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Review

Deciphering the mechanisms of carcinogens: Unravelling the pathways of cancer initiation and progression: An insight into DNA damage, genotoxicity, and epigenetic changes

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Abstract: Carcinogens are substances known to induce cancer by altering the genetic material and cellular processes within the human body. Understanding the mode of action of carcinogens is critical for developing effective prevention and intervention strategies against cancer. Cancer remains a significant global health challenge, with carcinogens posing a continuous threat to human well-being. This study explores into the intricate mechanisms by which carcinogens induce cancer, focusing on the interplay of DNA damage, genotoxicity, and epigenetic alterations. Through an analysis of direct and indirect-acting carcinogens, the study elucidates how these agents disrupt cellular DNA, leading to mutations and chromosomal abnormalities. Additionally, the role of genotoxicity in driving oncogenesis is explored, highlighting the importance of assessing carcinogenic risk through cytogenetic genotoxicity methods. The study focused into the direct and indirect DNA damage, genotoxicity, epigenetic changes, inflammation, hormonal effects, and immune system suppression induced by different carcinogens. It intends insight on the intricate interplay between environmental factors and the molecular foundation of carcinogenesis by thoroughly investigating these pathways. By comprehensively examining these pathways, which hope to focus on the complex interplay of carcinogenesis. By understanding these mechanisms, this study aims to inform preventive strategies and therapeutic interventions, ultimately mitigating the global burden of cancer.

Keywords: carcinogenesis; genetic erosion; genotoxicity

1. Background

"The measurements make the poison" may be an essential rule of toxicology. Coined by Paracelsus, who was a 15th century Swiss researcher, doctor, chemist, and secretive mastermind. Known as "the father of toxicology" since of this celebrated state. "The saying implies that any chemical can be harm in case the dose is past a certain edge additionally that any harm can be non-toxic on the off chance that the dosage is underneath a certain threshold" [1]. A universal association for the creation of pharmaceutical directions is the Worldwide Committee for Harmonisation (ICH [2]) Specialised Prerequisites for Pharmaceuticals for Human Utilise. The ICH rules known as M7 are utilised to assess and oversee DNA receptive (mutagenic) contaminants in pharmaceuticals in arrange to decrease the chance of cancer. The center of this rule is on DNA receptive compounds that have the potential to specifically cause DNA harm when display at moo levels, coming about to transformations and possibly causing cancer, agreeing to Segment 3 (common standards). The International Council for Harmonisation (ICH), a global organisation, is tasked with establishing regulations that govern the use of pharmaceuticals. The

guidelines outlined in the M7 document aim to mitigate the risk of cancer by assessing and controlling DNA-reactive (mutagenic) impurities in pharmaceuticals. Section 3 of this guideline emphasises its focus on substances capable of inducing DNA damage at minimal concentrations, potentially resulting in mutations and cancer development [2]. Cancer is a complex and multifaceted disease that continues to be a significant global health challenge. The identification of carcinogens and the elucidation of their mechanisms of action are of paramount importance in the field of oncology. Carcinogens can be found in various environmental, dietary, and occupational settings, posing a continuous threat to human health. Understanding the diverse pathways by which these agents initiate and promote cancer is crucial for implementing effective preventive measures and developing targeted therapies.

To lower the chance of getting cancer, it's critical to limit exposure to recognised carcinogens through dietary decisions, environmental safeguards, and workplace safety measures. To guide public health practises and policies, regulatory authorities and research organisations continuously investigate and evaluate potential carcinogens.

Carcinogens are substances that have the potential to cause cancer by destroying DNA and encouraging unchecked cell division. Carcinogens' modes of action might vary based on their chemical and physical characteristics, but generally speaking, they cause cancer by the following mechanisms:

1) Direct DNA damage: Some carcinogens can directly interact with the DNA in cells, causing mutations or chemical changes to the DNA sequence. These alterations can disrupt the normal cellular processes that control cell growth and division, leading to the formation of cancerous cells.

2) Indirect DNA damage: Indirect-acting carcinogens are not inherently cancer-causing but can become carcinogenic once they are metabolised in the body. Enzymes in the body can convert these substances into reactive intermediates that damage DNA. This can happen through processes such as: oxidation or chemical modification of the carcinogen.

