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Hepatocellular Carcinoma and Possible Chemical and Biological Causes: A Review

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ABSTRACT: The development of hepatocellular carcinoma (HCC) is a multistep process. In HCC, progressive and morphologically distinct preneoplastic lesions/alterations associated with chronic liver injury, inflammation, hepatocellular degeneration/regeneration, necrosis, and small-cell dysplasia can be observed. The incidence of HCC exhibits regional and ethnic differences. Several cytotoxic and DNA-damaging chemicals are suggested to be the underlying causes of HCC—for example, acrylamide, perfluorooctanoic acid (PFOA), polychlorinated biphenyls (PCBs), benzo(a)pyrene (BaP), perfluorinated chemicals (PFCs), vinyl chloride monomer (VCM), and dietary contaminants (aflatoxins, ochratoxins). Also suggested are substances of abuse (alcohol) and biological agents, such as hepatitis B and C and human immunodeficiency virus 1 (HIV-1). These can act through genetic and/or epigenetic mechanisms. This review will shortly address the genetic and epigenetic mechanisms of HCC and focus on cytotoxic and DNA-damaging chemicals and biological agents, exposure to which are suggested to lead to HCC initiation, promotion, and/or progression.

KEY WORDS: hepatocellular carcinoma, DNA-damaging chemicals, dietary contaminants, alcohol, hepatitis

I. INTRODUCTION

Primary liver cancers include hepatocellular carcinoma (HCC), intrahepatic choloangiocarcinoma (bile duct cancer), angiosarcoma/hemangiosarcoma, and hepatoblastoma.[1] Angiosarcoma and hemangiosarcoma are rare cancers. About 10% to 20% of liver cancers encountered in clinics are intrahepatic cholangiocarcinoma, which starts in the small bile ducts.[2] Hepatoblastoma is a very rare liver cancer that develops especially in children who are younger than four years old. HCC is the most common type of liver cancer in adults, the sixth most common cancer worldwide, and the third most common factor leading to cancer deaths worldwide because of its high malignancy and lack of effective medical therapy. The incidence of HCC exhibits a regional divergence.[3] Additionally, diverse etiological factors may affect HCC incidence.[4]

Hepatocellular carcinoma typically results from chronic liver inflammation followed by fibrosis or cirrhosis.[5,6] Studies have demonstrated that several risk factors may cause liver cancers (Table 1).[2,7] Symptoms of fatty liver, liver fibrosis, liver cirrhosis, and liver cancer are given in Fig. 1.

This review focuses on mechanisms involved in the development of HCC and the association of HCC with exposure to cytotoxic and DNA-damaging chemicals, dietary contaminants, and biological agents.

II. DEVELOPMENT OF HEPATOCELLULAR CARCINOMA

The development of HCC is a multistep process and determined with progressive and morphologically distinct preneoplastic lesions/alterations. These lesions are associated with chronic liver injury, inflammation, hepatocellular degeneration/regeneration, necrosis, and small-cell dysplasia which can be followed by low- and high-grade dysplastic nodules.[8]

Reactive oxygen species (ROSs) are generated constantly as a consequence of metabolic and other

TABLE 1: Risk factors for liver cancers

Biological agents (hepatitis B, hepatitis C)
Chronic infections (viral hepatitis, infections with parasites, etc.)
Cirrhosis, primary biliary cirrhosis
Cytotoxic and DNA-damaging chemicals (vinyl chloride, arsenic, acrylamide, polychlorinated biphenyls, perfluorinated chemicals, etc.)
Dietary contaminants (aflatoxins, ochratoxins)
Gender
Inherited metabolic diseases
Nonalcoholic fatty liver disease
Obesity
Race/ethnicity
Substances of abuse (heavy alcohol use)
Tobacco use
Type 2 diabetes

biochemical reactions and external factors. On the other hand, it is apparent that chronic inflammation, fibrosis, and cirrhosis cause the generation of ROSs within the liver. ROSs can additionally enhance the damaging effects of carcinogenetic xenobiotics. During the progression stage of carcinogenesis, ROSs can directly induce cell growth because of cytotoxicity and later uncontrolled proliferation.[9] Moreover, a variety of chronic inflammatory diseases and chronic exposure to chemicals may contribute to the oxidative stress-induced DNA lesions that can lead to cancer susceptibility.[10] For example, both hepatitis B and hepatitis C infections are linked with enhanced ROS/RNS levels and decreased antioxidant levels.[9]

Studies have shown that the development of HCC is caused by complex polygenetic, multipathway, and/or epigenetic alterations.[4] Genetic and

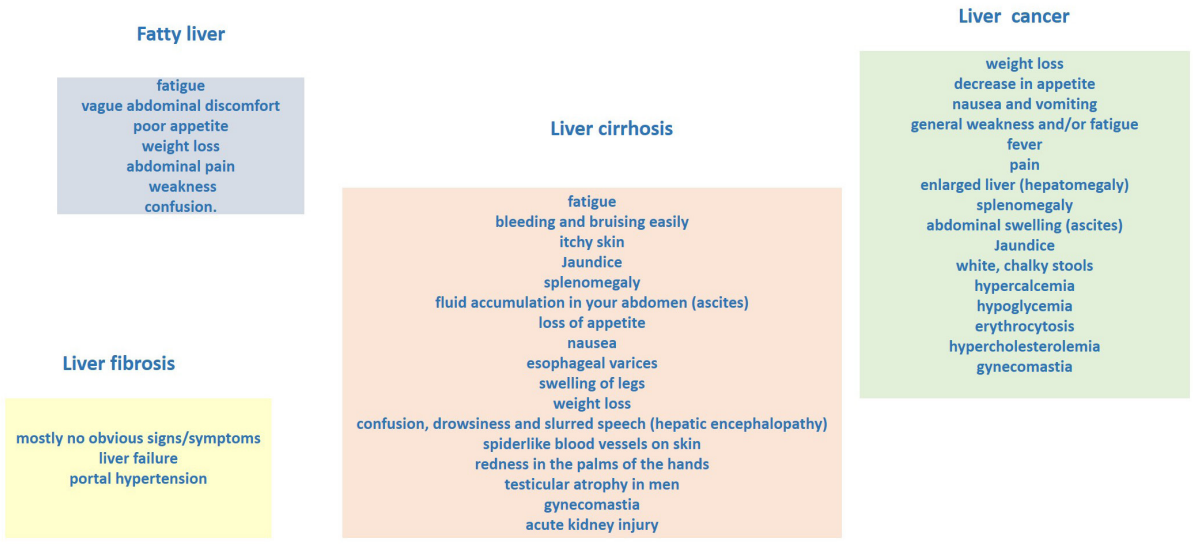

FIG. 1: Symptoms of fatty liver, liver fibrosis, liver cirrhosis, and liver cancer

epigenetic pathways in the development of HCC are briefly discussed next.

