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Understanding chemical carcinogenesis: Mechanisms and mutagenicity testing

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Abstract
This comprehensive review examines the multifaceted realm of chemical carcinogenesis, emphasizing the diverse types of chemical carcinogens, the intricate process of carcinogenesis, and the evolving methods for testing mutagenicity and carcinogenicity both *in vitro* and *in vivo*. Through an in-depth analysis, the review categorizes chemical carcinogens into various classes, encompassing environmental pollutants, industrial compounds, and dietary agents. Each class is scrutinized for its distinct mechanisms of carcinogenic action and potential health impacts. Furthermore, the review elucidates the stepwise progression of chemical carcinogenesis, from initiation, through promotion, to malignant transformation, elucidating the critical molecular events underlying tumor development. Innovative advancements in testing methodologies for mutagenicity and carcinogenicity are also explored, including sophisticated *in vitro* assays and animal models that enhance accuracy and efficiency in assessing the carcinogenic potential of chemicals. Overall, this review provides a comprehensive understanding of chemical carcinogenesis, from the identification of different types of chemical carcinogens to the elucidation of the underlying processes and the latest advancements in testing methodologies, thus contributing to the development of effective strategies for cancer prevention and intervention.

Keywords: Chemical, carcinogenesis mechanisms, mutagenicity testing

Introduction
In the era of rapid industrialization and technological advancement, the marvels of science have indeed made seemingly impossible feats achievable. However, alongside the benefits, a shadow looms over our modern world—a consequence unforeseen by many. The dawn of the COVID-19 pandemic has unveiled the grim reality of our situation: mandatory mask-wearing, virtual classrooms and restrictions on gatherings have become the norm. Yet, this crisis extends beyond a mere viral outbreak; it sheds light on the pervasive threat posed by the deliberate release of toxic chemicals into our environment.

As of June 2015, the Chemical Abstracts Service reported a staggering hundred millionth chemical, with registrations now surpassing the 200 million mark (Honma D 2020) [31]. This incessant production of chemicals poses a dire risk to both environmental integrity and human health. Among these compounds, many harbour carcinogenic potential, contributing to the alarming rise in cancer cases among both humans and animals.

Cancer, arising from a single aberrant cell, undergoes clonal proliferation, evades apoptosis, and accumulates genetic aberrations, culminating in the formation of neoplastic masses (Trosko 2001) [27]. While factors such as aging, genetics, and diet contribute to cancer risk, exogenous factors-ranging from chemical pollutants to dietary habits-play a substantial role. Chemical carcinogenesis, shared between humans and animals, underscores the universal threat posed by environmental toxins. These carcinogens pervade our surroundings, contaminating food, air, and water sources, as well as arising endogenously from metabolic processes and pathophysiological conditions. Shockingly, it is estimated that a significant portion of human cancers-approximately 90% stems from chemical exposure, with tobacco alone accounting for 30% of cases (Warburg & Nguyen 2015) [29]. Additionally, occupational hazards further exacerbate cancer risk, underscoring the multifaceted etiology of this disease.

In this context, it becomes imperative to confront the pervasive menace of chemical

carcinogenesis, devising strategies to mitigate its impact and safeguard public health.

History of Chemical carcinogenesis

The historical narrative of chemical carcinogenesis unfolds with Hippocrates' early observations of 'karkinos' and Galeno's conceptualization of neoplasia (Gutiérrez and Salsamendi 2001) [32]. Yet, it wasn't until the Industrial Revolution that the concept of chemical carcinogenesis began to crystallize. Prior to this era, occupational hazards, such as those documented by Paracelsus among miners in the 16th century, hinted at the potential link between environmental exposures and cancer. In the 18th century, Sir Percival Pott's seminal discovery of scrotal skin cancer among chimney sweepers highlighted the carcinogenic potential of chemical agents. This pivotal observation paved the way for subsequent investigations into industrial carcinogens, including the notorious 'aniline bladder cancer' associated with naphthylamines and the 'paraffin cancer' linked to shale oil exposure.