3) Genotoxicity: Carcinogens that exhibit genotoxicity can cause direct damage to the genetic material (DNA) of cells. This damage can lead to mutations, chromosomal rearrangements, and other genetic abnormalities that may promote cancer development. A genotoxin is a substance or agent that has the potential to damage DNA or chromosomes either directly or indirectly.

4) Epigenetic changes: Some carcinogens can induce epigenetic changes in the cells. Epigenetic modifications do not alter the DNA sequence but can affect gene expression patterns. Altered gene expression can lead to abnormal cellular behaviour and potentially contribute to the development of cancer.

5) Inflammation and chronic irritation: Some carcinogens can cause chronic inflammation or irritation in tissues. Prolonged inflammation can stimulate cell proliferation and create an environment conducive to cancer development.

6) Hormonal effects: Certain carcinogens can disrupt hormonal balance in the body, affecting the regulation of cell growth and potentially promoting the development of hormone-related cancers.

7) Immune system suppression: Some carcinogens can suppress the immune system's ability to detect and eliminate abnormal cells, allowing cancer cells to grow unchecked.

Cancer remains a significant global health challenge, characterised by the accumulation of genetic and epigenetic changes leading to uncontrolled cell growth and tumor formation. Carcinogens, whether natural or synthetic, are pivotal in triggering these alterations. Understanding how carcinogens operate at the molecular level is essential for identifying high-risk exposures, implementing prevention strategies, and developing targeted therapies.

The diverse mechanisms by which carcinogens act on cellular and molecular processes, leading to the initiation and progression of cancer. The distinction between direct and indirect-acting carcinogens and how they impact cellular DNA. Additionally, study delve into the concept of genotoxicity and its role in causing DNA damage and mutations. Further, epigenetic changes induced by carcinogens will be examined, as they can influence gene expression patterns and contribute to oncogenesis.

Further, this study will explore the link between inflammation and cancer development, as chronic inflammation can provide a conducive environment for tumour growth. The hormonal effects of certain carcinogens will also be discussed, as they can disrupt the delicate balance of hormones and contribute to hormone-related cancers. Lastly, the role of carcinogens in suppressing the immune system's surveillance and defense mechanisms against cancer will be addressed.

1) Direct and indirect-acting carcinogens: Impact on cellular DNA:

Carcinogens can be broadly categorised as direct-acting and indirect-acting agents based on their ability to interact directly with cellular DNA. Direct-acting carcinogens, such as: certain alkylating agents and U.V. radiation, directly damage the DNA structure, causing DNA adducts and breaks. Indirect-acting carcinogens, on the other hand, require metabolic activation within the body to become reactive intermediates that can damage DNA. We will delve into the various mechanisms of DNA damage induced by both types of carcinogens and discuss the consequences of such alterations on genomic stability.

2) Genotoxicity: A key driver of carcinogenesis:

The term "genotoxicity" describes an agent's capacity to harm DNA, which can result in mutations and chromosomal abnormalities. Alternatively, genotoxicity describes a substance's capacity to contaminate a cell's genetic material. Exposure to chemical and biological substances can cause changes in the epigenome and/or genomic instability, which can lead to a number of disorders, including cancer [3]. A genotoxin refers to a substance or element capable of inducing DNA or chromosomal harm. When such harm occurs in a germ cell, it holds the potential to trigger an inheritable modified characteristic (germline mutation). Conversely, DNA damage within a somatic cell might give rise to a somatic mutation, potentially leading to the development of cancer through malignant transformation [4].

Carcinogens often exhibit genotoxic properties, contributing to their cancer-causing potential. Through this section, we will explore the concept of genotoxicity and its significance in initiating oncogenic events. We will also discuss various assays and methodologies used to assess genotoxicity and their relevance in identifying potential carcinogens.

