker of aging,⁷⁰ which can be investigated using buccal cells. This approach enhances our understanding of aging and age-related diseases and aids in designing future immune therapies targeting NK cells in the elderly population.⁶⁹ Identifying reliable biomarkers is crucial for accurate risk stratification and exploring antiaging interventions.³ Given the challenge of pinpointing a single biomarker that reflects the intricate interaction between environmental exposures and aging, further studies are needed to identify biomarkers sensitive to mixed or low-dose exposures. In addition, a combination of biomarkers reflecting various levels of cellular organization, such as DNA, RNA, and proteins, may provide the most effective approach.

6. Interaction between molecular biomarkers and aging

Genomic damages after exposure to chemicals can occur in two forms: (i) point mutations at the nucleotide level in a gene and (ii) structural changes in chromosomes, such as chromosomal aberrations.⁹⁰ Two major perspectives on senescence include: (i) a specific genetic program for senescence and (ii) evolutionary forces, particularly reproductive success, that suggest a genetic basis for senescence.⁵⁸ Senescence results from the coordinated actions of multiple genes, and mutations in these genes can lead to premature aging.⁶⁷

Genetic analysis of aging helps identify regulatory links between gene expressions and lifespan,⁹¹ involving pathways such as insulin/IGF-1 signaling, PI3K, TOR, MAPK, AMPK, PKC, NF- κ B, TGF- β , Notch, WNT, S6K,⁹²⁻⁹⁶ and the p53/p21 and p16INK4A/pRb pathways.⁶⁴ The p16INK4A/pRb pathway can lead to premature senescence or stress-induced premature senescence. Oncogene-induced senescence is characterized by the activation of pathways involving p53 and DNA damage and is marked by tumor progression restriction without telomere shortening.⁶⁴

DNA repair pathways play an important role in correcting damage caused by mutagens, such as DNA damage⁷³ and chromosomal alterations,⁷⁹ which pose significant threats to living cells. These repair processes involve cell cycle regulation⁹⁷ and mechanisms such as the SOS response,⁷⁶ utilizing ATP and NADH to remove DNA lesions.⁶² It is estimated that over 130 genes in the human genome are involved in DNA repair,⁹⁸ with more than 70 genes contributing directly to the major nuclear DNA repair pathways.⁹⁹ Mutations in these repair genes

can trigger premature aging syndromes, such as Werner syndrome, Cockayne syndrome, Bloom's syndrome, and ataxia telangiectasia, suggesting possible roles of replicative senescence genes in the aging process.⁶² *CDK6*, *CCND1*, and *p16* are examples of such genes that contribute to aging.¹⁰⁰ The *CDK6* and *CCND1* genes play crucial roles in regulating the cell cycle through the retinoblastoma pathway¹⁰¹ and in growth arrest alongside the *p16* and *Rb* genes. During senescence, the *Rb* and *p53* genes are activated but gradually lose their ability to regulate cell proliferation.⁹¹

Many genes involved in aging are primarily located on chromosomes 1, 4, 6, 7, and 20. Telomerase suppressor genes are found on chromosomes 3 and 4, whereas the *WRN* gene, which is associated with Werner syndrome and premature aging, is localized on chromosome 8.⁶² Several genes also serve as biomarkers for susceptibility to environmental damage.¹⁰² This group includes genes from the CYP and GST families, which are crucial for the metabolism and detoxification of environmental hazards and xenobiotics.^{103,104} CYP enzymes contribute to phase I xenobiotic metabolism by oxidizing compounds, leading to the formation of active substances and highly reactive mutagenic metabolites.¹⁰⁵ Key members of this group include CYP1A1, CYP1A2, and CYP2E1. While CYP1A1¹⁰⁴ and CYP1A2¹⁰⁵ have been documented to potentially offer protective effects against DNA damage and aging, some reports suggest paradoxical effects, particularly for CYP1A1.¹⁰⁶ For instance, polymorphisms in *CYP2E1* have been shown to impact aging^{24,107} by inducing DNA damage.¹⁰⁷⁻¹⁰⁹ The GST family, also involved in xenobiotic metabolism, detoxifies xenobiotics.¹¹⁰ Notable members include the *GSTT1*, *GSTM1*, and *GSTP1* genes, which have

been studied for their effects on aging. For instance, the absence of *GSTM1*¹⁰³ and *GSTT1*^{83,102} has been associated with aging, and polymorphisms in the *GSTP1* gene have demonstrated both progressive^{24,111,112} and non-progressive effects^{24,113} on the aging process.

Another significant activator of cellular senescence is telomere dysfunction, which operates through the G1 DNA damage checkpoint and the upregulation of p21/p16. Telomeres are complex DNA-protein structures at the ends of eukaryotic chromosomes, and their shortening serves as a marker of DNA damage in cells.⁸⁸ By capping chromosome ends, telomeres prevent nucleolytic degradation, end-to-end fusion, irregular recombination, and other lethal cellular events.¹¹⁴ Telomere length is inversely correlated with age¹¹⁴ and shortens in all replicating somatic cells,⁸⁸ including NK cells, which are crucial for recognizing and eliminating senescent cells. As telomere shortening progresses with age, it gradually impairs NK cell function.¹¹⁵ The functional activities of NK cells, such as cytokine production in CD56^{bright} cells and cytotoxic activity in CD56^{dim} cells, shift with aging – CD56^{bright} cells decrease whereas CD56^{dim} cells increase.¹¹⁶ {Weber, 2024 #118} This shift results in impaired immunity and a heightened risk of age-related diseases.¹¹⁷ These changes are associated with declines in biomarkers such as IL-2, IL-15, and CD56^{bright}, alongside increases in others such as IL-6,⁷⁰ IFN- γ ,¹¹⁸ and CD38,¹¹⁹ all of which contribute to accelerated aging and age-related health issues. Consequently, these changes lead to a weakened immune response and increased inflammation associated with aging, making NK cells a significant target for interventions aimed at mitigating age-related immune decline. In addition, the function of NK

