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Review- on Risk of Genotoxic agents and its effects on Aging, Sterility and Cancer

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Abstract:

Genotoxicity is one among the major cause of mutations leading to cancer. Genotoxins are substances that can play role in damaging DNA or chromosomal structure either by direct or indirect mechanism. This damage in germ cells or somatic cells may lead to various diseases such as aging, sterility, cancer, cardiovascular disease and multifractional disease. In this review we have made an attempt to understand and expecting to provide depth understanding about the risk of genotoxic agents and their mechanism involved in causing aging, sterility and cancer.

Keywords: Aging, Cancer, DNA damage, Genotoxins, Sterility

INTRODUCTION:

Genotoxicity is the ability of a substance/agent to produce a destructive effect on a cell's genetic material (DNA, RNA), thus affecting its integrity. These agents are known as Genotoxins. They may be radiation or chemical genotoxins, or mutagens; causing mutations [1]. Genetic toxicology is a branch of science that deals with the study of agents or substances that can damage the cell's genetic material and chromosomes [2].

A "genotoxic carcinogen" is an agent that induce cancer by directly altering the DNA of target cells while a "non-genotoxic carcinogen" is an agent that causes cancer by a secondary mechanism not related to direct genetic material damage [3].

Measurement of DNA and protein adducts, along with assessment of biological effects including chromosomal alterations and genetic mutations indicates DNA damage in a human population exposed to genotoxins [4]. Genotoxicity testing of new chemical entities (NCE) finds importance in hazard identification, DNA damage and its fixation [5]. In a eukaryotic cell, DNA damage in somatic cells may result in malignancy. In germ cells it may adversely affect reproduction or provoke heritable mutations, thus causing birth defects [6]. These permanent, hereditary mutations can affect either somatic cells or germ cells of the organism and can be passed to future generations [7]. The development of genetic toxicology began in the midst of increasing awareness of human exposure to toxic chemicals in the environment due to modernization of life [8]. For example, majority of the anticancer drugs are extremely cytotoxic, as well as mutagenic and carcinogenic [9].

Examination of DNA damage provides information regarding the exposure of organisms to genotoxins [10]. Genetic damage has also been implicated in the aging process, as evidenced by the study regarding ionizing radiation exposure, which has detrimental effect on longevity of laboratory animals. These effects are more profound in older and younger age groups, as compared to intermediate age groups [10]. The aging process is heavily attributed to accumulation of DNA damage in somatic cells. Stem cell systems play a vital role in regulating homeostasis and are consistently threatened by genotoxic stress, induced by reactive metabolites and environmental mutagens. The genetic integrity of mankind is increasingly threatened by industrial activity, which is primarily responsible for exposure to genotoxins [11].

Pollution caused by heavy metals; lead and mercury or chlorinated organic compounds (pesticides) have hazardous effects on health. Both groups are implicated in various cancers, mainly those of the reproductive system and immune system, as well as in depression, birth defects, sterility, and neurobehavioral problems [12].

IMPORTANCE OF PERFORMING GENOTOXIC STUDIES:

In vivo and *In vitro* genotoxicity tests provide information regarding a compounds potential mutagenicity and carcinogenicity, as well as reveal the molecular mechanism underlying the chemicals genotoxic and carcinogenic effects, identify hazards in risk assessment within molecular epidemiologic research with regards to occupational and environmental chemicals, determine toxicity profiles of chemicals, monitor the diseases and effectiveness of clinical treatments, develop regulations concerning medical, cosmetic and industrial chemicals according to international and national guidelines [13].

As a part of safety evaluation process, regulatory authorities around the world need data on the genotoxic potential of modern drugs. Genetic toxicity is important component for safety analysis of industrial chemicals, agricultural chemicals, Pharmaceuticals, food additives, colorants and chemicals [6]. Pre-clinical studies are conducted to get the toxicological data of new chemical entities (NCE), which is used to evaluate the safety and efficacy of the NCE and in return help in evaluating drug risk and benefit assessment in New Drug Application (NDA) process. Thus, genotoxic assays are fundamental regulatory requirements. However, people in India are not familiar with genotoxicity and that it has now become mandatory to include it in drug master file required by European and United States regulatory authorities [1].