Meanwhile, the tobacco-cancer link emerged, prompting intensive research efforts. Experimental models, notably the mouse skin model, facilitated the identification of potent carcinogens like benzo [a] pyrene, a polycyclic aromatic hydrocarbon found in coal tar (Iversen & Iversen 2018) [13]. These discoveries underscored the carcinogenicity of chemicals, reshaping our understanding of cancer etiology. Since then, hundreds of chemicals have been implicated in carcinogenesis, reflecting the heightened environmental hazards of the industrial age. This historical journey underscores the imperative to confront and mitigate the risks posed by chemical carcinogens in both human and animal populations, safeguarding public health in an ever-evolving landscape of industrialization.

Classification of chemical carcinogens

Chemical carcinogens represent a diverse array of compounds, encompassing both naturally occurring substances and synthetics. Their classification has been a subject of ongoing refinement, reflecting the complexity of their mechanisms and effects on biological systems. Initially, scientists classified these carcinogens based on their mechanism of action, broadly categorizing them into genotoxic and non-genotoxic compounds. Genotoxic agents were considered complete carcinogens, directly forming DNA adducts, while non-genotoxic compounds acted as promoters, facilitating cell proliferation. However, this classification proved inadequate due to the overlapping nature of genotoxicity and chromosomal damage caused by certain chemicals (Butterworth 2006) [5]. To address this, Bolt *et al*. (2004) [4] proposed a more nuanced division of genotoxic compounds. They distinguished between agents that directly interact with DNA and those that induce genotoxic effects at a chromosomal level. Further stratification identified initiators with unlimited doses, borderline cases, and weak genotoxic compounds, offering a refined framework for understanding their carcinogenic potential (Rossetti *et al*. 2009) [26].

Alternatively, a simpler classification based on metabolic activation has gained traction. This scheme categorizes carcinogens into direct-acting agents, exemplified by certain anticancer drugs like cyclophosphamide and chlorambucil, which do not necessitate metabolic transformation to induce carcinogenesis (Riddick *et al*. 2005) [25]. In contrast, indirect-acting agents such as polycyclic and heterocyclic aromatic hydrocarbons, as well as azo dyes, require metabolic conversion to become active. Termed 'procarcinogens', these substances give rise to 'ultimate carcinogens' upon metabolic activation, with the majority of chemical carcinogens falling into this category (Fishbein 2011) [8]. This evolving understanding underscores the intricate interplay between chemical structure, metabolic pathways, and carcinogenic potential. By delineating distinct mechanisms and modes of action, researchers continue to refine our comprehension of chemical carcinogenesis, informing strategies for risk assessment and mitigation (Faustino-Rocha & Oliveira 2020) [7].

Steps of Chemical carcinogenesis

Understanding the process of chemical carcinogenesis has evolved through landmark studies. Beremblum and Shubik's pioneering work in 1947 demonstrated the multifaceted nature of cancer development using polycyclic aromatic hydrocarbons and croton oil (Beremblum and Shubik, 1947) [2]. Their findings highlighted the stages of initiation and promotion, wherein neoplasia arose only when specific compounds were administered sequentially (Beremblum and Shubik, 1947) [2]. Subsequent research by Foulds in 1954 [10] introduced the concept of progression, further elucidating the intricacies of carcinogenesis (Foulds, 1954) [10]. Initially perceived as interacting with proteins (Miller and Miller, 1952) [33], the correlation between carcinogens and DNA became apparent following Watson and Crick's elucidation of DNA's structure (Luch, 2005) [17]. This comprehensive understanding led to the classification of chemical carcinogenesis into initiation, promotion, and progression phases.