3) Epigenetic changes induced by carcinogens: implications for oncogenesis:

Beyond direct DNA damage, carcinogens can induce epigenetic changes that alter gene expression patterns without altering the DNA sequence. These epigenetic alterations, including DNA methylation, histone modifications, and microRNA dysregulation, can significantly impact cellular functions and contribute to tumourigenesis. This section will focus on the epigenetic changes caused by carcinogens and their potential role in cancer initiation and progression. Additionally, we will highlight emerging epigenetic therapies as promising avenues for cancer treatment.

2. Introduction

In our contemporary society, exposure to chemical substances is unavoidable, with certain agents posing risks to human health. The impact of chemical carcinogens is a significant concern globally, prompting the establishment of guidelines by international bodies like the World Health Organisation for their regulation. Carcinogens are presently divided into two groups: genotoxic and non-genotoxic carcinogens, each governed by distinct regulatory measures [1].

2.1. Rationale of the study

Cancer is a significant global health burden, affecting millions of individuals and causing substantial morbidity and mortality worldwide. The identification and understanding of the mechanisms by which carcinogens exert their cancer-causing effects are critical for developing effective prevention strategies and targeted therapeutic interventions. Additionally, unraveling the concept of genotoxicity, a key driver of carcinogenesis, holds immense potential for early detection and risk assessment of cancer.

Despite extensive research on carcinogens and genotoxicity, there are still gaps in our knowledge of the intricate molecular and cellular processes that underlie cancer initiation and progression. Existing studies have highlighted the role of direct and indirect-acting carcinogens, the impact of DNA damage and mutations, and the influence of epigenetic changes on oncogenesis. However, comprehensive and up-to-date insights into the various mechanisms of carcinogens and genotoxicity remain essential for advancing cancer research and clinical practices.

The findings from this study will have significant implications for cancer prevention, early detection, and treatment. By identifying high-risk exposures and understanding the molecular basis of cancer initiation, public health efforts can be better directed towards reducing carcinogenic exposures and improving lifestyle choices. Moreover, this study will contribute to the development of targeted therapies that can disrupt the specific mechanisms utilised by carcinogens and halt tumour growth and progression.

The rationale for this study is to address these gaps and provide a thorough examination of the diverse mechanisms by which carcinogens induce cancer and the role of genotoxicity in the carcinogenic process. By investigating the direct and indirect-acting carcinogens' distinct pathways and their specific effects on cellular DNA, we aim to uncover the molecular events that drive cancer development. Understanding the complex relationships between environmental variables and cancer

pathogenesis will help us to better understand how carcinogens interact with cellular DNA and how these interactions result in genetic changes and abnormal cell activity and cancer pathogenesis.

2.2. A mechanistic overview of DNA damage

Genotoxic substances directly interact with cellular DNA, inducing stress that, if not managed properly, can lead to mutations [5]. Genotoxic carcinogens are chemicals that cause cancer by inducing mutations [1].

Genotoxic substances can change DNA in a variety of potentially harmful ways. Double-strand breaks (DSBs)—simultaneous breaks on both DNA strands—occur seldom and only when extremely potent DNA-damaging agents are present. DSBs are grave occurrences that split chromosomes and, if left unrepaired, can be fatal. Single-strand breaks (SSBs) are more common and can happen under normal physiological circumstances during transcription and replication processes. However, if these breaks occur at a higher rate than the cell's repair capacity, they can result in detrimental effects [6]. Genotoxic carcinogens are regulated upon the presumption that, even at very low levels, they represent a cancer risk to people. On the other hand, non-genotoxic carcinogens are believed to have a permissible exposure limit or dose, allowing their use in society as long as the exposure or intake remains below this threshold. These carcinogens induce cancer through pathways unrelated to mutations, such as: hormonal impacts, cytotoxicity, cell proliferation, or epigenetic alterations [1].

2.3. A study of commonly affecting, well-known carcinogenic compounds

The consensus is that genotoxic carcinogens like benzo[a]pyrene and aflatoxin B1 trigger tumours by causing DNA damage and mutations, whereas non-genotoxic carcinogens like phenobarbital, carbon tetrachloride, or diethylstilbestrol lead to tumour formation through mechanisms that do not involve DNA damage, such as promoting cell proliferation [1].

Benzo[a]pyrene: Found in tobacco smoke, charred food, and exhaust fumes.