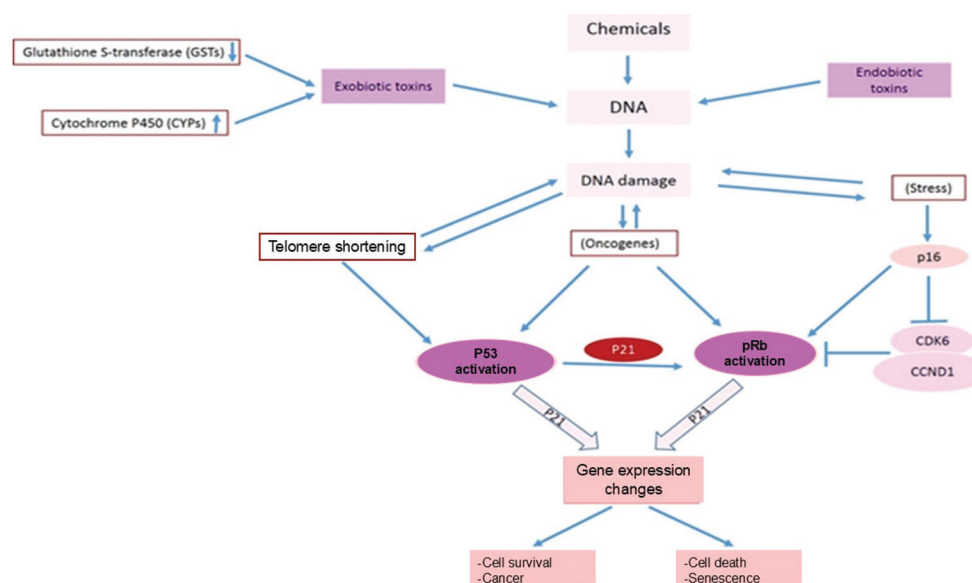


Figure 4. Consequences of DNA damage following chemical exposure

Table 3. Examples of biomolecular changes in aging

Type	Effect	Examples	References
Pathways	Mediated cell survival, growth, and DNA repair	AKT1, ATM, FOXO1, FOXO3, GHR, HIF1A, IGF1, IGF1R, PIK3CA, and PIK3CB	96
	Mediated apoptosis/senescence signaling pathway	BAX, BCL2, CDK4, CDK6, CDKN1A (P21), CDKN2A (P16), CDKN2B (P15), FAS, and TP53	96
	Autophagy/Survival	AMPK subunits (PRKAA1, PRKAA2, PRKAB2, PRKAG1, PRKAG2), SIRT1, RPS6KB1 (S6K), and TSC2	96
	Cross-talk between the aging pathways	KL, MYC, NFKB1, NFKB2, PPARGC1A, PTEN, TGFB1, and TGFBR2	96
	Sirtuins are involved in metabolic control, apoptosis, cell survival, development, inflammation, and healthy aging	Sir2, SIRT1, PGC-1 α , Ku70, NF- κ B, AceCS1, MEF2 and p53, FOXO transcription factors	127
	Lifespan regulation through mitochondrial DNA	PGC-1 α , PGC-1 β , NRF-1, and ERR α	127
	Stable housekeeping genes in aging	18s, HPRT1, ACTB, and TMEM199	128,129
Epigenetics	DNA methylation, histone modifications, non-coding RNAs		130
Natural killer cells in the buccal mucosa (CD56 ^{dim} cells and CD56 ^{bright} cells)	Biomarkers modifications during aging	IFN- γ , TNF- α , TNF- β , GM-CSF, CXCR3, CCR6, CCR9, CCR4, IL-10, IL-4, IL6, IL-13, IL-2, IL-15, and IL-12	69,70,117,131-137

Abbreviations: IFN- γ : Interferon- γ ; TNF- α : Tumor necrosis factor- α ; TNF- β : Tumor necrosis factor- β ; IL: Interleukin.

cells¹²⁰ and the aging process¹²¹ can be regulated through epigenetic changes in the body.

Epigenetics, including DNA methylation, histone modifications, and non-coding RNAs,¹²² plays a crucial role in the regulation of aging. These epigenetic modifications are key regulators of gene expression¹²² and influence cellular responses to environmental exposures,⁹⁹ as well as changes associated with aging.¹²² Various environmental factors, such as diet, exposure to toxins, stress, smoking, and childhood trauma, can significantly impact individual epigenetic clocks and gene regulation.¹²³ These changes are directly linked to longevity across a wide range of organisms, from yeast to humans.¹²⁴

Exposure to environmental chemicals can lead to changes in epigenetic biomarkers and genome function,^{125,126} which accelerate aging by influencing caloric restriction, mitochondrial function, the activity of housekeeping genes, and metabolic enzyme control.¹²² Figure 4 illustrates how these factors contribute to cellular senescence. Despite these findings, further efforts are needed to identify the genetic and epigenetic factors that contribute to aging and to determine their precise effects, thereby expanding the list of contributing factors (Table 3).

7. Conclusion

Given the profound impact of aging on mortality and disease susceptibility, researchers are continually developing non-

invasive methods and identifying molecular biomarkers to monitor cellular damage caused by both external and internal factors. Environmental hazards are well-documented contributors to aging, leading to DNA damage and premature aging, which, in turn, increase the incidence of diseases and premature mortality. As a result, molecular biomarkers are being investigated for their potential to assess DNA damage in cells, including buccal and NK cells found in the oral mucosa, to enable the screening and diagnosis of individuals at risk of premature aging. This review aims to clarify the intricate relationship between environmental hazards, cellular senescence, and DNA damage, thereby aiding in the development of diagnostic and preventive measures. Further research is essential to fully understand the role of biomarkers in assessing DNA damage during aging and to identify new targets for strategies aimed at preventing and treating premature aging and age-related health issues.