A. Genetic Mechanisms

Oxidative stress occurs when ROS levels exceed the cell's natural antioxidant defense mechanism. Increased intracellular ROS concentrations may contribute to hepatic DNA lesions. The main oxidative DNA lesions are base alterations (deletions, insertions, transversions, and transions), abasic sites, single-strand DNA breaks (SSBs), double-strand DNA breaks (DSBs), DNA adducts, sugar moiety modifications, frame shift mutations, and chromosomal aberrations.[11] These changes can all be underlying factors for different diseases and cancers, including cancers of liver.[12,13] ROSs can also damage specific genes, such as those related to cell growth and tumor suppression. Moreover, oxidative stress can lead to alterations in other macromolecules, such as proteins and lipids.[14,15]

DSBs are the most dangerous type of DNA damage because they can induce gene mutations, chromosomal aberrations, and cell transformation.[15] They can occur spontaneously or can be induced by chemical agents, ionizing radiation, radiomimetic chemicals, or ROSs.[16,17] DSBs are major causes of some cancer types. In liver cancer, DSB repair defects have particular importance. DSB repair gene XRCC7 polymorphisms seem to be substantial in the development of HCCs.[18]

Cellular signaling alterations are also very important in the outcome of HCCs. Different signaling cascades have been identified in HCC by experimental studies and signaling pathway–based research. Retinoblastoma 1 (Rb1), p53, epidermal growth factor (EGF), and WNT/β-catenin are the most affected pathways in this process. The p53/ARF pathway controls mechanisms such as cell cycle arrest, apoptosis, and DNA repair.[16–18] Tang et al. (1998) suggested that the p53/CDKN2 mutation or overexpression of H-Ras or epidermal growth factor receptor (EGFR) is associated with the invasiveness and reoccurrence of HCC.[19] Somatic mutations in the β-catenin gene can induce liver tumorigenesis and dysregulation of the Wnt/β-catenin pathway. Additionally, during hepatic tumor progression, β-catenin activation can generate a special genetic modification in cooperation with several oncogenes such as Myc and Ras.[20]

Different researchers have reported that the overexpression of members of the Ras oncogene family, such as HRas, are the common mutations in HCC.[21,22] Experimental studies have demonstrated that genes such as Myc, EGF, transforming growth factor α (TGFA), and phosphatase and tensin homolog (PTEN) also have major roles in HCC initiation. TGFA is an important paracrine activator of EGF signaling in HCC and contributes to proliferation and invasion of tumor cells.[22] EGF signaling, on the other hand, is one of the key drivers in HCC. Evidence has shown that the specific single nucleotide polymorphism of the EGF gene has a major role in HCC.[23]

B. Epigenetic Mechanisms

Several studies have reported that genetic and epigenetic events affect each other and trigger different types of tumorigenesis.[4] Major tumor drivers in HCC are the following: global changes in DNA methylation (genome-scale alterations in the DNA methylation landscape, loci-specific DNA hypermethylation, DNA hypomethlation, dysfunction of histone-modifying enzymes, and alterations in CpG island methylation profiles); histone modifications and alterations in the chromatin structure (chromatin remodeling and compaction); and alterations in transcription factors, microRNAs (miRNAs), and noncoding RNAs (ncRNAs).[24] The importance of epigenetics in HCC has been revealed in the last decade, and a limited number of studies (compared to genetic studies) have been performed. Most of these studies have focused on DNA methylation, whereas histone modifications and changes in RNA profiles have yet to be widely studied.

DNA hypomethylation, especially progressive loss of cytosine DNA methylation, is substantial in the conversion of normal cells to tumor cells.[25] Chappell et al. (2014) showed that epigenetic changes are crucial in the early development

of HCC, particularly if fibrosis and/or cirrhosis is present.[8] Yamada et al. (2005) showed that DNA hypomethylation causes the development of multiple liver tumors in mice. Thus, hypermethylation of promoter CpG islands and global hypomethylation of repeats can cause additional tumor-promoting changes.[26] Shen et al. (2012) performed a genome-wide methylation study mainly on HBV-induced HCC (79% of tumors studied) and compared tumor and adjacent tissue DNA methylation profiles. They identified 1,640 hypomethylated and 684 hypermethylated CpGs in the tumors.[27] Using a similar study approach, Song et al. (2015) reported that 62,692 loci displayed differential methylation between HCC and surrounding tissue, of which 61,058 were hypomethylated (CCL20, ATK3, SCGB1D1, WFDC6, and PAX4) in HCC patients (n = 27), while a small number of genes (DAB2IP, BMP4, ZFP41, SPDY1, and CDKN2A) were found to be hypermethylated.[28] In a more comprehensive work, Nishida et al. (2012) identified eight hypermethylated tumor suppressor genes (HIC1, GSTP1, SOCS1, RASSF1, CDKN2A, APC, RUNX3, and PRDM2) in the early stages of HCC, which were associated with a shorter period for the occurrence of HCC.[29]

A limited number of studies in the literature have reported histone methylation alterations in HCC. Cai et al. (2011) showed that high levels of trimethylated histone H3 lysine 4 (H3K4me3), which is associated with transcriptional repression, correlate with worse prognosis of HCC.[30] In addition, H3K4me3 levels were closely associated with aggressive tumor features (vascular invasion, large tumor size, multiplicity of tumors, and poor differentiation). He et al. (2012) also determined that reduced overall survival and poor prognosis were associated with high levels of H3K4me3.[31]

EZH2 is a methyltransferase that mediates gene silencing by trimethylating histone H3 lysine 27 (H3K27).[32] Elevated expression of EZH2 has been reported in different cancers (e.g., breast, prostate).[33,34] EZH2 knockdown in liver cancer cell lines was shown to reduce the repressive H3K27me3 marker, leading to re-expression of a distinct subpopulation of tumor suppressor miRNAs (miR-139-5p, miR-125b, miR-101, let-7c, and miR-200b), which control motility and adhesion.[35] Another study showed that EZH2 knockdown strongly inhibits the proliferation of Dlk+ hepatic progenitor cells, promoting and fastening their differentiation into hepatocytes.[36] Additionally, Wang et al. (2013) reported that c-Myc together with EZH2 silenced tumor-suppressive miRNAs. This silencing targeted the PRC2 complex in turn and caused the overexpression of EZH2 in HCC.[37]

III. AGENTS THAT MAY CAUSE HEPATOCELLULAR CARCINOMA

HCC most often occurs as a result of chronic liver inflammation, fibrosis, cirrhosis, impairment of metabolism, viral infections, and toxic damage. Several environmental chemicals and biological and physical agents can be underlying causes of liver cancer because of their ability to induce genetic and epigenetic modifications.[4,14]

Epidemiological studies show that the prominent risk factors for HCC are the following: (1) chemical agents such as acrylamide, polychlorinated biphenyls (PCBs), benzo(a)pyrene (BaP), perfluorinated chemicals (PFCs) including perfluorooctanoic acid (PFOA), and vinyl chloride; (2) dietary contaminants (aflatoxins, ochratoxins); (3) alcohol abuse; and (4) chronic infections such as hepatitis B and C virus and human immunodeficiency virus 1 (HIV-1).[4,38,39] The agents and/or conditions that cause ROSs and their consequences are given in Fig. 2.