Brief information of the carcinogenic substances acting mechanism has been described below:

Benzo[a]pyrene: Benzo[a]pyrene and similar endocrine disruptors impact the growth and behaviour of terrestrial animals, while aquatic species can be affected by the presence of human female urine, which contains residues of oral contraceptives and other substances [7]. The levels of benzo[a]pyrene in sidestream cigarette smoke have been documented to vary from 52 to 95 nanograms per cigarette, surpassing the concentration found in mainstream smoke by over threefold. The primary origins of polycyclic aromatic hydrocarbons (PAHs) in the air, whether indoors or outdoors, encompass residential and commercial activities involving wood, coal, or other biomass combustion for heating purposes. Additionally, indoor sources like cooking and tobacco smoke contribute to PAH levels, while outdoor emissions from motor vehicles, particularly diesel engines, industrial discharges, and forest fires, also play significant roles.

Mechanism of action: Benzo[a]pyrene is a polycyclic aromatic hydrocarbon (PAH) found in tobacco smoke, charred food, and exhaust fumes [8–10]. PAHs are

classified as procarcinogens, implying that they need metabolic activation to become carcinogenic. Occupational exposure to PAHs predominantly happens through inhalation and skin contact. Following entry into the body, enzymes metabolise Benzo[a]pyrene into reactive compounds, like epoxides, which can attach to DNA, forming DNA adducts. These adducts have the potential to cause mutations in crucial genes, including tumor suppressor genes and oncogenes. The modified DNA can disrupt typical cellular functions, resulting in unregulated cell proliferation and the onset of cancer [8].

Diolepoxide mechanism: The metabolic pathway of benzo[a]pyrene involving diolepoxide comprises several stages: benzo[a]pyrene undergoes transformation into benzo[a]pyrene-7,8-oxide through the action of enzymes CYP1A1 and CYP1B1, then further converts into benzo[a]pyrene-7,8-diol by epoxide hydrolase. Finally, this compound is metabolised into benzo[a]pyrene-7,8-diol-9,10-epoxides by enzymes CYP1A1 and CYP1B1. Each category of metabolic intermediate has been demonstrated to possess genotoxic and carcinogenic properties [10].

Radical-cation mechanism: The radical-cation mechanism of benzo[a]pyrene has been studied primarily concerning tumorigenesis in mouse skin. When benzo[a]pyrene undergoes one-electron oxidation by enzymes like CYPs or peroxidases, it generates a radical cation primarily localised at carbon 6 due to its geometric arrangement and ionisation potential. The radical cation results in the creation of covalent bonds with guanine (at the C8 carbon and N7 nitrogen) and adenine (at the N7 nitrogen) in the skin of mice. These adducts, which are unstable, are believed to induce the formation of apurinic sites in mouse skin. However, no studies have yet shown an increase in apurinic sites in lung tissues treated with benzo[a]pyrene, and only minimal levels of apurinic sites were observed in the epidermis of mice treated with the compound. Two in vivo studies demonstrated the excretion of 7-(benzo[a]pyrene-6-yl)-N7-guanine in the urine and feces of rats treated with benzo[a]pyrene, while the same adduct was detected in the lung tissue of mice. Additionally, alterations were observed at guanine and/or adenine in codons 12, 13, and 61 of the Ha-Ras oncogene in skin papillomas from mice treated topically with benzo[a]pyrene [10].

Synonyms: BaP; benzo[def]chrysene; 3,4-benzopyrene*; 6,7-benzopyrene*; benz[a]pyrene; 3,4-benz[a]pyrene*; 3,4-benzpyrene*; 4,5-benzpyrene* (*alternative numbering conventions). $C_{20}H_{12}$

Description: Yellowish plates, needles from benzene/methanol; crystals may be monoclinic or orthorhombic [8,10].