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Conflict of interest

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Authors contributions

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Not applicable.

Consent for publication

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References

- Rodríguez-Rodero S, Fernández-Morera JL, Menéndez-Torre E, Calvanese V, Fernández AF, Fraga MF. Aging genetics and aging. *Aging Dis.* 2011;2(3):186-195.
- Tulchinsky TH, Varavikova EA. Environmental and occupational health. In: *The New Public Health*. Amsterdam: Elsevier; 2014. p. 471.
- Chen R, Wang Y, Zhang S, *et al.* Biomarkers of ageing: Current state-of-art, challenges, and opportunities. *MedComm Futur Med.* 2023;2:e50.
doi: 10.1002/mef2.50
- Cherrie JW, Semple S, Christopher Y, Saleem A, Hughson GW, Philips A. How important is inadvertent ingestion of hazardous substances at work? *Ann Occup Hyg.* 2006;50(7):693-704.
doi: 10.1093/annhyg/mel035
- Groeger S, Meyle J. Oral mucosal epithelial cells. *Front Immunol.* 2019;10:208.
doi: 10.3389/fimmu.2019.00208
- Farah R, Haraty H, Salame Z, Fares Y, Ojcius DM, Sadier NS. Salivary biomarkers for the diagnosis and monitoring of neurological diseases. *Biomed J.* 2018;41(2):63-87.
doi: 10.1016/j.bj.2018.03.004
- Langie SA, Moisse M, Declerck K, *et al.* Salivary DNA methylation profiling: Aspects to consider for biomarker identification. *Basic Clin Pharmacol Toxicol.* 2017;121:93-101.
doi: 10.1111/bcpt.12721
- Li H, Reeves RK. Functional perturbation of classical natural killer and innate lymphoid cells in the oral mucosa during SIV infection. *Front Immunol.* 2013;3:417.
doi: 10.3389/fimmu.2012.00417
- Handajani J, Tabtila U, Auliawati NR, Rohman A. Characterization of buccal cell DNA after exposure to azo compounds: A cross-sectional study. *F1000Res.* 2020;9:1053.
doi: 10.12688/f1000research.25798.2
- Celik A, Cavas T, Ergene-Gozukara S. Cytogenetic biomonitoring in petrol station attendants: Micronucleus test in exfoliated buccal cells. *Mutagenesis.* 2003;18(5):417-421.
doi: 10.1093/mutage/geg022
- Surrallés J, Autio K, Nylund L, *et al.* Molecular cytogenetic analysis of buccal cells and lymphocytes from benzene-exposed workers. *Carcinogenesis.* 1997;18(4):817-823.
doi: 10.1093/carcin/18.4.817
- Holland N, Bolognesi C, Kirsch-Volders M, *et al.* The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps. *Mutat Res.* 2008;659(1):93-108.
doi: 10.1016/j.mrrev.2008.03.007
- Papagerakis S, Pannone G, Zheng L, *et al.* Oral epithelial stem cells - Implications in normal development and cancer metastasis. *Exp Cell Res.* 2014;325(2):111-129.
doi: 10.1016/j.yexcr.2014.04.021
- Eshkoora SA, Ismail P, Rahman SA, Moin S, Adon MY. The association of DNA damage level with early age at the occupational exposure in the mechanical workshops workers. *Asian J Biotechnol.* 2012;4(2):83-91.
doi: 10.3923/ajbkr.2012.83.91
- Motgi AA, Chavan MS, Diwan NN, Chowdhery A, Channe PP, Shete MV. Assessment of cytogenic damage in the form of micronuclei in oral epithelial cells in patients using smokeless and smoked form of tobacco and non-tobacco users and its relevance for oral cancer. *J Cancer Res Ther.* 2014;10(1):165-170.
doi: 10.4103/0973-1482.131454
- Pradeep M, Guruprasad Y, Jose M, Saxena K, Deepa K, Prabhu V. Comparative Study of genotoxicity in different tobacco related habits using micronucleus assay in exfoliated buccal epithelial cells. *J Clin Diagn Res.* 2014;8(5):ZC21-ZC24.
doi: 10.7860/JCDR/2014/8733.4357
- Spivack SD, Hurteau GJ, Jain R, *et al.* Gene-environment interaction signatures by quantitative mRNA profiling in exfoliated buccal mucosal cells. *Cancer Res.* 2004;64(18):6805-6813.
doi: 10.1158/0008-5472.CAN-04-1771
- Chen C, Arjomandi M, Qin H, Balmes J, Tager I, Holland N. Cytogenetic damage in buccal epithelia and peripheral lymphocytes of young healthy individuals exposed to ozone. *Mutagenesis.* 2006;21(2):131-137.