A. Chemical Agents

Chemical and biological agents that may cause fatty liver, liver fibrosis, liver cirrhosis, and liver cancer are given in Fig. 3. Some of these agents are explained next.

1. Acrylamide

Acrylamide is an industrial chemical that is produced in high volumes. It is used in wastewater treatment; in adhesive agents, cement slurry, and

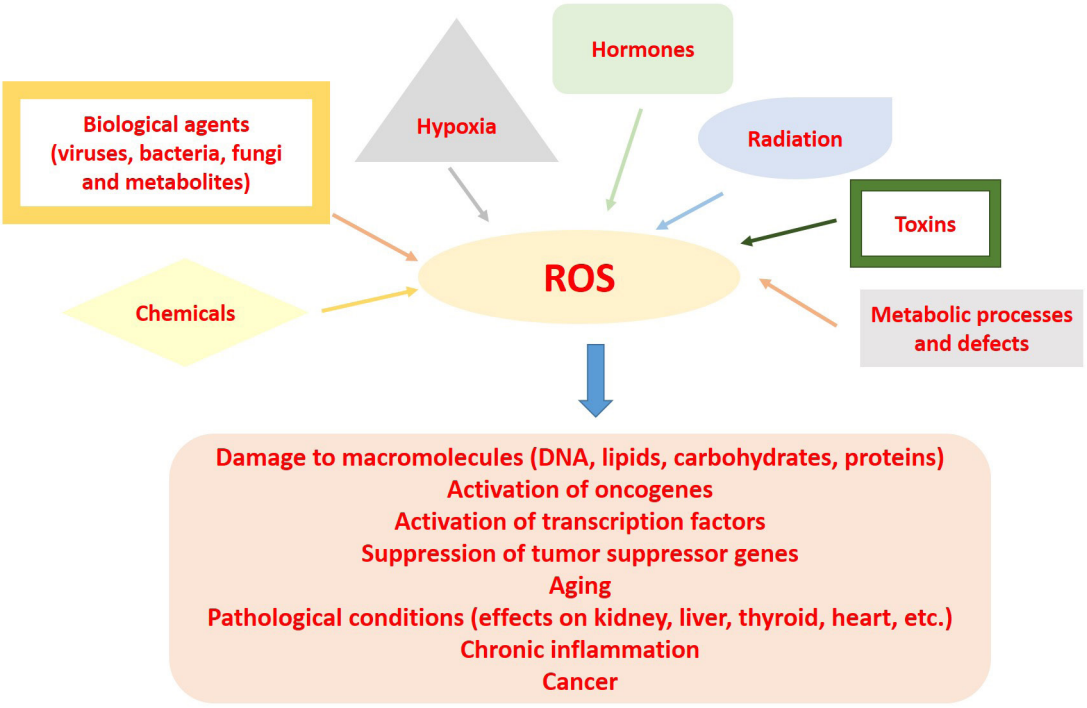

FIG. 2: Agents and/or conditions that cause ROS generation and its consequences

cosmetics; and in laboratories.[40] Additionally, acrylamide can be formed from food components during heat treatment as a result of the Maillard reaction between amino acids and reducing sugars.[41] High levels of acrylamide have been detected in several foods, such as baked potatoes, french fries, and coffee.[40,42,43]

Acrylamide is neurotoxic to human and laboratory animals and is classified as a group 2A carcinogen by the International Agency for Research on Cancer (IARC).[42,44] Following oral intake, the liver is the initial site of acrylamide metabolism; thus acrylamide is found at high concentrations in the liver. Acrylamide is metabolized to glycidamide by cytochrome p450 2E1 (CYP2E1).[38] Several researchers have reported that acrylamide and glycidamide have clastogenic and mutagenic properties.[45]

Jiang et al. (2007) studied the genotoxicity of acrylamide in HepG2 cells and measured the level of intracellular ROSs. They observed that acrylamide induced a significant, dose-dependent increase in intracellular ROS generation. They also found that the nuclei of acrylamide-treated cells exhibited strong positive staining for 8-hydroxy-2'-deoxyguanosine (8-OHdG). The staining of 8-OHdG was more prominent when the acrylamide dose was increased. The researchers also determined DNA damage in HepG2 cells by Comet assay and showed that acrylamide induced DNA damage in a dose-dependent manner.[38]

The upregulation of CYP2E1 can lead to an increase in acrylamide biotransformation. It is evident that ethanol can lead to an increase in CYP2E1 expression and activity. Lamy et al. (2008) evaluated the genotoxicity of acrylamide in HepG2 cells using Comet assay and examined the modulatory effects of ethanol on acrylamide-induced DNA migration. They observed that acrylamide induced significant increases in DNA migration. Additionally, after ethanol treatment and subsequent acrylamide exposure, DNA migration was enhanced almost twofold compared to cells treated with acrylamide alone. Consequently, the researchers