GENOTOXIC RISK:

Genetic material damage in somatic cells causes aging, cardiovascular diseases and cancer, whereas in germ cells

causes sterility, multifactorial diseases such as diabetes, psychoses and cardiovascular diseases. It also leads to genetic diseases, such as, sickle cell anaemia, haemophilia and cystic fibrosis [3].

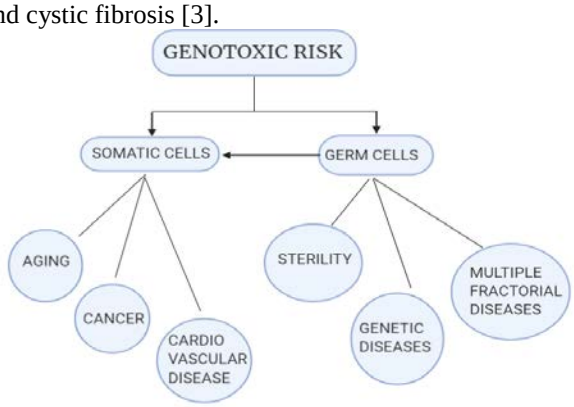

Figure 1: Genotoxic risk

MECHANISM OF GENOTOXICITY:

Direct mechanism:

In case of the former, genotoxins directly act on DNA, leading to formation of lesions comprising of adducts and breaks that damage the overall genetic material, which ultimately produces mutations in gene, chromosome and genome, thus increasing uncontrolled cell proliferation i.e. carcinogenesis.

Indirect Mechanism:

In case of the latter, genotoxins or carcinogens indirectly act on non-DNA targets, including mitotic spindle formation, process of cell cycle, apoptosis and enzymes of DNA repair, which lead to carcinogenesis through uncontrolled cell proliferation.

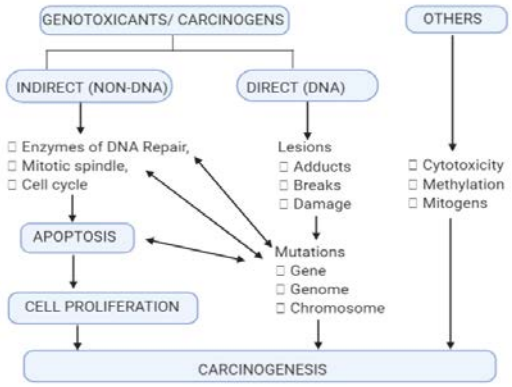

Figure 2: Genotoxicity mechanism

GENETIC MATERIAL DAMAGE AND ITS EFFECTS ON AGING:

Aging is a series of time-related processes that occur in adults till death, which is coordinated by genes [14]. Stem cells play a major role in long term repair of any damaged tissue or cell that contributes to production of cells that can repair or replace tissues throughout its life [15].

At organism level, aging is a chronological clock. At the cellular level, aging can be divided into two parts chronological and replicative aging. Chronological aging is age of intracellular constituents of stem cells. Replicative aging is proliferative output of stem cells during their history [16]. Martin Alexzander et al, elaborated on concentration of pesticides in soil and how long-term exposure leading to pesticide toxicity in invertebrates and plants, further damaging human genome [17].

Christopher et al, also described that ecotoxicological studies for DNA damage and repair is important for two reasons: DNA is an important unit of inheritance and reproduction. The effect of genotoxins on genetic material leads to aging process. For example, long term exposure to ionizing radiation, a physical genotoxin increases risk of aging through genetic damage [10].

Mechanisms of aging:

a. p53 and p16 ^{Ink4a} pathway:

Aging is attributed to accumulation of DNA in somatic or stem cells. Stem cell senescence or apoptosis are considered the two important cellular mechanisms for stem cell depletion following DNA damage [18]. p53andp16^{Ink4a}are the best mark regulators of senescence and apoptosis in response to DNA damage, including oncogenic mutations, and have been implicated as ‘‘gatekeepers’’ for tumour suppression and tissue aging [16].