- doi: 10.1093/mutage/gel007
19. Manikantan P, Balachandar V, Sasikala K, Mohanadevi S. DNA damage in viscose factory workers occupationally exposed to carbon di-sulfide using buccal cell comet assay. *Braz J Oral Sci.* 2009;8(4):197-200.
doi: 10.20396/bjos.v8i4.8642078
 20. Rajkokila K, Shajithanoop S, Usharani MV. Nuclear anomalies in exfoliated buccal epithelial cells of petrol station attendants in Tamilnadu, South India. *J Med Genet Genomics.* 2010;2(2):18-22.
 21. Lorenzoni DC, Pinheiro LP, Nascimento HS, *et al.* Could formaldehyde induce mutagenic and cytotoxic effects in buccal epithelial cells during anatomy classes? *Med Oral Patol Oral Cir Bucal.* 2017;22(1):e58.
doi: 10.4317/medoral.21492
 22. Salama SA, Serrana M, Au WW. Biomonitoring using accessible human cells for exposure and health risk assessment. *Mutat Res.* 1999;436(1):99-112.
doi: 10.1016/s1383-5742(98)00021-0
 23. Eshkoor SA, Ismail P, Rahman SA, Moin S, Adon MY. Occupational exposure as a risk factor to enhance the risk of early ageing. *Asian J Biotechnol.* 2011;3(6):573-580.
doi: 10.3923/ajbkr.2011.573.580
 24. Heuser VD, Erdtmann B, Kvitko K, Rohr P, da Silva J. Evaluation of genetic damage in Brazilian footwear-workers: Biomarkers of exposure, effect, and susceptibility. *Toxicology.* 2007;232(3):235-247.
doi: 10.1016/j.tox.2007.01.011
 25. Borthakur G, Butryee C, Stacewicz-Sapuntzakis M, Bowen PE. Exfoliated buccal mucosa cells as a source of DNA to study oxidative stress. *Cancer Epidemiol Biomarkers Prev.* 2008;17(1):212-219.
doi: 10.1158/1055-9965.EPI-07-0706
 26. Hearn R, Arblaster K. DNA extraction techniques for use in education. *Biochem Mol Biol Educ.* 2010;38(3):161-166.
doi: 10.1002/bmb.20351
 27. Toy E, Yuksel S, Ozturk F, Karatas OH, Yalcin M. Evaluation of the genotoxicity and cytotoxicity in the buccal epithelial cells of patients undergoing orthodontic treatment with three light-cured bonding composites by using micronucleus testing. *Korean J Orthod.* 2014;44(3):128-135.
doi: 10.4041/kjod.2014.44.3.128
 28. Franzke B, Schober-Halper B, Hofmann M, *et al.* Chromosomal stability in buccal cells was linked to age but not affected by exercise and nutrients-Vienna Active Ageing Study (VAAS), a randomized controlled trial. *Redox Biol.* 2020;28:101362.
doi: 10.1016/j.redox.2019.101362
 29. Michalczyk A, Varigos G, Smith L, Ackland ML. Fresh and cultured buccal cells as a source of mRNA and protein for molecular analysis. *Biotechniques.* 2004;37(2):262-269.
doi: 10.2144/04372RR03
 30. Martino-Roth MG, Viegas J, Amaral M, Oliveira L, Ferreira FLS, Erdtmann B. Evaluation of genotoxicity through micronuclei test in workers of car and battery repair garages. *Genet Mol Biol.* 2002;25(4):495-500.
doi: 10.1590/S1415-47572002000400021
 31. Herber RFM, Duffus JH, Christensen JM, Olsen E, Park MV. Risk assessment for occupational exposure to chemicals. A review of current methodology (IUPAC technical report). *Pure Appl Chem.* 2001;73(6):993-1031.
doi: 10.1351/pac200173060993
 32. Fairhurst S. Hazard and risk assessment of industrial chemicals in the occupational context in Europe: Some current issues. *Food Chem Toxicol.* 2003;41(11):1453-1462.
doi: 10.1016/S0278-6915(03)00193-5
 33. Hassim MH, Hurme M. Occupational chemical exposure and risk estimation in process development and design. *Process Saf Environ Prot.* 2010;88(4):225-235.
doi: 10.1016/j.psep.2010.03.011
 34. Dinman BD, Dinman JD. The mode of absorption, distribution, and elimination of toxic materials. In: Harris RL, editor. *Patty's Industrial Hygiene*. New York: John Wiley & Son Inc.; 2000.
doi: 10.1002/0471435139.hyg003
 35. Borm PJA, Robbins D, Haubold S, *et al.* The potential risks of nanomaterials: A review carried out for ECETOC. *Part Fibre Toxicol.* 2006;3:11.
doi: 10.1186/1743-8977-3-11
 36. Nielsen GD, Ovrebo S. Background, approaches and recent trends for setting health-based occupational exposure limits: A minireview. *Regul Toxicol Pharmacol.* 2008;51(3):253-269.
doi: 10.1016/j.yrtph.2008.04.002
 37. Artik S, Haarhuis K, Wu X, Begerow J, Gleichmann E. Tolerance to nickel: Oral nickel administration induces a high frequency of anergic T cells with persistent suppressor activity. *J Immunol.* 2001;167(12):6794-6803.
doi: 10.4049/jimmunol.167.12.6794
 38. Rustemeyer T, de Groot J, von Blomberg BME, Frosch PJ, Scheper RJ. Induction of tolerance and cross-tolerance to methacrylate contact sensitizers. *Toxicol Appl Pharmacol.* 2001;176(3):195-202.
doi: 10.1006/taap.2001.9266.
 39. Moller P, Knudsen LE, Loft S, Wallin H. The Comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors.