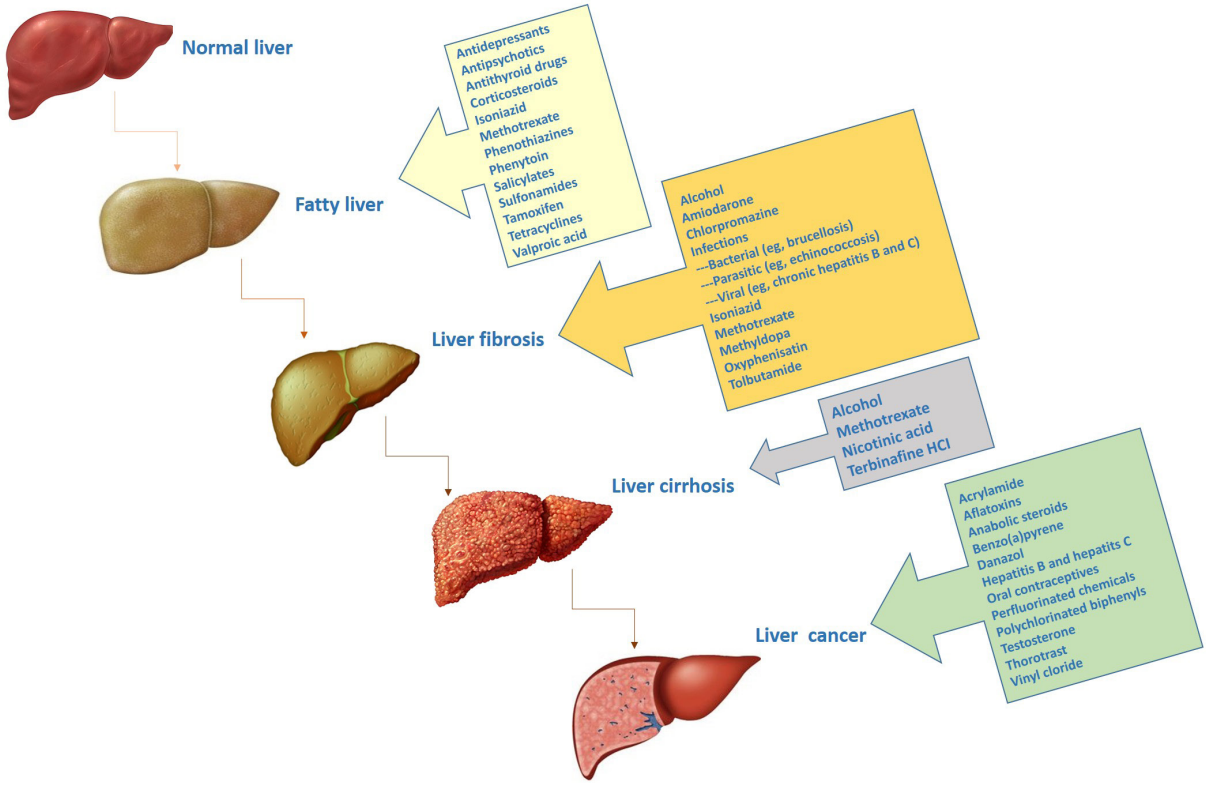

FIG. 3: Chemical and biological agents that may cause fatty liver, liver fibrosis, liver cirrhosis, and liver cancer

suggested that ethanol exposure of HepG2 cells results in increased of CYP2E1 activity and that CYP2E1-mediated transformation of acrylamide to glycidamide might increase DNA damage in HepG2 cells.[40]

2. Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are environmental pollutants that are commonly used in industrial applications. However, their use was banned by the U.S. Congress in 1979 and by the Stockholm Convention on Persistent Organic Pollutants in 2001 because of their high toxicity and high persistence in the environment.[46] Studies have demonstrated that PCBs cause serious toxic effects such as cardiotoxicity, immunotoxicity, reproductive toxicity, neurotoxicity, and hepatotoxicity.[47,48] The IARC classified PCBs as group I carcinogens in humans.[49] According to the U.S. Environmental Protection Agency (EPA), PCBs cause cancer in animals and are probable human carcinogens.[50]

PBCs are metabolized by CYP450 and by peroxidases. Their metabolism leads to generation of quinone-type metabolites such as quinones, semiquinones, and hydroquinones.[47–49] These metabolites undergo oxidation reduction cycles which cause the formation of ROSs. PCB biotransformation and the effects of PCB metabolites on apoptosis are schematized in Fig. 4.

Dong et al. (2014) treated HepG2 cells with 2,3,5-6-phenyl-(1,4)benzoquinone (PCB29-pQ) (0–10 μM). They demonstrated that PCB29-pQ caused generation of 8-OHdG in a dose- and time-dependent manner. In addition, it led to high levels of γ-H2AX, which is an indicator of DSBs. The researchers suggested that the genotoxicity of PCB29-pQ was associated with the generation of ROSs in HepG2 cells.[48]

Rocha de Oliveira et al. (2014) conducted a

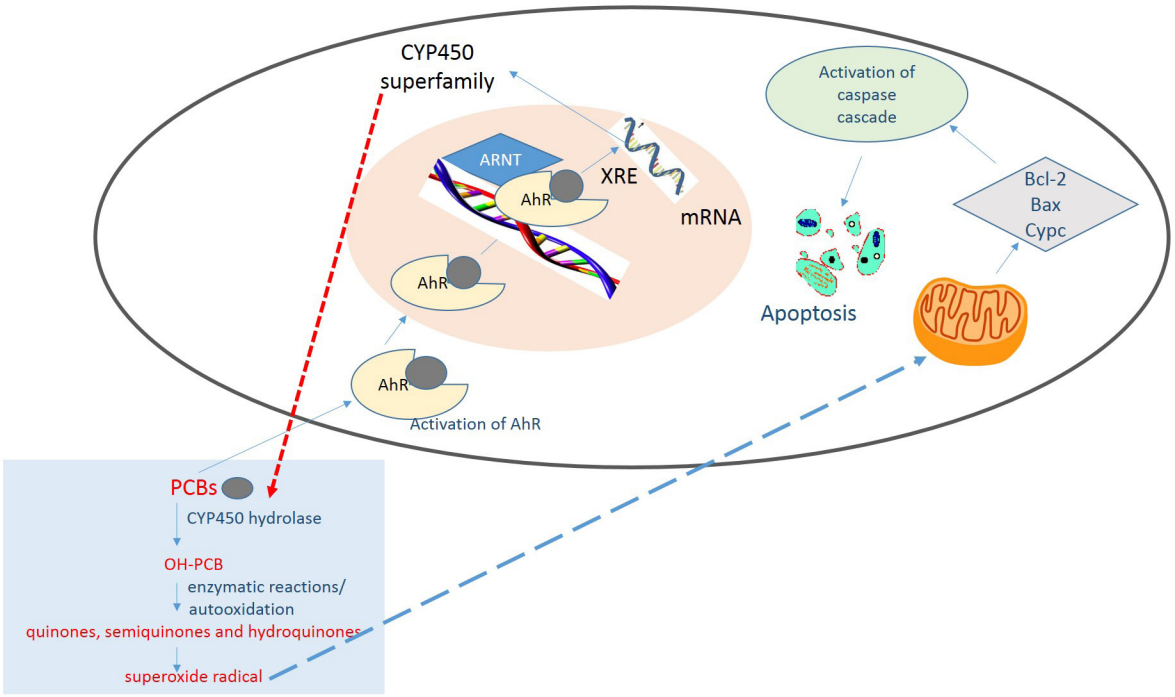

FIG. 4: PCB biotransformation and the effects of PCB metabolites on apoptosis. ARNT, aryl hydrocarbon receptor nuclear translocator; AhR, aryl hydrocarbon receptor; CYP450, cytochrome p450; XRE, xenobiotic response element.