Initiation

Initiation marks the onset of chemical carcinogenesis, characterized by irreversible alterations in DNA (Beremblum and Shubik, 1947) [2]. Both directly and indirectly acting agents, possessing electron-deficient atoms, predominantly interact with DNA, forming DNA adducts (Bertram, 2000) [3]. While repair mechanisms can mitigate damage, extensive or unrepaired lesions lead to permanent DNA alterations, defining initiated cells (Frowein, 2000) [11]. These cells, harboring latent genetic changes, may remain quiescent or undergo autonomous growth, heralding the initial step towards neoplasia (Player *et al*., 2004) [23]. Stem cells, susceptible due to high proliferation rates and ubiquity, are particularly prone to initiating rapid tumorigenesis (Williams, 2001) [30]. Initiation's dose-dependent nature underscores its additive process, with mutational transfer to daughter cells facilitating neoplastic transformation (Trosko, 2003) [28]. Notably, initiation's outcomes hinge not only on mutation occurrence but also on alterations in genes governing terminal differentiation.

Promotion

Promotion as elucidated by Beremblum and Shubik in 1947 [2], delineates a process wherein certain compounds, termed promoters, augment carcinogenic potential in experimental settings (Beremblum and Shubik, 1947) [2]. Unlike initiators, promoters do not directly damage DNA but bolster cell proliferation, facilitate mutation fixation, and induce alterations in gene expression and growth control (Gutiérrez and Salsamendi, 2001) [32]. Initially attributed to epigenetic modifications, promotion now encompasses genetic changes as well (Hanahan and Weinberg, 2016) [12]. Mitogenic rather than genotoxic, promoters sustain prolonged cell proliferation

by impeding quiescence entry, fostering a conducive environment for neoplastic progression (Pitot and Dragan, 1991) [22]. Unlike initiation, promotion is reversible, with cessation leading to regression via apoptosis, governed by physiological processes (Trosko, 2001) [27]. Participation in promotion is selective, involving undifferentiated, apoptosis-resistant cells, driving the delicate balance between growth and cell death towards malignancy.

Progression

Progression, a critical stage in carcinogenesis, signifies the transformation from benign to malignant neoplasia (Gutiérrez and Salsamendi, 2001; Klaunig *et al*., 2008) [32, 15]. This process, underscored by genetic and epigenetic alterations (Shacter and Weitzman, 2002), manifests as autonomous cell proliferation (Lutz, 2000) [18]. Irreversible and characterized by genetic instability, rapid growth, invasion and metastasis, progression heralds profound changes in cellular phenotype and function (Flavahan *et al*., 2017) [9]. Notably, angiogenesis, pivotal for tumor growth and key epigenetic modifications are hallmark features of this malignant transition (Flavahan *et al*., 2017) [9].

Epigenetics involved in Chemical carcinogenesis

In the intricate landscape of chemical carcinogenesis, alongside genetic mutations, epigenetic modifications play a pivotal role. Predominantly, DNA methylation and histone modifications such as acetylation, demethylation, and phosphorylation emerge as prominent players (Dixon and Kopras, 2004) [6]. These alterations render DNA more susceptible to mutations while activating proto-oncogenes, ultimately fostering tumorigenesis.

Molecular targets for chemical carcinogenesis

In the intricate process of chemical carcinogenesis, molecular targets are numerous, with particular emphasis on proto-oncogenes, tumor suppressor genes, and cell cycle regulators. Central to tumor formation is the interplay between these genes, especially during critical checkpoints of the cell cycle aimed at preventing replication of damaged DNA (Klaunig *et al*. 2000) [34]. Key players like the tumor suppressor gene p53 and Ras gene act to halt cell progression at the G1 phase, safeguarding against flawed DNA replication (Khan & Dipple, 2000) [14]. However, chemical carcinogens wield the power to induce mutations in these genes, disrupting their function and permitting aberrant cell replication despite DNA defects. This perpetuates irreversible damage, which is perpetuated through successive cell divisions, ultimately fostering tumorigenesis (Oliveira *et al*. 2007) [21].