- Cancer Epidemiol Biomarkers Prev.* 2000;9(10):1005-1015.
40. Keshava N, Ong TM. Occupational exposure to genotoxic agents. *Mutat Res.* 1999;437(2):175-194.
doi: 10.1016/s1383-5742(99)00083-6
 41. Benites CI, Amado LL, Vianna RAP, Martino-Roth MG. Micronucleus test on gas station attendants. *Genet Mol Res.* 2006;5(1):45-54.
 42. Conaway CC, Schreiner CA, Cragg ST. *Mutagenicity Evaluation of Petroleum Hydrocarbons*. Vol. 7. New Jersey: Princeton Scientific Publishers Inc.; 1984. p. 1-302.
 43. Loury DJ, Smith-Oliver T, Strom S, Jirtle R, Michalopoulos G, Butterworth BE. Assessment of unscheduled and replicative DNA synthesis in hepatocytes treated *in vivo* and *in vitro* with unleaded gasoline or 2,2,4-trimethylpentane. *Toxicol Appl Pharmacol.* 1986;85(1):11-23.
doi: 10.1016/0041-008x(86)90383-2
 44. Nylander PO, Olofsson H, Rasmuson B, Svahlin H. Mutagenic effects of petrol in *Drosophila melanogaster* I. Effects of benzene and 1, 2-dichloroethane. *Mutat Res.* 1978;57(2):163-167.
doi: 10.1016/0027-5107(78)90263-4
 45. Pitarque M, Carbonell E, Xamena N, Creus A, Marcos R. Genotoxicity of commercial petrol samples in cultured human lymphocytes. *Rev Int Contam Ambient.* 1997;13(1):15-21.
 46. Garcia PV, Linhares D, Amaral AFS, Rodrigues AA. Exposure of thermoelectric power-plant workers to volatile organic compounds from fuel oil: Genotoxic and cytotoxic effects in buccal epithelial cells. *Mutat Res.* 2012;747(2):197-201.
doi: 10.1016/j.mrgentox.2012.05.008
 47. Thomas RD. Age-specific carcinogenesis: Environmental exposure and susceptibility. *Environ Health Perspect.* 1995;103(Suppl 6):45-48.
doi: 10.1289/ehp.95103s645
 48. Yin D, Chen K. The essential mechanisms of aging: Irreparable damage accumulation of biochemical side-reactions. *Exp Gerontol.* 2005;40(6):455-465.
doi: 10.1016/j.exger.2005.03.012
 49. Tsao T, Tsai M, Hwang J, *et al.* Health effects of a forest environment on natural killer cells in humans: An observational pilot study. *Oncotarget.* 2018;9(23):16501-16511.
doi: 10.18632/oncotarget.24741
 50. De Celis R, Feria-Velasco A, Bravo-Cuellar A, *et al.* Expression of NK cells activation receptors after occupational exposure to toxics: A preliminary study. *Immunol Lett.* 2008;118(2):125-131.
doi: 10.1016/j.imlet.2008.03.010
 51. Unnikrishnan S, Hegde DS. An analysis of cleaner production and its impact on health hazards in the workplace. *Environ Int.* 2006;32(1):87-94.
doi: 10.1016/j.envint.2005.05.023
 52. Stokinger HE. Concepts of thresholds in standards setting. An analysis of the concept and its application to industrial air limits (TLVs). *Arch Environ Health.* 1972;25(3):153-157.
doi: 10.1080/00039896.1972.10666154
 53. Zielhuis RL, Notten WRF. Permissible levels for occupational exposure; Basic concepts. *Int Arch Occup Environ Health.* 1979;42(3-4):269-281.
doi: 10.1007/BF00377781
 54. Henschler D. Exposure limits: History, philosophy, future developments. *Ann Occup Hyg.* 1984;28(1):79-92.
doi: 10.1093/annhyg/28.1.79
 55. Hunter WJ, Aresini G, Haigh R, Papadopoulos P, von der Hude W. Occupational exposure limits for chemicals in the European Union. *Occup Environ Med.* 1997;54(4):217-222.
doi: 10.1136/oem.54.4.217
 56. Semsei I. On the nature of aging. *Mech Ageing Dev.* 2000;117(1):93-108.
doi: 10.1016/s0047-6374(00)00147-0
 57. Baker GT 3rd, Sprott RL. Biomarkers of aging. *Exp Gerontol.* 1988;23(4-5):223-239.
doi: 10.1016/0531-5565(88)90025-3
 58. Crews DE. *Human Senescence Evolutionary and Biocultural Perspectives*. Columbus, Ohio, U.S.A: Ohio State University, Cambridge University Press; 2004. p. 302.
 59. Bowen RL, Atwood CS. Living and dying for sex. A theory of aging based on the modulation of cell cycle signaling by reproductive hormones. *Gerontology.* 2004;50(5):265-290.
doi: 10.1159/000079125
 60. Mattson MP, Duan W, Maswood N. How does the brain control lifespan? *Ageing Res Rev.* 2002;1(2):155-165.
doi: 10.1016/s1568-1637(01)00003-4
 61. Kowald A, Kirkwood TB. Towards a network theory of ageing: A model combining the free radical theory and the protein error theory. *J Theor Biol.* 1994;168(1):75-94.
doi: 10.1006/jtbi.1994.1089
 62. Wojda A, Witt M. Manifestations of ageing at the cytogenetic level. *J Appl Genet.* 2003;44(3):383-399.
 63. Yadav JS, Chhillar AK. Cytogenetic damage in individuals exposed to vehicular pollution. *Int J Hum Genet.* 2002;2(2):113-117.
doi: 10.1080/09723757.2002.11885797
 64. Shawi M, Autexier C. Telomerase, senescence and ageing. *Mech Ageing Dev.* 2008;129(1-2):3-10.