study on four groups of male Wistar rats (n = 36): control, quercetin (50 mg/kg/day), PCB mixture (0.4 mL/kg/day), and PCBs + quercetin. After 25 days, the animals were euthanized. Thiobarbituric acid reactive substances (TBARS) as an indicator of lipid peroxidation, activities of antioxidant enzymes, DNA damage (micronucleus assay), and histological liver damage were determined. Liver TBARS concentrations and superoxide dismutase (SOD) activities were significantly higher in the PCB group versus the PCB + quercetin group . Hepatic catalase (CAT) and glutathione peroxidase (GPx) activities decreased in the PCB group and increased in the PCB + quercetin group. The histological analysis showed that PCB exposure caused hepatic damage but that quercetin was protective against the damage. The micronucleus test showed an increase in the production of microclenuclei compared to control, which quercetin was able to reduce. The researchers concluded that PCBs lead to increased lipid peroxidation and DNA damage and that the use of antioxidant quercetin is effective in reducing PCB-induced liver injury.[51]

Al-Anati et al. (2015) analyzed the genotoxic effects of PCB180 both *in vitro* and *in vivo*. Rats were exposed to ultrapure PCB180 (10–1,000 mg/kgBW) for 28 days to investigate the induction of hepatic genotoxicity. DNA damage–signaling proteins (pChk1Ser317 and γH2AXSer319) were found to be increased in female rats. This increase was compatible with increasing levels of the metabolite 3'-OH-PCB180. The most sensitive marker was pChk1. In *in vitro* studies, HepG2 cells were exposed to PCB180 (1 μM) and 3'-OH-PCB180 (1 μM) or the positive control benzoapyrene (BaP, 5 μM). It was discovered that 3'-OH-PCB180, but not PCB180, induced CYP1A1 mRNA and formation of a phosphorylated form of histone H2AX (γH2AX). CYP1A1 mRNA induction was seen at 1 h, and γH2AX was observed after 3 h of exposure. The researchers concluded that PCB180 metabolized to its hydroxyl metabolite and that

the subsequent induction of CYP1A1 led to DNA damage in vivo. [52]

Song et al. (2015) investigated the effects of a synthetic PCB metabolite, PCB29-pQ, on DNA damage checkpoint activation, cell cycle arrest, and death receptor–related extrinsic apoptosis in HepG2 cells. They found that PCB29-pQ increased the S-phase cell population by down-regulating cyclins A/D1/E, cyclin-dependent kinases (CDK 2/4/6), and cell division cycle 25A (CDC25A), and by up-regulating p21/p27 protein expressions. PCB29-pQ also induced apoptosis via the up-regulation of Fas/FasL and the activation of caspase 8/caspase 3. Moreover, p53 was suggested to play a pivotal role in PCB29-pQ–induced cell cycle arrest and apoptosis via the activation of ATM/Chk2 and ATR/Chk1 checkpoints. Cell cycle arrest and apoptotic cell death were attenuated by pretreatment with antioxidant N-acetyl-cysteine (NAC). The researchers concluded that PCB29-pQ induces oxidative stress and promotes p53-dependent DNA damage checkpoint activation, S-phase cycle arrest, and extrinsic apoptosis in HepG2 cells.[53]

3. Benzo(a)pyrene

Benzo(a)pyrene (BaP) is a polycyclic aromatic hydrocarbon (PAH) found in circumferential and indoor air from motor vehicle emissions, cigarette smoke, and burning stoves.[54] It has been classified as a group I carcinogen by IARC.[55] Benzo(a)pyrene diol epoxide (BPDE) is a highly reactive metabolite of BaP, which studies have demonstrated can induce mutagenesis, carcinogenesis, and immunosuppression.[56,57]

Tung et al. (2014) identified alterations caused by BaP exposure in the CHO 3-6 cell line and in a pKZ1 mouse model. *In vitro* assessment of homologous recombination (HR) showed significantly increased HR frequency following exposure to BaP (10 μM). When BaP-induced DSB repair was evaluated, positive staining for intrachromosomal recombination events, which are associated with nonhomologous end joining (NHEJ), were observed in the lung and thymus of exposed animals, with the stainings statistically significant in the thymus. Gene expression analyses from mouse tissues showed significantly decreased expression of ATM and Xrcc6 in BaP-treated liver and lung. In addition, BaP exposure significantly reduced the expression of Xrcc5, p53 and DNA-protein kinases (PKcs) in the lung. The researchers also showed that BPDE can induce DNA damage by formation of bulky adducts in the liver. These results demonstrate that BaP increases DSB repair both *in vitro* and *in vivo* and induces changes in the expression of DNA repair pathway genes. As DSB repair is not error-free, aberrant DNA repair may be contributing to the mechanism of BaP-induced toxicity.[57]

4. Perfluorinated Chemicals

Perfluorinated chemicals (PFCs) are industrial products. They have antiwetting and surfactant properties. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) are the most widely used. Several studies have shown that PCFs cause oxidative stress and oxidatively damaged DNA, and it has been shown that chronic exposure to PFOA and PFOS cause liver, pancreas, and testis tumor development in rats.[58,59]

PFOA is extremely resistant to degradation. It is also bioaccumulative and thus is considered a danger to both the environment and humans. It is classified as a possible human carcinogen (group IIB) by IARC.[60] The EPA has not officially classified PFOA as to its carcinogenicity. In a draft report, the EPA's Scientific Advisory Board examined the evidence on PFOA, mainly from studies in laboratory animals, concluded that evidence of its carcinogenicity, although conclusive could not definitively prove it to be a human carcinogen. The board agreed that new evidence would be considered as it becomes available.[61,62]

High doses of PFOA have caused oxidative stress in Vero cells, most probably because of cell cycle arrest in the G1 phase and induction of apoptosis.[63] In addition, it has been reported that this compound perturbs the cell cycle, induces apoptosis, and exerts genotoxic effects (such as DNA breaks) in HepG2 cells.[64,65] Yao and Zhong