Mutagenesis

Mutagenesis denotes the enduring alteration in genetic material, capable of being inherited across successive cell divisions. These changes may encompass individual genes, gene segments, or even entire chromosomes, culminating in mutations caused by mutagens. While genotoxicity parallels mutagenicity, not all genotoxic effects necessarily culminate in mutations. Although all mutagens exhibit genotoxicity, the converse is not always true, signifying a nuanced distinction (Ames *et al*. 1975) [1]. Mutations can transpire in either germ cells or somatic cells, with germline mutations engendering heritable effects while somatic mutations may precipitate cancer A myriad of mutagenic agents, including radioactive substances, x-rays, ultraviolet radiation, and certain chemicals, possess the capacity to induce mutations (Klaunig *et al*. 2010) [16]. Consequently, while all mutagenic chemicals wield carcinogenic potential, not every mutagen necessarily fosters carcinogenesis, underscoring the complex interplay between mutagenicity and carcinogenicity (Luch 2005) [17].

Evaluation of mutagenicity of chemicals

The evaluation of the mutagenicity of chemicals has become increasingly urgent with the influx of new substances into the market and our daily lives. To address this need rapidly, a variety of testing methods are available, encompassing both *in vitro* and *in vivo* approaches. Among the *in vitro* tests is the bacterial reverse mutation test, commonly known as the Ames test (OECD 471), which assesses the mutagenic potential of chemicals by detecting their ability to induce mutations in bacterial strains (Maron and Ames, 1983) [19]. Additionally, *in vitro* mammalian tests such as the chromosome aberration test (OECD 473) and the mammalian cell gene mutation test (OECD 476) offer valuable insights into the genotoxicity of substances at the cellular level (OECD Guidelines for the Testing of Chemicals). Furthermore, the *in vitro* micronucleus test (OECD 474) provides a means to evaluate chromosomal damage and genotoxicity in mammalian cells (OECD Guidelines for the Testing of Chemicals). Complementing these *in vitro* methods are a suite of *in vivo* tests designed to assess mutagenicity in whole organisms. The mammalian erythrocyte micronucleus test (OECD 474) and the mammalian bone marrow chromosome aberration test (OECD 475) offer comprehensive assessments of genotoxicity and chromosomal damage in living organisms (OECD Guidelines for the Testing of Chemicals). Moreover, the rodent dominant lethal mutation test (OECD 478) and the transgenic rodent somatic and germ cell gene mutation assay (OECD 488) provide insights into the heritable effects of mutagenic substances (OECD Guidelines for the Testing of Chemicals). By employing this array of tests, researchers and regulatory agencies can effectively evaluate the mutagenic potential of chemicals and safeguard public health.

In the realm of mutagenicity assessment, certain compounds exhibit the capability to induce chromosomal aberrations, which encompass both structural and numerical alterations in chromosomes (OECD 473). Structural aberrations entail deletions, inversions, duplications, and ring formations, while numerical aberrations include aneuploidy and polyploidy. The fundamental principle underlying the *in vitro* mammalian chromosome aberration test involves exposing cell cultures to the test chemicals for a predetermined duration, allowing for the manifestation of structural chromosomal aberrations (OECD 473). These aberrations are subsequently discerned under a microscope following the addition of a metaphase-arresting agent to the cell culture.

***In vitro* mammalian chromosome aberration test (OECD 473)**

To conduct this test, a variety of mammalian cell lines can be employed, such as Chinese hamster ovary or Chinese hamster lung cell cultures (OECD 473). The cell lines are subjected to the test compound, often supplemented with an external source of metabolic activation, such as rat liver extract. Following exposure, a metaphase-arresting substance like colchicine is introduced, facilitating the harvest of cells for subsequent staining and microscopic examination to identify structural chromosomal abnormalities (OECD 473). However, it is pertinent to note that while this test effectively detects structural aberrations, it is unable to discern numerical abnormalities, presenting a limitation (OECD 473).