- doi: 10.1016/j.mad.2007.11.007
65. Getof N. Anti-aging and aging factors in life. The role of free radicals. *Radiat Phys Chem.* 2007;76(10):1577-1586.
doi: 10.1016/j.radphyschem.2007.01.002
 66. Beckman KB, Ames BN. The free radical theory of aging matures. *Physiol Rev.* 1998;78(2):547-581.
doi: 10.1152/physrev.1998.78.2.547
 67. Vijg J. Somatic mutations and aging: A re-evaluation. *Mutat Res.* 2000;447(1):117-135.
doi: 10.1016/s0027-5107(99)00202-x
 68. da Costa JP, Vitorino R, Silva GM, Vogel C, Duarte AC, Rocha-Santos T. A synopsis on aging-Theories, mechanisms and future prospects. *Ageing Res Rev.* 2016;29:90-112.
doi: 10.1016/j.arr.2016.06.005
 69. Qi C, Liu Q. Natural killer cells in aging and age-related diseases. *Neurobiol Dis.* 2023;183:106156.
doi: 10.1016/j.nbd.2023.106156
 70. Brauning A, Rae M, Zhu G, *et al.* Aging of the immune system: Focus on natural killer cells phenotype and functions. *Cells.* 2022;11(6):1017.
doi: 10.3390/cells11061017
 71. Wojcik A, Brzeski Z, Kolodziej K, Lojko W, Letkiewicz D, Sieklucka-Dziuba M. Evaluation of toxicological threat to health caused by some carcinogenic factors in the working environment of an industrial plant. *Pol J Environ Stud.* 2000;9(6):531-536.
 72. Adamus T, Mikulenkova I, Dobias L, Havrankova J, Pek T. Cytogenetic methods and biomonitoring of occupational exposure to genotoxic factors. *J Appl Biomed.* 2006;4:197-203.
doi: 10.32725/jab.2006.022
 73. Verger A, Crossley M. Chromatin modifiers in transcription and DNA repair. *Cell Mol Life Sci.* 2004;61(17):2154-2162.
doi: 10.1007/s00018-004-4176-y
 74. Powell CL, Swenberg JA, Rusyn I. Expression of base excision DNA repair genes as a biomarker of oxidative DNA damage. *Cancer Lett.* 2005;229(1):1-11.
doi: 10.1016/j.canlet.2004.12.002
 75. Friedberg EC, McDaniel LD, Schultz RA. The role of endogenous and exogenous DNA damage and mutagenesis. *Curr Opin Genet Dev.* 2004;14(1):5-10.
doi: 10.1016/j.gde.2003.11.001
 76. Hoeijmakers JHJ. Genome maintenance mechanisms for preventing cancer. *Nature.* 2001;411(6835):366-374.
doi: 10.1038/35077232
 77. Bolognesi C, Lando C, Forni A, *et al.* Chromosomal damage and ageing: Effect on micronuclei frequency in peripheral blood lymphocytes. *Age Aging.* 1999;28:393-397.
doi: 10.1093/ageing/28.4.393
 78. Ono T, Uehara Y, Saito Y, Ikehata H. Mutation theory of aging, assessed in transgenic mice and knockout mice. *Mech Ageing Dev.* 2002;123(12):1543-1552.
doi: 10.1016/s0047-6374(02)00090-8
 79. Zhang L, Eastmond DA, Smith MT. The nature of chromosomal aberrations detected in humans exposed to benzene. *Crit Rev Toxicol.* 2002;32(1):1-42.
doi: 10.1080/20024091064165
 80. Yadav AS, Jaggi S. Buccal micronucleus cytome assay-A biomarker of genotoxicity. *J Mol Biomarkers Diagn.* 2015;6:236.
doi: 10.4172/2155-9929.1000236
 81. Smith MT, Zhang L. Biomarkers of leukemia risk: Benzene as a model. *Environ Health Perspect.* 1998;106:937-946.
doi: 10.1289/ehp.98106s4937
 82. Nordberg GF. Biomarkers of exposure, effects and susceptibility in humans and their application in studies of interactions among metals in China. *Toxicol Lett.* 2010;192(1):45-49.
doi: 10.1016/j.toxlet.2009.06.859
 83. Dusinska M, Collins AR. The comet assay in human biomonitoring: Gene-environment interactions. *Mutagenesis.* 2008;23(3):191-205.
doi: 10.1093/mutage/gen007
 84. Ada AO, Yilmazer M, Suzen S, *et al.* Cytochrome P450 (CYP) and glutathione S-transferases (GST) polymorphisms (CYP1A1, CYP1B1, GSTM1, GSTP1 and GSTT1) and urinary levels of 1-hydroxypyrene in Turkish coke oven workers. *Genet Mol Biol.* 2007;30(3):511-519.
doi: 10.1590/S1415-47572007000400002
 85. Bukvic N, Gentile M, Susca F, *et al.* Sex chromosome loss, micronuclei, sister chromatid exchange and aging: A study including 16 centenarians. *Mutat Res.* 2001;498(1):159-167.
doi: 10.1016/s1383-5718(01)00279-0
 86. Offer T, Ho E, Traber MG, Bruno RS, Kuypers FA, Ames BN. A simple assay for frequency of chromosome breaks and loss (micronuclei) by flow cytometry of human reticulocytes. *FASEB J.* 2005;19(3):485-487.
doi: 10.1096/fj.04-2729fj
 87. Mussali-Galante P, Tovar-Sánchez E, Valverde M, Rojas del Castillo E. Biomarkers of exposure for assessing environmental metal pollution: From molecules to ecosystems. *Rev Int Contam Ambient.* 2013;29:117-140.
 88. Morla M, Busquets X, Pons J, Sauleda J, MacNee W, Agusti AGN. Telomere shortening in smokers with and without COPD. *Eur Respir J.* 2006;27(3):525-528.