Study of carcinogenic activity: Following the administration of benzo[a]pyrene either through gavage or dietary intake to mice of various strains, elevated tumour responses were observed in lymphoid and hematopoietic tissues, as well as in multiple organs including the lung, forestomach, liver, oesophagus, and tongue. When benzo[a]pyrene was orally administered to XPA–/– mice, a significantly higher incidence of lymphomas was observed compared to XPA+/– and XPA+/+ mice under similar treatment conditions. Moreover, in XPA–/–/p53+/– double-transgenic mice, tumours (mainly splenic lymphomas and forestomach tumours) occurred earlier and at higher rates when benzo[a]pyrene was administered via gavage compared to their single transgenic and wild-type counterparts. These cancer-prone XPA–/– or XPA–/–/p53+/– mice also developed a high frequency of tumours (mainly in the forestomach)

when benzo[a]pyrene was included in their diet. Additionally, oral gavage of benzo[a]pyrene in rats led to an increased occurrence of mammary gland adenocarcinomas [8]. The IARC Monograph Volume 3 revealed that exposure to benzo[a]pyrene through various routes (oral, dermal, inhalation, intratracheal, intrabronchial, subcutaneous, intraperitoneal, and intravenous) led to tumour formation across all tested species, including mice, rats, hamsters, guinea pigs, rabbits, ducks, newts, and monkeys. Prenatal and transplacental exposure to benzo[a]pyrene resulted in both local and systemic carcinogenic effects in single-dose studies. Moreover, it induced skin cancer in mice. Repeatedly applying benzo[a]pyrene to the buccal pouch mucosa of male hamsters resulted in a notable increase in forestomach papilloma occurrence. In mice, intravaginal application of benzo[a]pyrene resulted in invasive cervical carcinoma, a phenomenon not observed in control groups [10]. Benzo[a]pyrene, a prototypical polycyclic aromatic hydrocarbon, belongs to the category of human genotoxic carcinogens (classified as IARC Group 1), demonstrating tumorigenic potential across various in vivo experimental animal models. Its carcinogenic activity is linked to the induction of interconnected genotoxic and nongenotoxic epigenetic changes. Specifically, exposure to benzo[a]pyrene leads to the extensive and selective formation of anti-7β, 8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE) adducts at key mutation sites, namely codons 157, 248 and 273 in the human tumour suppressor P53 gene, and at codon 14 in the human KRAS oncogene. CpG methylation at these sites significantly enhances the formation of these genotoxic benzo[a]pyrene-DNA adducts. The presence of BPDE-DNA adducts disrupts both global and gene-specific DNA methylation by impeding the activity of DNA methyltransferases. Consequently, hypermethylation of critical cancer-related genes such as cyclin-dependent kinase inhibitor 2A (CDKN2A; p16INK4A), retinoic acid receptor β2 (RARβ2), hypermethylated in cancer 1 (HIC1), and glutathione-S-transferase genes is frequently observed following exposure to benzo[a]pyrene [11] (**Figure 1–3**).

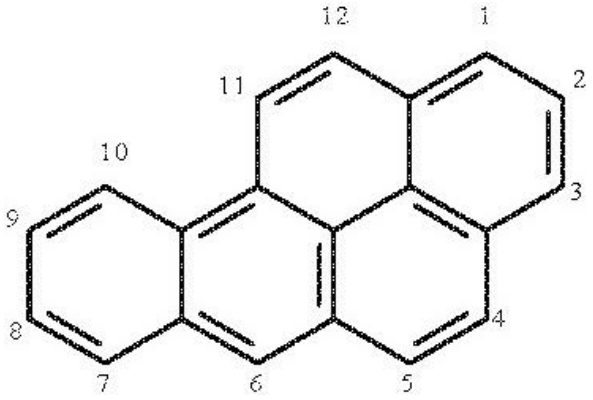

Figure 1. Benzo[a]pyrene structure [8–10].