***In vitro* micronucleus test (OECD 487)**

The *in vitro* micronucleus test (OECD 487) assesses genotoxicity by detecting micronuclei formation, resulting from chromosome fragments or whole chromosomes failing to incorporate into daughter nuclei during cell division due to DNA damage. This assay, widely regarded as one of the most reliable for genotoxicity evaluation, employs human or mammalian cell cultures exposed to test chemicals (OECD 487). Enhanced sensitivity is achieved through fluorescence in situ hybridization (FISH), facilitating precise damage visualization. Two versions of this assay exist: *in vivo* and *in vitro* micronucleus tests, with micronuclei primarily observed in erythrocytes but applicable to other cell types as well (OECD 487). The principle involves exposing cultured primary human or mammalian peripheral blood lymphocytes to test chemicals, supplemented with an external source of metabolic activation. Following cell division, CytoB is added to block cytokinesis, allowing visualization of bi-nucleated cells indicative of micronuclei presence. Notably, this test detects both clastogenic and aneugenic effects of mutagens (OECD 487).

***In vitro* mammalian cell gene mutation test (OECD 476)**

The *in vitro* mammalian cell gene mutation test, also known as the HPRT assay (OECD 476), evaluates mutagenic potential by assessing the activity of the hypoxanthine guanine phosphoribosyl transferase (HPRT) gene located on the X chromosome. HPRT regulates the enzymatic formation of purines, including the cytostatic purine analogue 6-thioguanine (TG) (OECD 476). Mutant cells deficient in HPRT activity exhibit resistance to the cytostatic effects of 6-thioguanine. Thus, if cells exposed to a test compound proliferate in the presence of TG, it indicates mutagenic activity (OECD 476). Common cell lines used in this assay include mouse lymphoma and Chinese hamster ovary cells. Following exposure to the test compound, mutant strains are isolated through subculturing, then treated with 6-thioguanine to assess cell growth arrest. The observation of cell growth despite the presence of the cytostatic agent indicates mutational activity.

In vivo tests serve as crucial tools for assessing mutagenicity and genotoxicity which involves following tests.

Mammalian Erythrocyte Micronucleus Test (OECD 474)

This test evaluates chromosome damage induced by test chemicals in laboratory animals such as rats and mice, focusing on micronuclei formation in erythroblasts. Treatment involves administering multiple doses of the test compound via oral or parenteral routes to young rodents, with bone marrow and peripheral blood samples collected for micronucleus analysis at specified intervals (OECD 474).

Rodent Dominant Lethal Mutation Test (OECD 478)

Test assesses germ cell mutations, crucial for predicting genetic disease risks. Sexually mature male mice or rats are treated with the test compound, followed by mating with virgin females to evaluate dominant lethality based on fetal and embryonic deaths. Observation for clinical signs, morbidity, and mortality ensues, with uterus examination at mid-pregnancy to determine implantation loss (OECD 478).

Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay (OECD 488)

This utilizes transgenic animals harboring reporter genes to detect mutations in both somatic and germ cells. Following exposure to the test compound, DNA extraction from sperm or somatic cells facilitates mutation detection through subsequent bacterial host assays, providing insights into mutagenic potential (OECD 488).

Carcinogenicity Studies

Two key *in vivo* tests are available to assess the carcinogenicity of chemicals: Carcinogenicity Studies (OECD TG 451) and Combined Chronic Toxicity/Carcinogenicity Studies (OECD TG 453). These studies aim to identify the carcinogenic properties of a compound by evaluating the incidence and nature of neoplasms, distinguishing target organs, and determining the time of neoplasm appearance. Furthermore, they characterize the dose-response relationship for tumor formation, establish a no-observed-adverse-effect level (NOAEL) or a point of departure for Benchmark Dose (BMD) estimation, and facilitate extrapolation of carcinogenic effects to low-dose human exposure levels (OECD TG 451; OECD TG 453). Histopathology serves as the primary endpoint for assessment in these studies, providing insights into the carcinogenic potential of the tested compounds.