The ATM (ataxia telangiectasia mutated) kinase and WRN (Werner syndrome ATP-dependent) helicase are necessary for genetic material repair. Deletion of anyone enzyme causes premature aging phenotype in mice [17]. These pathways are employed in a celland organismspecific pattern. In MEFs (Mouse Embryonic Fibroblasts), activation of p19^{ARF} leads to stabilization of p53. In certain human cell types (e.g., fibroblasts and keratinocytes), telomere attrition can activate p53 via the ATM and (potentially) ATR (Ataxia telangiectasia and Rad-3 related protein) kinases. Senescence can also be triggered in human cells via p16^{Ink4a}expression.

Activation of p53 gene causes senescence through a complex gene expression that also induces p21^{KIP}. The role of Rb (retinoblastoma) gene in senescence takes part in repression of E2F target genes alsocauses alterations in chromatin structure. Senescent cells may contribute to aging via depletion of stem cell pools and/ or elaboration of factors that interfere with tissue function. Factors elaborated by senescent cells may also stimulate the growth of epithelial tumors.

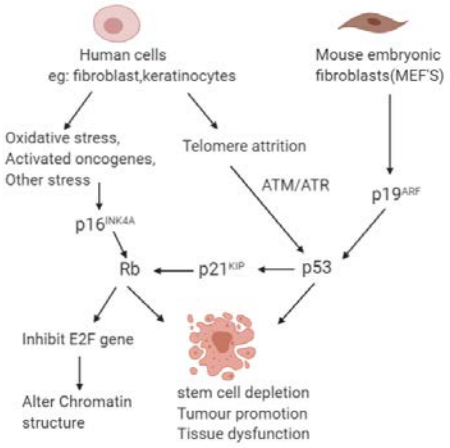

Figure 3: Mechanism of aging through p53 and p16^{Ink4a}

b. Reactive oxygen speceies (ROS) pathway:
Reactive oxygen species which include superoxide anion, hydroxyl radical, hydrogen peroxide, nitric oxide and others are majorly seen in mitochondria, peroxisomes, cytochrome p450 and antimicrobial oxidative brust of phagocytic cells. ROS cause DNA lesions (single and double stand breaks), lipid peroxidation, protein damage, DNA adducts and cross-links [17].
c. Cell intrinsic and extrinsic factors:
Multiple components drive stem cell aging. It occurs through changes in both cell-extrinsic regulators and intrinsic effectors.Cell extrinsic factors includes changes from systemic blood and also from stem cell niche induces signalling pathway thus alters stem cell function. hence can modify genetic and epigenetic signature of stem cells. The venerable stem cells can directly alter niche.
Aging occurs by deregulation of cell-intrinsic effectors that manage metabolic system, proteostasis, mitochondrial function. These includes ROS, mitochondrial DNA (mtDNA) [17]. Many of these modifications are interrelated, thus implicating mutual impact on each other, which culminates in stem cell failure at multiple levels.

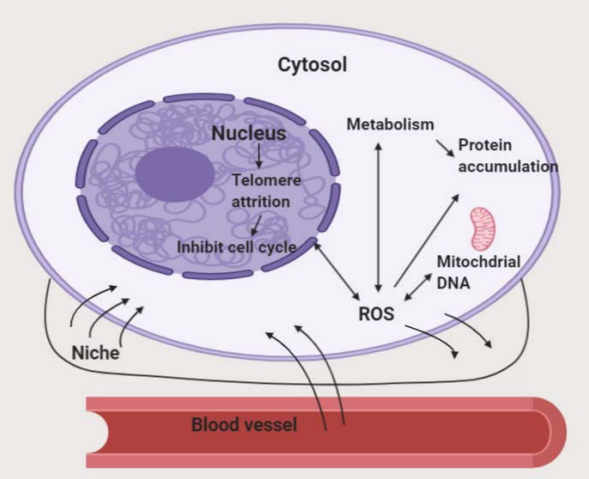

Figure 4: Mechanism of aging through ROS and cell intrinsic and extrinsic factors.