Carcinogenesis:

Radical cation pathway:

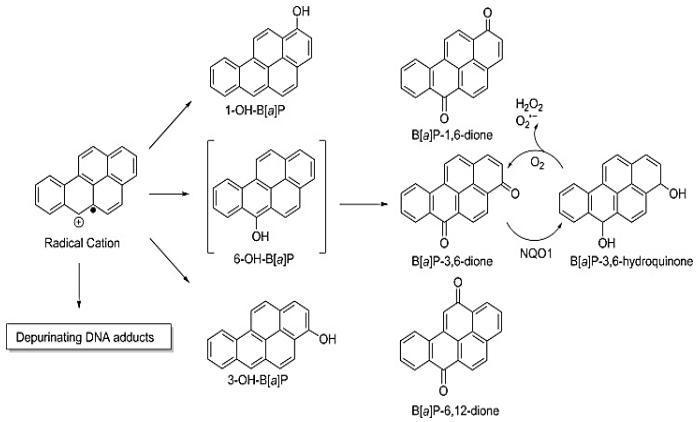

Figure 2. The radical cation mechanism of polycyclic aromatic hydrocarbon (PAH) activation, specifically with benzo[a]pyrene (B[a]P) [12].

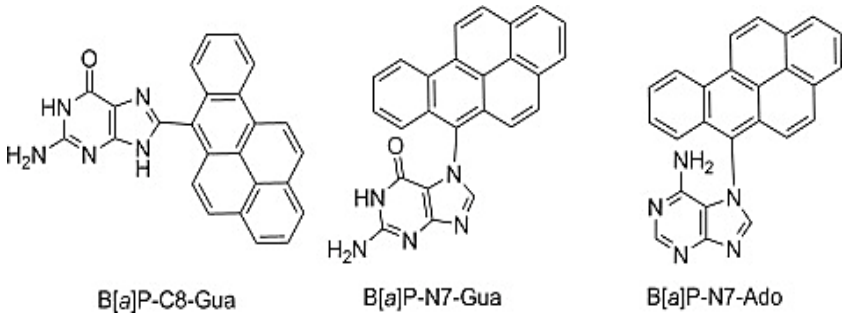

Figure 3. Depurinating B[a]P adducts derived from radical cation pathway [12].

B[a]P undergoes metabolic activation through the action of P450 peroxidase, acting as a co-reductant of Complex 1 $[Fe^{4+} = O]^{+\bullet}$, similar to perferryl-oxygen ($FeV^{+} = O$). In this process, Complex 1 returns to its resting state (Fe^{3+}) by extracting electrons from the C6 atom of B[a]P. Cavalieri proposed that B[a]P's relatively low ionisation potential enables the formation of a relatively stable radical cation capable of traveling to the nucleus and binding to DNA. Cavalieri and colleagues also identified peroxidases within the nucleus, suggesting that the radical cation may be formed locally. Other peroxidases, like horseradish peroxidase, prostaglandin H synthase (PHS), or myeloperoxidase, could generate the radical cation through a similar mechanism. This radical cation pathway also generates hydroxylated metabolites. Oxygen transfer in the peroxidase reaction to C6, the most electron-deficient carbon, produces 6-hydroxy-B[a]P (6-OH-B[a]P), which is highly unstable but can be detected by measuring the formation of stable polynuclear quinones like B[a]P-1,6-dione, -3,6-dione, and -6,12-dione. Oxygen may also transfer to C1 or C3, leading to the formation of 1-OH-B[a]P and 3-OH-B[a]P. Reactive metabolites like polynuclear quinones, such as B[a]P-1,6-dione, B[a]P-3,6-dione, and B[a]P-6,12-dione, are formed. Quinones are highly reactive and can be enzymatically reduced to hydroquinones via a two-electron reduction catalysed by NAD(P)H:quinone oxidoreductase (NQO1) or two one-electron reductions catalysed by NADPH P450 oxidoreductase, or non-enzymatically by reductants like NAD(P)H and glutathione. The hydroquinones then undergo rapid autoxidation to form semiquinone anion radicals and regenerate the quinones. These futile cycles are linked to molecular

oxygen, generating superoxide anion radicals ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2) [12].

Genotoxicity assays play a crucial role in differentiating between the two categories of carcinogens. Nevertheless, certain carcinogens may produce negative outcomes in in vitro bacterial mutation tests but show positive results in in vivo transgenic rodent gene mutation assays [1]. Typically, the test employs four strains of Salmonella typhimurium and one strain of Escherichia coli to identify various point mutations. Named after its developer, Dr. Bruce N. Ames, the assay is commonly referred to as the Ames test, which initially utilised Salmonella strains [13]. Following positive findings in transgenic assays, methyleugenol, estragole, and madder colour were identified as genotoxic carcinogens. Conversely, citrinin, flumequine, ginkgo biloba extract, and 3-monochloropropane-1,2-diol esters exhibited unfavourable results in the organs targeted for carcinogenicity and were thus categorised as non-genotoxic carcinogens [14–17] (**Figure 4**).