Disadvantages of current approaches

While the tests outlined earlier are valuable for detecting mutagenicity and carcinogenicity, they come with inherent limitations. No single test can comprehensively detect all mutations induced by chemicals, highlighting the need for multiple assays to provide a comprehensive assessment. Additionally, the high sensitivity of *in vitro* tests may lead to false negatives when translated to *in vivo* conditions. Moreover, conducting these battery of tests is both time-consuming and costly, posing practical challenges for regulatory agencies and industries alike. The reliance on laboratory animals for *in vivo* testing raises ethical concerns, contributing to calls for alternative testing methods. In certain regions, such as certain European countries, *in vivo* testing is prohibited for cosmetic product approval, further complicating regulatory processes. Additionally, the scale-up of chemical production may render *in vivo* testing impractical or incompatible. Hence, while current approaches are valuable, efforts are needed to address these drawbacks and develop more efficient and ethically sound testing methods.

Recent advancements

Recent advancements in genotoxicity screening include the development of high-throughput screening (HTS) techniques aimed at replicating animal systems in a cost-effective and rapid manner. These methods, such as the fluctuation method for the Ames test and flow cytometry-based assays for the *in vitro* micronucleus test, enable the evaluation of mutagenicity for multiple compounds simultaneously. However, challenges remain in establishing systems that accurately mimic human physiological and histological conditions, while also navigating ethical and regulatory considerations. Simplified systems with genetic resemblance to mammalian systems may offer a more practical approach for HTS genotoxicity screening, avoiding the labour-intensive and time-consuming nature of complex models (Ranganatha *et al*. 2016) [24].

Conclusion

In our daily lives, we encounter numerous toxic chemicals that can lead to various adverse effects, including chemical carcinogenesis, a complex multistep process. To assess the mutagenicity and carcinogenicity of these chemicals,

extensive testing is conducted using a battery of *in vitro* and *in vivo* assays. However, these tests have limitations, highlighting the need for more advanced and efficient testing methods that can analyze a large number of samples quickly and affordably. Ultimately, prevention remains paramount in safeguarding both human and animal health from the harmful effects of these toxic substances.