- doi: 10.1183/09031936.06.00087005
89. Pacheco KA. Epigenetics mediate environment: Gene effects on occupational sensitization. *Curr Opin Allergy Clin Immunol.* 2012;12(2):111-118.
doi: 10.1097/ACI.0b013e328351518f
 90. Kasuba V, Rozgaj R, Sentija K. Cytogenetic changes in subjects occupationally exposed to benzene. *Chemosphere.* 2000;40(3):307-310.
doi: 10.1016/s0045-6535(99)00265-9
 91. Cakir S. Genetics and some aging-related mechanisms. *Turk J Zool.* 2000;24(2):183-190.
 92. Yu M, Zhang H, Wang B, *et al.* Key signaling pathways in aging and potential interventions for healthy aging. *Cells.* 2021;10(3):660.
doi: 10.3390/cells10030660
 93. Mantovani C, Terenghi G, Magnaghi V. Senescence in adipose-derived stem cells and its implications in nerve regeneration. *Neural Regen Res.* 2014;9(1):10-15.
doi: 10.4103/1673-5374.125324
 94. Lu W, Tang S, Li A, *et al.* The role of PKC/PKR in aging, Alzheimer's disease, and perioperative neurocognitive disorders. *Front Aging Neurosci.* 2022;14:973068.
doi: 10.3389/fnagi.2022.973068
 95. Balistreri CR, Madonna R, Melino G, Caruso C. The emerging role of Notch pathway in ageing: Focus on the related mechanisms in age-related diseases. *Ageing Res Rev.* 2016;29:50-65.
doi: 10.1016/j.arr.2016.06.004
 96. Ukraintseva S, Duan M, Arbeev K, *et al.* Interactions between genes from aging pathways may influence human lifespan and improve animal to human translation. *Front Cell Dev Biol.* 2021;9:692020.
doi: 10.3389/fcell.2021.692020
 97. Ronen A, Glickman BW. Human DNA repair genes. *Environ Mol Mutagen.* 2001;37(3):241-283.
doi: 10.1002/em.1033
 98. Wood RD, Mitchell M, Sgouros J, Lindahl T. Human DNA repair genes. *Science.* 2001;291(5507):1284-1289.
doi: 10.1126/science.1056154
 99. Kirsch-Volders M, Fenech M. Inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes. *Mutagenesis.* 2001;16(1):51-58.
doi: 10.1093/mutage/16.1.51
 100. LLeonart ME, Carnero A, Paciucci R, Wang ZQ, Shomron N. Cancer, senescence, and aging: Translation from basic research to clinics. *J Aging Res.* 2011;2011:692301.
doi: 10.4061/2011/692301
 101. Eshkoora SA, Ismail P, Abdul Rahman S. Gene expression of CDK6 and CCND1 genes in basal cell carcinoma. *J Med Biol Sci.* 2008;2(2):1-9.
 102. Han X, Zheng T, Foss FM, *et al.* Genetic polymorphisms in the metabolic pathway and non-Hodgkin lymphoma survival. *Am J Hematol.* 2010;85(1):51-56.
doi: 10.1002/ajh.21580
 103. Eshkoorb SA, Marashi SJ, Ismail P, *et al.* Association of GSTM1 and GSTT1 with ageing in auto repair shop workers. *Genet Mol Res.* 2012;11(2):1486-1496.
doi: 10.4238/2012.May.21.5
 104. Eshkoor SA, Ismail P, Rahman SA. Does CYP1A1 gene polymorphism affect cell damage biomarkers and ageing? *Turk J Biol.* 2014;38:219-225.
doi: 10.3906/biy-1308-61
 105. Eshkoora SA, Ismail P, Rahman SA, Moin S. Role of the CYP1A2 gene polymorphism on early ageing from occupational exposure. *Balkan J Med Genet.* 2013;16(2):45-52.
doi: 10.2478/bjmg-2013-0031
 106. Smith GB, Harper PA, Wong JM, *et al.* Human lung microsomal cytochrome P4501A1 (CYP1A1) activities: Impact of smoking status and CYP1A1, aryl hydrocarbon receptor, and glutathione S-transferase M1 genetic polymorphisms. *Cancer Epidemiol Biomarkers Prev.* 2001;10:839-853.
 107. Eshkoorb SA, Ismail P, Rahman SA, Adon MY, Devan RV. Contribution of CYP2E1 polymorphism to aging in the mechanical workshop workers. *Toxicol Mech Methods.* 2013;23(4):217-222.
doi: 10.3109/15376516.2012.743637
 108. Lucas D, Ferrara R, Gonzales E, Albores A, Manno M, Berthou F. Cytochrome CYP2E1 phenotyping and genotyping in the evaluation of health risks from exposure to polluted environments. *Toxicol Lett.* 2001;124(1):71-81.
doi: 10.1016/s0378-4274(00)00287-3
 109. Guengerich FP, Shimada T. Activation of procarcinogens by human cytochrome P450 enzymes. *Mutat Res.* 1998;400(1):201-213.
doi: 10.1016/s0027-5107(98)00037-2
 110. Pande M, Amos CI, Osterwisch DR, *et al.* Genetic variation in genes for the xenobiotic-metabolizing enzymes CYP1A1, EPHX1, GSTM1, GSTT1, and GSTP1 and susceptibility to colorectal cancer in Lynch syndrome. *Cancer Epidemiol Biomarkers Prev.* 2008;17(9):2393-2401.
doi: 10.1158/1055-9965.EPI-08-0326
 111. Da Silva J, Moraes CR, Heuser VD, *et al.* Evaluation of genetic