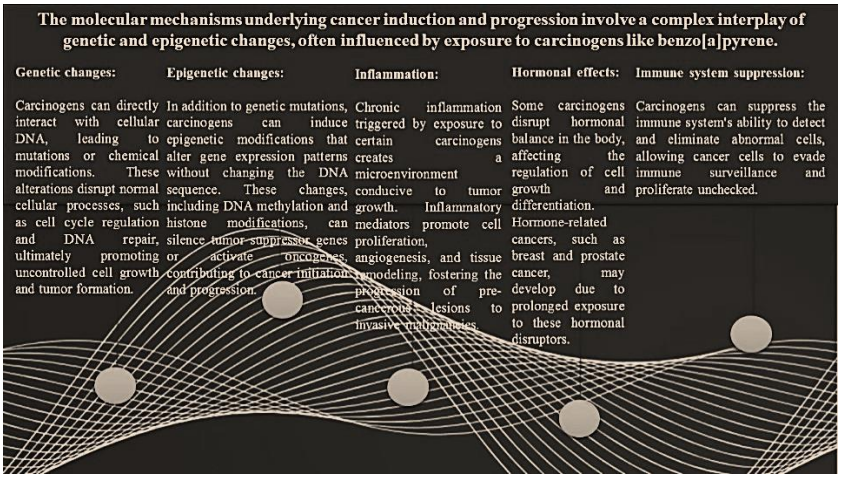

Figure 4. Mechanism of action of carcinogens.

3. Conclusion

To safeguard the public during acute chemical occurrences, it is crucial to provide credible, authoritative, evidence-based, and timely scientific advice that carefully considers the toxicological aspects of dangerous compounds.

The quality of the final response will be influenced by partnership functioning and expert input, among other important considerations. The mode of action of carcinogens encompasses a complex interplay of various cellular and molecular processes that lead to cancer development. From direct DNA damage to epigenetic modifications, inflammation, and immune system suppression, carcinogens utilise diverse mechanisms to induce oncogenesis. Understanding these pathways is vital for implementing effective preventive strategies and developing targeted therapies against cancer.

By identifying high-risk exposures and adopting stringent environmental and occupational safety measures, we can significantly reduce the burden of cancer caused by carcinogens. Moreover, advancements in research, including genomics, epigenomics, and proteomics, will pave the way for personalised approaches to cancer prevention and treatment.

In conclusion, the study highlights on the intricate mechanisms by which carcinogens exert their cancer-causing effects. Continued research into the mode of action of carcinogens is essential for improving cancer risk assessment, management, and overall public health.

The mechanisms of carcinogens encompass a complex interplay of cellular and molecular events that drive cancer initiation and progression. The distinction between direct and indirect-acting carcinogens, the role of genotoxicity in promoting mutations, and the influence of epigenetic changes on oncogenesis are fundamental aspects of understanding cancer development. This comprehensive study focused on these mechanisms and their significance in cancer research and public health. Continued research in this field is vital for advancing cancer prevention, diagnosis, and treatment, ultimately alleviating the global burden of cancer. Cytogenetic genotoxicity methods like the chromosome aberration assay or the micronucleus assay often yield positive outcomes even in the presence of non-DNA damaging agents like spindle poisons or topoisomerase inhibitors. To discern between genotoxic and non-genotoxic carcinogens, researchers rely on mechanistic analyses of tumour formation derived from the findings of these genotoxicity tests.