References

1. Ames BN, McCann J, Yamasaki E. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 1975 Dec;31(6):347-364.
2. Berenblum I, Shubik P. The role of croton oil applications, associated with a single painting of a carcinogen, in tumour induction of the mouse's skin. British journal of cancer. 1947 Dec;1(4):379.
3. Bertram JS. The molecular biology of cancer. Molecular aspects of medicine. 2000 Dec 1;21(6):167-223.
4. Bolt HM, Foth H, Hengstler JG, Degen GH. Carcinogenicity categorization of chemicals - new aspects to be considered in a European perspective. Toxicology letters. 2004 Mar 7;151(1):29-41.
5. Butterworth BE. A classification framework and practical guidance for establishing a mode of action for chemical carcinogens. Regulatory Toxicology and Pharmacology. 2006 Feb 1;45(1):9-23.
6. Dixon K, Kopras E. Genetic alterations and DNA repair in human carcinogenesis. Seminars in cancer biology. 2004 Dec 1;14(6):441-448.
7. Faustino-Rocha AI, Oliveira PA. Chemical carcinogens; c2020.
8. Fishbein L. Potential industrial carcinogens and mutagens. Elsevier; c2011.
9. Flavahan WA, Gaskell E, Bernstein BE. Epigenetic plasticity and the hallmarks of cancer. Science. 2017 Jul 28;357(6348).
10. Foulds LJC. The experimental study of tumor progression: A review. Cancer research. 1954 May 1;14(5):327-339.
11. Frowein J. Hypothesis: Chemical carcinogenesis mediated by a transiently active carcinogen receptor. Cytogenetic and Genome Research. 2000;91(1-4):102-104.
12. Hanahan D, Weinberg RA. Biological hallmarks of cancer. Holland-Frei Cancer Medicine; c2016. p. 1-10.
13. Iversen PL, Iversen PL. Chemicals in the Environment. Molecular Basis of Resilience: Adapting to a Changing Environment; c2018. p. 141-68.
14. Khan QA, Dipple A. Diverse chemical carcinogens fail to induce G1 arrest in MCF-7 cells. Carcinogenesis. 2000 Aug 1;21(8):1611-1168.
15. Klaunig JE. Acrylamide carcinogenicity. Journal of agricultural and food chemistry. 2008 Aug 13;56(15):5984-5988.
16. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicologic Pathology. 2010 Jan;38(1):96-109.
17. Luch A. Nature and nurture-lessons from chemical carcinogenesis. Nature Reviews Cancer. 2005 Feb;5(2):113-125.
18. Lutz WK. A true threshold dose in chemical carcinogenesis cannot be defined for a population, irrespective of the mode of action. Human & experimental toxicology. 2000 Oct;19(10):566-568.
19. Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutation Research/Environmental Mutagenesis and Related Subjects. 1983 Nov 1;113(3-4):173-215.
20. OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development.
21. Oliveira PA, Colaço A, Chaves R, Guedes-Pinto H, De-La-Cruz P, Luis F, *et al*. Chemical carcinogenesis. Anais da academia brasileira de ciências. 2007 Jun;79:593-616.
22. Pitot HC, Dragan YP. Facts and theories concerning the mechanisms of carcinogenesis. The FASEB Journal. 1991 Jun;5(9):2280-2286.
23. Player A, Barrett JC, Kawasaki ES. Laser capture microdissection, microarrays and the precise definition of a cancer cell. Expert review of molecular diagnostics. 2004 Nov 1;4(6):831-840.
24. Ranganatha R, Chakravarthy S, Sukumaran S. High-throughput approaches for genotoxicity testing in drug development: recent advances. International Journal of High Throughput Screening. 2016;6:1-12.
25. Riddick DS, Lee C, Ramji S, Chinje EC, Cowen RL, Williams KJ, *et al*. Cancer chemotherapy and drug metabolism. Drug Metabolism and Disposition. 2005 Aug 1;33(8):1083-1096.
26. Rossetti S, Ren M, Somenzi G, Tagliavacca L, Sacchi N. Silencing of the RARA epigenetic network reveals the tumor-promoting activity of vitamin A. Journal of Nutrigenetics and Nutrigenomics. 2009;2(4-5):218-218.
27. Trosko JE. Commentary: is the concept of tumor promotion a useful paradigm? Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center. 2001 Nov;30(3):131-137.
28. Trosko JE. The role of stem cells and gap junctional intercellular communication in carcinogenesis. BMB Reports. 2003 Feb 28;36(1):43-48.
29. Warburg O, Nguyen T. The Prime Cause of Cancer. EnCognitive.com, 2015, 2.
30. Williams GM. Mechanisms of chemical carcinogenesis and application to human cancer risk assessment. Toxicology. 2001 Jan 2;166(1-2):3-10.
31. Shirai N, Tsubaki A, Morishita S, Honma D, Isobe S, Ikarashi K, *et al*. The association between time spent in performing physical activity and physical function in outpatients with type 2 diabetes who may have diabetic neuropathy. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2020 Nov 1;14(6):2111-2116.
32. Bello Gutiérrez J, López de Cerain Salsamendi A. Fundamentos de Ciencia Toxicológica. Ediciones Díaz de Santos; c2001.
33. Miller FA, Wilkins CH. Infrared spectra and characteristic frequencies of inorganic ions. Analytical chemistry. 1952 Aug 1;24(8):1253-1294.
34. Isenberg JS, Klaunig JE. Role of the mitochondrial membrane permeability transition (MPT) in rotenone-induced apoptosis in liver cells. Toxicological Sciences. 2000 Feb 1;53(2):340-351.