- damage in a Brazilian population occupationally exposed to pesticides and its correlation with polymorphisms in metabolizing genes. *Mutagenesis*. 2008;23(5):415-422.
doi: 10.1093/mutage/gen031
112. Pérez-Cadahía B, Laffon B, Valdiglesias V, Pásaro E, Méndez J. Cytogenetic effects induced by Prestige oil on human populations: The role of polymorphisms in genes involved in metabolism and DNA repair. *Mutat Res*. 2008;653(1):117-123.
doi: 10.1016/j.mrgentox.2008.04.002
 113. Eshkoorb SA, Ismail P, Rahman SA, Moin S. Does GSTP1 polymorphism contribute to genetic damage caused by ageing and occupational exposure? *Arh Hig Rada Toksikol*. 2011;62(4):291-298.
doi: 10.2478/10004-1254-62-2011-2088
 114. Wu X, Amos CI, Zhu Y, *et al*. Telomere dysfunction: A potential cancer predisposition factor. *J Natl Cancer Inst*. 2003;95(16):1211-1218.
doi: 10.1093/jnci/djg011
 115. Li J, Zhang Y, You Y, *et al*. Unraveling the mechanisms of NK cell dysfunction in aging and Alzheimer's disease: Insights from GWAS and single-cell transcriptomics. *Front Immunol*. 2024;15:1360687.
doi: 10.3389/fimmu.2024.1360687
 116. Weber S, Menees KB, Park J, *et al*. Distinctive CD56^{dim} NK subset profiles and increased NKG2D expression in blood NK cells of Parkinson's disease patients. *NPJ Parkinsons Dis*. 2024;10(1):36.
doi: 10.1038/s41531-024-00652-y
 117. Chidrawar SM, Khan N, Chan YLT, Nayak L, Moss PAH. Ageing is associated with a decline in peripheral blood CD56^{bright} NK cells. *Immun Ageing*. 2006;3:10.
doi: 10.1186/1742-4933-3-10
 118. Hazeldine J, Lord JM. The impact of ageing on natural killer cell function and potential consequences for health in older adults. *Ageing Res Rev*. 2013;12(4):1069-1078.
doi: 10.1016/j.arr.2013.04.003
 119. Chini CC, Peclat TR, Warner GM, *et al*. CD38 ecto-enzyme in immune cells is induced during aging and regulates NAD⁺ and NMN levels. *Nature Metab*. 2020;2(11):1284-1304.
doi: 10.1038/s42255-020-00298-z
 120. Xia M, Wang B, Wang Z, Zhang X, Wang X. Epigenetic regulation of NK cell-mediated antitumor immunity. *Front Immunol*. 2021;12:672328.
doi: 10.3389/fimmu.2021.672328
 121. Lv L, Chen Q, Lu J, *et al*. Potential regulatory role of epigenetic modifications in aging-related heart failure. *Int J Cardiol*. 2024;401:131858.
doi: 10.1016/j.ijcard.2024.131858
 122. Moskalev AA, Aliper AM, Smit-McBride Z, Buzdin A, Zhavoronkov A. Genetics and epigenetics of aging and longevity. *Cell Cycle*. 2014;13:1063-1077.
doi: 10.4161/cc.28433
 123. Colită CI, Udristoiu I, Ancuta DL, *et al*. Epigenetics of ageing and psychiatric disorders. *J Integr Neurosci*. 2024;23(1):13.
doi: 10.31083/j.jin2301013
 124. Johnson AA, Akman K, Calimport SRG, Wuttke D, Stolzing A, de Magalhaes JP. The role of DNA methylation in aging, rejuvenation, and age-related disease. *Rejuvenation Res*. 2012;15(5):483-494.
doi: 10.1089/rej.2012.1324
 125. Olden K, Freudenberg N, Dowd J, Shields AE. Discovering how environmental exposures alter genes could lead to new treatments for chronic illnesses. *Health Aff (Millwood)*. 2011;30(5):833-841.
doi: 10.1377/hlthaff.2011.0078
 126. Kanherkar RR, Bhatia-Dey N, Csoka AB. Epigenetics across the human lifespan. *Front Cell Dev Biol*. 2014;2:49.
doi: 10.3389/fcell.2014.00049
 127. Haigis MC, Yankner BA. The aging stress response. *Mol Cell*. 2010;40(2):333-344.
doi: 10.1016/j.molcel.2010.10.002
 128. Zampieri M, Ciccarone F, Guastafierro T, *et al*. Validation of suitable internal control genes for expression studies in aging. *Mech Ageing Dev*. 2010;131(2):89-95.
doi: 10.1016/j.mad.2009.12.005
 129. Hernandez-Segura A, Rubingh R, Demaria M. Identification of stable senescence-associated reference genes. *Aging Cell*. 2019;18(2):e12911.
doi: 10.1111/accel.12911
 130. Pal S, Tyler JK. Epigenetics and aging. *Sci Adv*. 2016;2(7):e1600584.
doi: 10.1126/sciadv.1600584
 131. Laroni A, Uccelli A. CD56^{bright} natural killer cells: A possible biomarker of different treatments in multiple sclerosis. *J Clin Med*. 2020;9(5):1450.
doi: 10.3390/jcm9051450
 132. Abel AM, Yang C, Thakar MS, Malarkannan S. Natural killer cells: Development, maturation, and clinical utilization. *Front Immunol*. 2018;9:1869.
doi: 10.3389/fimmu.2018.01869
 133. Shi FD, Ljunggren HG, La Cava A, Van Kaer L. Organ-specific features of natural killer cells. *Nat Rev Immunol*. 2011;11(10):658-671.

- doi: 10.1038/nri3065
134. Almeida-Oliveira A, Smith-Carvalho M, Porto LC, *et al.* Age-related changes in natural killer cell receptors from childhood through old age. *Hum Immunol.* 2011;72(4):319-329.
doi: 10.1016/j.humimm.2011.01.009
135. Campos C, Pera A, Pita-Lopez ML, *et al.* *Natural Killer Cells in Human Aging.* Cham: Springer; 2018.
doi: 10.1007/978-3-319-64597-1_27-1
136. Poli A, Michel T, Thérésine M, Andrès E, Hentges F, Zimmer J. CD56bright natural killer (NK) cells: An important NK cell subset. *Immunology.* 2009;126(4):458-465.
doi: 10.1111/j.1365-2567.2008.03027.x
137. Berahovich RD, Lai NL, Wei Z, Lanier LL, Schall TJ. Evidence for NK cell subsets based on chemokine receptor expression. *J Immunol.* 2006;177(11):7833-7840.
doi: 10.4049/jimmunol.177.11.7833