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Abbreviations

RARβ2	The retinoic acid receptor beta 2 (RARβ2)
BPDE	benzo[a]pyrene diol epoxide
CDKN2A	CDKN2A, also known as cyclin-dependent kinase inhibitor 2A, is a gene which in humans is located at chromosome 9, band p21.3.
CYP1A1	Cytochrome P450 Family 1 Subfamily A Member 1
CYP1B1	gene provides instructions for producing an enzyme that is a member of the cytochrome P450 family of enzymes.
CYPs	Cytochromes P450 (P450s or CYPs)
IARC	International Agency for Research on Cancer
KRAS	Kirsten rat sarcoma virus
p16(INK4A)	proteins
p14(ARF)	proteins
PAH	Benzo[a]pyrene.
XPA	DNA repair protein complementing XP-A cells is a protein that in humans is encoded by the XPA gene.

References

1. Nohmi T. Thresholds of Genotoxic and Non-Genotoxic Carcinogens. Toxicological Research. 2018; 34(4): 281-290. doi: 10.5487/tr.2018.34.4.281
2. ICH Harmonised Tripartite Guideline. Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk. PMDA. Available online: https://www.pmda.go.jp/files/000208234.pdf (accessed on 1 January 2023).

3. Ren N, Atyah M, Chen WY, et al. The various aspects of genetic and epigenetic toxicology: testing methods and clinical applications. Journal of Translational Medicine. 2017; 15(1). doi: 10.1186/s12967-017-1218-4
4. Luch A. Molecular, Clinical and Environmental Toxicology. Birkhäuser Basel; 2009.
5. Móricz ÁM, Ott PG. Effects-directed detection. Instrumental Thin-Layer Chromatography. Published online 2023: 259-296. doi: 10.1016/b978-0-323-99970-0.00012-0
6. Piña B, Barata C. Ecotoxicology, Genetic. Encyclopedia of Toxicology. Published online 2014: 295-300. doi: 10.1016/b978-0-12-386454-3.00497-8
7. Csaba G. Chapter 19—Transgenerational Effects of Perinatal Hormonal Imprinting. In: Tollefsbol T (editor). Transgenerational Epigenetics. Academic Press; 2014. pp. 255-267. doi: 10.1016/B978-0-12-405944-3.00019-2
8. Lyon. Chemical Agents and Related Occupations. Available online: https://www.ncbi.nlm.nih.gov/books/NBK304415/ (accessed on 10 January 2023).
9. NIH (National Cancer Institute). NCI Dictionary of Cancer Terms. Available online: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/benzoapyrene (accessed on 16 January 2023).
10. IARC. BENZO[a]PYRENE monographs. Available online: https://monographs.iarc.who.int/wp-content/uploads/2018/06/mono100F-14.pdf (accessed on 29 December 2022).
11. Pogribny IP. Environmental Exposures and Epigenetic Perturbations. Available online: https://www.sciencedirect.com/science/article/pii/B9780128012383650626 (accessed on 9 January 2023).
12. Murray JR, Penning TM. Carcinogenic Polycyclic Aromatic Hydrocarbons. Comprehensive Toxicology. Published online 2018: 87-153. doi: 10.1016/b978-0-12-801238-3.95691-5
13. Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res. 1983; 113: 173–215. doi: 10.1016/0165-1161(83)90010-9
14. Suzuki Y, Umemura T, Hibi D, et al. Possible involvement of genotoxic mechanisms in estragole-induced hepatocarcinogenesis in rats. Archives of Toxicology. 2012; 86(10): 1593-1601. doi: 10.1007/s00204-012-0865-8
15. Jin M, Kijima A, Hibi D, et al. In Vivo Genotoxicity of Methyleugenol in gpt Delta Transgenic Rats Following Medium-Term Exposure. Toxicological Sciences. 2012; 131(2): 387-394. doi: 10.1093/toxsci/kfs294
16. Kuroda K, Ishii Y, Takasu S, et al. Cell cycle progression, but not genotoxic activity, mainly contributes to citrinin-induced renal carcinogenesis. Toxicology. 2013; 311(3): 216-224. doi: 10.1016/j.tox.2013.07.003
17. Kuroiwa Y, Umemura T, Nishikawa A, et al. Lack of in vivo mutagenicity and oxidative DNA damage by flumequine in the livers of gpt delta mice. Archives of Toxicology. 2006; 81(1): 63-69. doi: 10.1007/s00204-006-0126-9