GENETIC MATERIAL DAMAGE AND ITS EFFECT ON STERILITY:

Over the decades studies shown man-made chemicals and environmental toxicants have attributed destruction of reproductive function in animals and in humans [19]. such as organophosphate pesticides, dichloro diphenyl trichloro ethane(DDT) and its metabolite dichloro diphenyl dichloro ethylene(DDE) causes infertility in males [20]. 60-80 million couples world-wide are suffering from infertility, failing to conceive after regular intercourse for about 12 months.
Farag et al., [21] revealed that 14 and 28mg/kg/day doses of acephate decreases sperm count and sperm motility in adult male mice. William W et al. [9], explained genotoxic effects of Adriamycin causes damage to spermatozoa and the mature sperm can remain in the cauda epididymides for a long time. Motility of the sperms were also drastically reduced during the event of low sperm count, thus implying that the surviving spermatozoa also succumbed to the genotoxic effects of the drug.
Avi Harley et al., [22] reported contents of cigarette smoke are present remarkably higher level in the spermatozoa DNA of smokers than in the spermatozoa DNA of age-matched non-smokers.

Mechanisms of sterility:

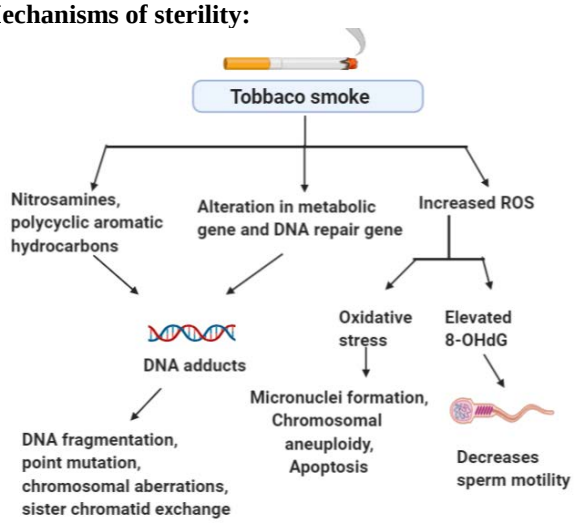

Figure 5: Tobacco smoke and its effects on sterility

The mechanisms mentioned in present figure-5 focus mainly on Tobacco smoke related to alterations in genetic material. Increased ROS (reactive oxygen species) level causes elevation of 8-hydroxyguanosine (8-OHdG).Guanine is the most important base in DNA to attack free radical is converted to various biomarkers like 8-hydroxy-2-deoxyguanosine (8-OHdG), 8-hydroxyguanosine,8-hydroxyguanine.8-OHdG adduct is abundant base excreted in urine and storage of such biomarker leads to oxidative DNA damage [23].Increase in the level of 8-OHdG decreases sperm quality and enhances oxidative genetic damage in human spermatozoa [24]. Concentration of cotinine from 400–800 ng/mL depresses sperm motility, membrane function, and their ability to endure capacitation [25].
Tobacco smoke contain 60 known carcinogens, low-volatile substances such as polycyclic aromatic hydrocarbons, aromatic amines and N-nitrosamines. Accumulation of these substances causes formation of DNA adducts, fragmentation of DNA, chromosomal aberrations, sister chromatid exchange, DNA strand breaks (single and double strand breaks) and point mutations [26]. Alteration in xenobiotic metabolism gene and DNA repair gene by tobacco smoke leads to oxidative stress and further produces micronuclei formation, chromosomal aneuploidy and apoptosis [27].

GENETIC MATERIAL DAMAGE AND ITS EFFECTS ON CANCER:

Over the past few decades worldwide, smoking tobacco is identified as main risk factor for lung cancer [28]. Carcinogens like asbestos, polycyclic aromatic hydrocarbons, silica or diesel fuel are known risk factors.

KRAS gene (Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) mutations are more frequently seen in smokers [29].

Mutations in cell cycle leads to uncontrolled proliferation or impaired death of cells. This is majorly because of loss of tumour suppressor gene function or an upregulation of oncogenes [30]. For human cancers Genome Wide Associate Studies (GWAS's) have identified hundreds of genetic risk variants. Majority of genetic risk variants cancer types are breast, colorectal and prostate cancer [31].