(2005) studied the genotoxic potential of PFOA in HepG2 cells. Following PFOA treatment, oxidative DNA damage levels were established by immunocytochemical analysis of 8-OhdG; also, it was found that the staining intensity of 8-OHdG after exposure to PFOA increased markedly in a dose-dependent manner. Moreover, significantly increased ROS levels were observed in these cells. The DNA damage in HepG2 cells was evaluated by Comet assay, and the results showed that PFOA (50–400 μM) also caused significant increases in DNA breaks in a dose-dependent manner. PFOA exposure (100–400 μM) increased micronuclei frequency in HepG2 cells as well, again dose-dependently. The researchers stated that PFOA exerted genotoxic effects on HepG2 cells, probably through oxidative DNA damage induced by high levels of intracellular ROSs.[39]

Eriksen et al. (2010) investigated the potential of five PFCs to generate ROSs and induce oxidative DNA damage in HepG2 cells. PFOA and PFOS increased intracellular ROS production 1.52-fold and 1.25-fold versus control, respectively. However, this increase was not concentration-dependent and the compounds did not generate DNA damage that could be detected by alkaline Comet assay as strand breakage, alkali-labile sites, or formamidopyrimidine-DNA-glycosylase (FPG) sites. On the other hand, perfluorobutane sulfonate (PFBS) and perfluorohexanoic acid (PFHxA) did not generate ROSs or DNA damage. Only exposure to perfluorononanoic acid (PFNA) caused a modest increase in DNA damage at a cytotoxic concentration level, which was detected as lactate dehydrogenase (LDH) release into the cell medium; moreover, LDH leakage was not related to ROS generation. The results of this study indicate that PFCs cause modest toxicity, as evidenced by moderate levels of ROS production and DNA damage in HepG2 cells.[58]

Florentin et al. (2011) investigated the cytotoxic and genotoxic effects of PFOA and PFOS using human HepG2 cells after 1 or 24 h of exposure. They observed that both PFOA and PFOS exerted cytotoxic effects after 24 h starting from concentrations of 200 μM and 300 μM, respectively. However, they did not observe an increase in DNA damage with the Comet assay or an increase in micronuclei frequency after exposure to either. Nor did they find any increase in intracellular ROS generation by both PFOA and PFOS after 1 h. These findings show that both PFOA and PFOS are cytotoxic but do not induce an increase in DNA damage (DNA strand breaks and micronucleus) or ROSs at the applied concentrations.[66]

5. Vinyl Chloride Monomer

Vinyl chloride monomer (VCM) is a colorless gas under room temperature. Polyvinyl chloride (PVC) is a polymerized form of VCM.[13] Thirteen billion kilograms of VCM are produced annually, of which PVC is widely used in the plastics industry. Small amounts of VCM are present in finished plastic products and in cigarette smoke.[67] VCM is highly toxic and flammable and so is categorized as a group I carcinogen by IARC.[68] Exposure causes so-called VCM disease, a multisystem disorder that includes hepatic, dermal, vascular, and neurological dysfunctions. Furthermore, VCM exposure is linked with the HCC and liver angiosarcomas.[69,70]

Walles and Holmberg (1984) reported that mice exposed to VCM developed liver SSBs and that this damage was dose-dependent.[71] In other studies performed on mice, the plot of liver angiosarcoma incidence over the entire range of VC doses showed an almost linear increase up to 1,000 ppm followed by a decrease starting at 2,500 ppm. The decrease in incidence was probably due to the development of other tumor types that cause mouse mortality (decreasing the number of animals at liver tumor risk), although the absence of a survival-adjusted tumor incidence analysis in any of the available studies precludes testing that possibility. Studies on mice exposed to VC up to a 1,000-ppm dose have detected a significant dose response in liver angiosarcoma incidence ($r = 0.89$; $p < 0.001$), whereas plots of HCC incidence versus VC dose levels have shown a flat pattern, with no significant dose response up to 1,000 ppm or in the entire dose range.[72–74]

Epidemiological studies have demonstrated that VCM exposure causes genotoxicity in humans such as chromosomal aberrations, micronuclei, sister chromatid changes, and DNA strand breaks.[75] A study performed on Italian workers[76] in a VC production facility—a subcohort of 1,658 workers included in a European multicenter study; 26-year follow-up)—reported 17 liver cancer cases (including 6 angiosarcomas and 12 HCCs, with one most likely carrying both neoplasms). All types of liver cancer showed a standardized mortality ratio (SMR) of 2.78 (90% CI, 1.86–4.14) associated with VC exposure. While the SMR of VC-associated HCC exposure was not reported, it was reported that rates of HCC increased with cumulative exposure according to a statistically significant trend test; however, risk estimates were based on only three cases in the reference category, one each in the low- and high-exposed categories, and seven in the intermediate-exposed category.[76] A study of Chinese workers exposed to VC (3,293 male PVC workers; 12-year follow-up; no reported exposure and no liver angiosarcomacases observed) included 25 liver cancer cases, five of which were histopathologically confirmed to be HCC and five were considered HCC based on extremely high serum levels of alpha-fetoprotein (>1000 μg/L), at least one positive image from angiography, sonography, liver scan, and/or computed tomography scan. No risk estimates were reported for HCC, whereas an SMR of 1.78 (95% CI, 1.15–2.62) was calculated for malignant neoplasms of the liver.[77]

B. Diet Contaminants: Mycotoxins

Mycotoxins, toxic fungal secondary metabolites, are common contaminants of human foods and animal feeds.[5] These toxicants are largely produced under conditions of high humidity and temperature.[78] Both aflatoxins (AFs) and ochratoxins have been linked to induction of carcinogenesis. Studies have documented that aflatoxins can be hazardous to human and animal health by impairing liver function and the immune system and enhancing oxidative stress. Aflatoxins mainly cause the development of HCC, while ochratoxins cause tumors in the kidneys.[79] The relationship between aflatoxin B1 (AFB1) exposure, DNA, protein adduct formation, aflatoxicosis, and HCC is shown in Fig. 5.