Cancer is mainly modulated by the Wingless and integrated or Int1 gene(Wnt) or(Wnt/β-catenin), Reactive Oxygen species (ROS) [32] [33]. Phosphatidylinositol-3-kinase/ Protein kinase B (PI3K/Akt) or (PI3K/Akt/mTOR) [34]. Microtubule associated protein kinase, originally known as extracellular signal- regulated kinases (MAPK/ERK) or (Ras-Raf- MEK - ERK) [35] and Hippo pathway [36] [37] [38]. In addition to these pathways, various protein modulators and alterations in genes result in the induction of cancer.

Mechanisms of cancer:

a) Wnt and ROS Pathway:

Tumors are the result of malfunctioning in metabolic pathways which include- amino acid, steroid and lipid metabolism, nucleotide biosynthesis, TCA cycle, Glycolysis etc., as evidenced by proteomic and metabolic studies on various carcinomas. These studies further suggest that the alterations in metabolic pathways are attributed to defective cell signaling, caused by oncogenic triggering, which control them [32].

In the case of Wnt pathway, which is responsible for cell proliferation, EMT induction, angiogenesis, migration and cell survival, is largely implicated as an important modulator in cancer as a result of oncogenic signaling in the pathway, affecting normal cellular metabolism [32]. Wnt also enhances glucose metabolism through direct modulation on the transcription of pyruvate dehydrogenase kinase 1 (PDK1) and lactate transporter, Monocarboxylate transporter (MCT -1), which causes the conversion of glucose-derived pyruvate to lactate, when shunted from the mitochondria [39]. The increased secretion of lactate, under the influence of MCT-1, supports angiogenesis in tumors. In addition to carbohydrate metabolism, Wnt is also triggers the generation of ROS, which is perpetuated in case of Adenomatous polyposis coli (APC) loss, resulting in the altered functions of the TIGAR and RAC1 proteins that regulate various types of cellular ROS [32].

TIGAR controls the regeneration of glutathione (GSH), an intracellular antioxidant, while RAC1, a component of the NADPH oxidase complex, functions to generate ROS which is of importance to cell signaling and proliferation. On oncogenic signaling, causing alteration in the Wnt pathway, results in loss of TIGAR and RAC1, which further causes increase in destructive ROS [40], while halting cell proliferation and Pro-proliferative ROS respectively. Thus, the combination of effects produced by loss of the ROS modulating proteins results in uncontrolled cell proliferation. ROS also plays a pivotal role in promoting apoptosis [32].

Mutations in the APC gene encoding the APC protein, results in proliferation and DNA damage, further enhancing carcinoma progression as evidenced by the study on CRC, wherein the integration of APC mutations and high fat diet increases progression of intestinal tumorigenesis [32].

b) KRAS gene pathway:

KRAS gene has a prominent role in modulating alteration in metabolism as a result of mutations in the gene, which is implicated in many types of cancer. It supports tumor growth by increasing glucose metabolism and glucose uptake by cancer cells through up-regulation of Glucose transporter (GLUT1), as well as regulating glutamine, amino acid and fatty acid metabolism thereby promoting cancer cell proliferation [32].

Mutated KRAS gene also assists cancer cell proliferation in the event of glutamine depletion, by up regulating asparagine synthase (ASNS), which supports de novo asparagine biosynthesis. Some studies have shown that cellular respiration is supported by high levels of fatty acid synthase(FASN) that increasing lipid oxidation, thus promoting cell adoption during metabolic stress [32].

c) Protein Modulators:

Studies have shown that various protein modulators promote tumorigenesis, in addition to altered genes triggered by oncogenic signaling. This is implicated in the study on CRC, where the serine biosynthetic pathway is used for glutamine consumption via a metabolic switch triggered by Protein Kinase delta (PKC) deficiency, which ultimately results in intestinal tumorigenesis [32].

d) Wnt/β-catenin signaling pathway:

Cancer stem cells have the hallmark properties of normal stem cells i.e. self- regeneration and differentiation, with the difference being, loss in homeostatic mechanisms that control cell division. The Wnt/β-catenin signaling pathway is responsible for the pivotal role in regulating the balance between stemness and differentiation in various adult stem cells locations- Hair follicles, mammary glands and intestinal glands. Mutations in genes encoding the Wnt signaling is the basis of tumorigenesis in the above stem cell locations [39] [41] [42] [33] [43].