Long-term exposure to low concentrations of aflatoxins, particularly AFB1, is teratogenic, mutagenic, immunotoxic, nephrotoxic, and hepatotoxic. AFB1 is categorized as a group I carcinogen by IARC.[80] It can stimulate the production of free radicals and lipid peroxides, which cause cell damage.[81] It is primarily metabolized to AFB1-8,9-exo-epoxide and AFB1-8,9-endo-epoxide in the liver. These metabolites induce different types of DNA damage, such as DNA adduct formation and DNA single-strand breaks. Additionally, one study found that cells exposed to AFB1 are unsuccessful in activating the p53 pathway, apoptosis, or cell cycle arrest, despite the formation of DNA adducts and the accumulation of DNA strand breaks.[82, 83] Yuzugullu et al. (2011) studied AFB1 toxicity in the HepG2 cell line and reported that the treatment of HepG2 cells with toxic doses of AFB1 induced DNA adducts, oxidative DNA damage (8-OHdG lesions), and DNA strand breaks. The researchers suggested that ABF1 could induce DNA damage and protein adducts, which could be associated with ineffective damage response and insufficient DNA damage repair so that DNA breaks could be observed.[83]

Ochratoxins are also suggested to be carcinogenic to both animals and humans. Ochratoxin A (OTA) is considered a group IIB carcinogen (possibly carcinogenic to humans) by IARC.[84] Aydin et al. (2013) investigated the possible protective effects of lycopene against the genotoxicity of OTA in rat tissues using alkaline Comet assay. Male Sprague-Dawley rats were administered OTA (0.5 mg/kgBW/day) by gavage for 14 days, whereas lycopene was applied on the last 7 days or for 14 days of the feeding period with OTA treatment. OTA caused marked increases in tail length, tail moment, and tail intensity versus control in both kidney and liver cells but not in lymphocytes. Lycopene administration alone for 7 and 14 days did not provide any significant change in DNA damage of lymphocytes or renal and hepatic cells ver-

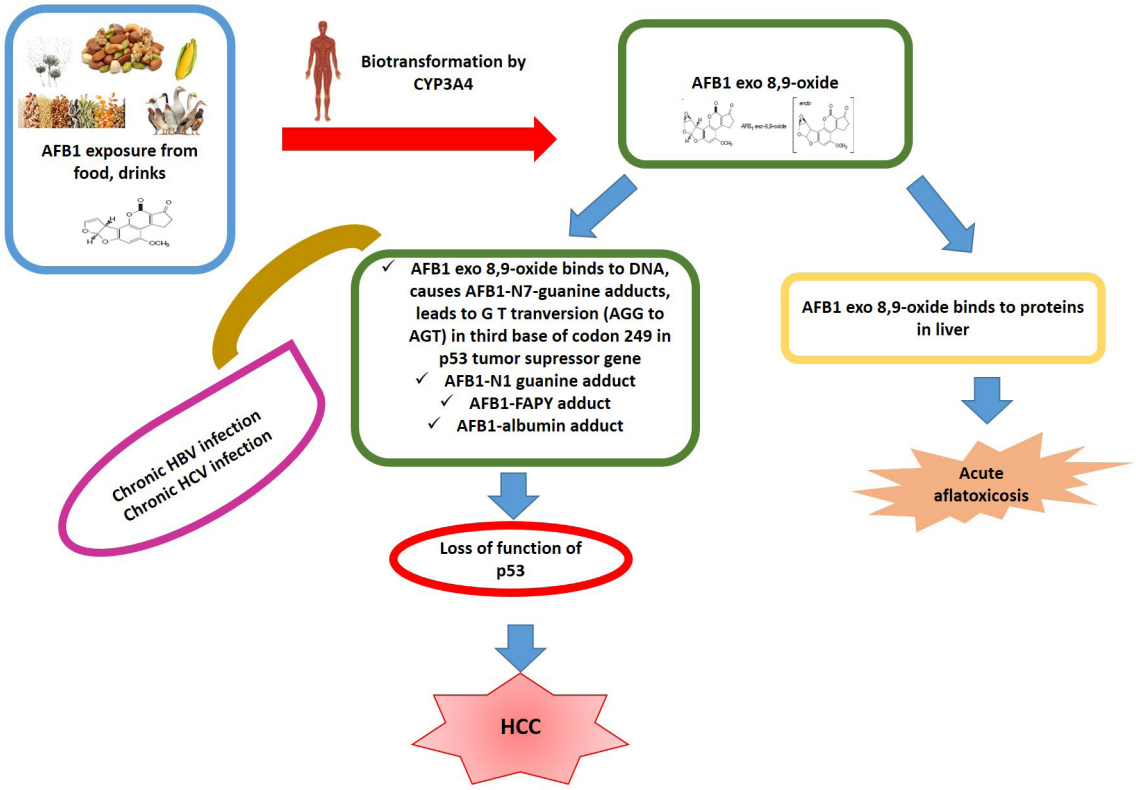

FIG. 5: Relationship between liver cancer and aflatoxin B1. AFB1, aflatoxin B1; CYP3A4, cytochrome P450 3A4; FAPY, formamidopyrimidine; HBV, hepatitis B; HCV, hepatitis C; p53, protein 53.

sus controls. However, lycopene for both 7 and 14 days, with OTA exposure in renal and hepatic cells, caused significant decreases in tail length, tail moment, and tail intensity versus OTA-exposed rats. The effect of 14 days supplementation seemed to be more protective, particularly against hepatic cells. Lycopene was found to be partially protective against hepatic and renal OTA-induced DNA damage.[85]

In another study, the researchers determined the apoptotic and necrotic effects of OTA in the liver of the same animals. OTA exposure was found to induce focal necrosis of hepatocytes and mononuclear cell infiltration. Also, exposure to OTA caused an imbalance in oxidant and antioxidant parameters in the rat liver, as evidenced by significant decreases in glutathione S-transferase activity and glutathione levels, and marked increases in concentrations of thiobarbituric acid reactive substances. Furthermore, TUNEL analysis revealed a significant ~2.7-fold increase in the number of TUNEL-positive liver cells of rats exposed to OTA compared to the control group. The results of this study showed that oxidative stress is at least one of the mechanisms underlying the hepatic toxicity of OTA, and that both necrosis and apoptosis are types of cell death in the hepatic toxicity of this mycotoxin.[86]

C. Substances of Abuse: Heavy Alcohol Use

The Word Health Organisation (WHO) has reported that alcohol-related diseases are the third most common cause of death in developing countries. Studies have shown that the major toxicity mechanism of ethanol is oxidative stress and later DNA damage and cell death (by either apoptosis