Most cancer cases are the result of loss of APC function or oncogenic β-catenin mutations. The presence of nuclear β-catenin, formed as a result of mutations, is a definitive indicator of tumor cells. The varying degrees of intracellular distributions of β-catenin in the tumor cells implicate the differing levels in oncogenic Wnt signaling, which attributes to different cellular activities- Cell proliferation, epithelial - mesenchymal transitions etc. This in turn promote tumorigenesis and malignancy. Studies have shown that various factors contribute to the differing oncogenic Wnt signaling in the tumor mass. These factors many linked to the cell intracellular components or the tumor's environment [39].

e) PI3K/Akt signaling pathway:

Phosphatidylinositol-3 kinases, PI3Ks are components of the lipid kinase family, which are notable for phosphorylating inositol ring 3′-OH group in inositol

phospholipids to produce the second messenger phosphatidylinositol-3,4,5-trisphosphate (PI-3,4,5-P3). PI3K are responsible for the release of PI(3,4,5)P3through the activation of RPTK in the inner plasma membrane, where Akt interacts with the above phospholipids, causing their phosphorylation and activation by PDK 1 and PDK2 [44].

Various studies have shown that the PI3K/Akt signaling pathway is consistently changed in various types of cancers [34] [45]. This is attributed to the ability of activated Akt molecules that regulate cell survival, cell cycle and cell growth [45]. The most common mechanism for treatment chemotherapy and gamma radiation has been through apoptosis of tumor cells, that have been reprogrammed as a result of altered oncogenic PI3K / Akt signaling.

This presents a problem since several studies have proven the resistance to apoptotic activation. Since the PI3K/ Akt pathway mediates survival signals, it is implicated in the resistance of tumor cells. Hence, various innovations in cancer treatment is aimed at this pathway [44] [46].

f) Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways:

Genetic alterations in Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways result in uncontrolled cell proliferation and reduced responsiveness to apoptotic agents. Hence this pathway is implicated in resistance of tumor cells to apoptosis. Alterations due to oncogenic triggering also support the survival cancer cells via the pathways [35] [47].

Studies have shown the immense importance of the pathways in regulating growth of stem cells, cellular senescence and aging, which is adversely affected due to mutations occurring in the components of the pathway [48].

g) Hippo pathway:

The Hippo pathway plays a pivotal role in maintaining organ size in various species. It has been implicated in inducing tumor's due to mutations in Hippo pathway genes, most notably neurofibromin (NF2), which is an important tumor suppressor gene. In addition to main tong organ size, it also controls cell proliferation and apoptosis, which are vital cell activities in mediating tumorigenesis. Hippo pathway is known to produce tumor's in mouse and flies due to mutations, as well as in the case of humans, as evidenced by various studies and hence it is considered as important target for cancer treatment [36] [37] [38].

CONCLUSION:

Genotoxins are agent that has ability to interact with DNA and damage its structure either by direct or indirect mechanism. Genetic toxicity is important for safety evaluation ofpharmaceuticals, agricultural chemicals, food, additives, colorants and industrial chemicals. DNA damage in somatic and germ cells causes certain diseases like aging, sterility and cancer. Aging is caused mainly by long term exposure to certain pesticides, ionizing radiations and other physical toxicants throughp53 and p16 ^{Ink4a} pathway, Cell intrinsic and extrinsic factors and ROS pathway. Sterility is caused by certain chemicals of tobacco smoke induces DNA damage, adducts formation, DNA fragmentation, micronuclei formation and chromosomal abnormalities. Cancer or mutagenesis is induced by certain carcinogens such as, polycyclic aromatic hydrocarbons, silica, diesel fuel or asbestos and the pathways involved areHippo pathway,PI3K/Akt signaling pathway,Wnt/β-catenin signaling pathway, Wnt and ROS Pathway, KRAS gene pathway and many more pathways.

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