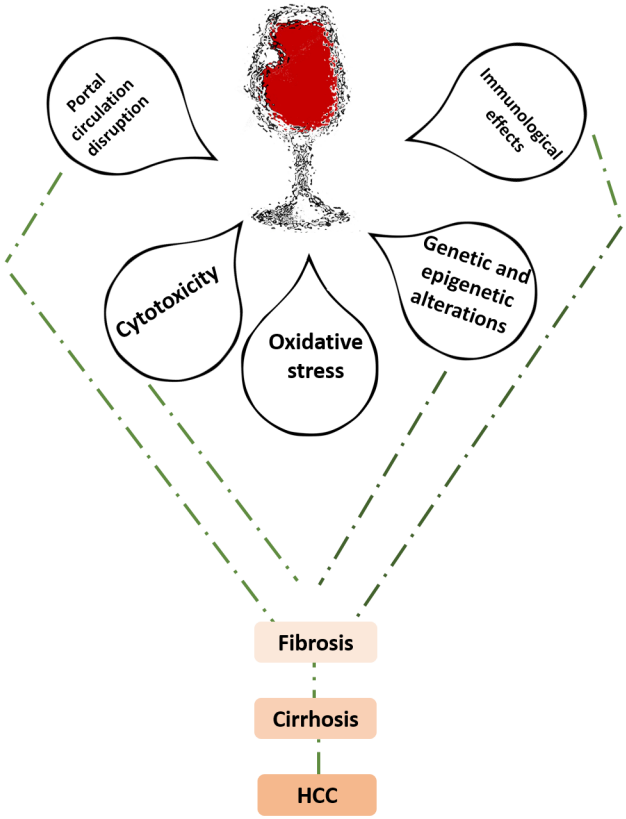

FIG. 6: Main effects of alcohol abuse on hepatic circulation, including portal circulation disruption and portal hypertension. Immunological effects include decreases in T and B cell production, higher secretion of tumor necrosis factor α (TNF-α), and cytokines. Heavy alcohol use causes hepatic oxidative stress, leading to alterations in antioxidant enzymes, increases in lipid peroxidation, and protein oxidation. Alcohol-induced hepatic cytotoxicity can be seen as apoptosis or necrosis, depending on time and dose. The primary metabolite of ethanol, acetaldehyde, can bind DNA, inhibit DNA repair systems, and cause carcinogenic exocyclic DNA ethenoadducts; thus it can directly induce DNA damage. Ethanol consumption can also cause epigenetic alterations in liver, leading to HCC.

or necrosis). Additionally, many systems facilitate alcohol's stimulation of oxidative stress.[9] The main toxic effects of alcohol abuse are summarized in Fig. 6.

Castañeda and Kinne (2000) studied the cytotoxic effects of ethanol in primary hepatocytes and liver cell lines. Primary cells and HepG2 cell cultures were incubated with increasing ethanol concentrations or without ethanol (control group) for 24 h and analyzed immediately (group I) or after an additional incubation time of 48 h without additional ethanol application (group II). Twenty-four-hour exposure to 1 mmol ethanol inhibited cell proliferation in HepG2 cells by 75% ($p < 0.05$), while cell proliferation remained unaltered in rat hepatocytes. The effect of ethanol persisted for another 48 h where cell proliferation was 5% of control in HepG2 cells and 70% of control in rat hepatocytes. After 24 h incubation with 1 mmol ethanol, 28% of HepG2 cells and 12% of rat hepatocytes showed DNA fragmentation as a sign of apoptosis ($p < 0.001$ versus control). In group II, 39% of HepG2 cells and 26% of rat hepatocytes were apoptotic. Caspase-3 activation progressively increased after ethanol treatment in HepG2 cells and rat hepatocytes.

The first significant difference was observed after 4 h (activity in HepG2 was 68% higher than in rat hepatocytes) and was maximum after 10–12 h, when the activity in HepG2 was 180% of the activity in rat hepatocytes. LDH release as a sign of necrosis into culture medium in HepG2 cells increased from 0.5% in group I to 12% in group II, and from 0.1% to 8% in rat hepatocytes ($p < 0.005$). Increasing ethanol concentration to 10 mmol increased necrosis to 75% in HepG2 cells and to 45% in rat hepatocytes, whereas the effects on cell proliferation and apoptosis were not significantly different.

Castañeda and Kinne concluded that small ethanol concentrations (equivalent to 1 mmol) inhibit cell proliferation and increase apoptosis more strongly in HepG2 cells than in normal rat hepatocytes. These findings suggest the use of 1 mmol ethanol as a treatment for hepatocellular carcinoma because this mainly affects tumor cells but not surrounding normal tissue.[87]

Chronic alcohol exposure causes hepatocyte hyper-regeneration by activating survival factors. The primary metabolite of ethanol, acetaldehyde, can bind DNA, inhibit DNA repair systems, cause carcinogenic exocyclic DNA ethenoadducts, and thus directly induce DNA damage.[6] Both *in vivo* and *in vitro* studies have demonstrated that ethanol intake may cause structural modification of mitochondrial DNA (mtDNA) due to increased oxidative stress in aging animals. Cahill et al. (1999) showed that consumption of an ethanol-containing diet for more than 1 year causes elevated levels of 8-OHdG and enhanced mtDNA strand breaks in experimental animals. It has been suggested that chronic ethanol consumption leads to increases in intracellular production of ROSs and selectively reduces mitochondrial glutathione (GSH) levels.[88]

D. Biological Agents

1. Chronic Hepatitis B and C Infections

Hepatitis B (HBV) and hepatitis C (HCV) viruses are still a major health problem worldwide. Epidemiological studies have suggested that over 50% of global HCC cases are associated with HBV and HCV infections.[89, 90] An estimated 240 million people are chronically infected with hepatitis B, with prevalence highest in Sub-Saharan Africa and East Asia, where 5%–10% of the adult population is chronically infected. High rates of chronic infections are also found in the Amazon and the southern parts of eastern and central Europe. In the Middle East and on the Indian subcontinent, an estimated 2%–5% of the general population is chronically infected. In Western Europe and North America that total is less than 1%. More than 686,000 people die every year because of complications of hepatitis B, including cirrhosis and liver cancer.[91,92]

Chronic hepatitis due to HBV infection results in an incidence of 0.1 per 100 people in Europe to 0.8 per 100 people in Japan, rising to 2.2 in Europe and 4.3 in Japan in the case of compensated cirrhosis throughout the years.[93] In 70%–90% of cases, HBV-associated cirrhosis leads to HCC. However, in the absence of cirrhosis, HBV is still a substantial risk factor.[94] As a consequence, three out of five primary liver cancers in Africa and Asia are attributable to HBV infection whereas in Japan, Europe, and the United States 20% of cases are a sequel of the disease.[95] Several case-control studies have confirmed the effect of HBV infection on HCC with high overall odds ratios (OR). The risk of liver cancer in individuals exposed to chronic HBV infection and AF is up to 30 times greater than the risk in individuals exposed to AF alone.[96,97] In a study from India, the OR was found to be 48.[98]

Studies on the molecular mechanisms of HBV-associated HCC have suggested that HCC is associated with multiple procarcinogenic processes that lead to the accumulation of genetic changes and complex chromosomal abnormalities.[90,93] It has been considered that long-term exposure to chronic inflammation induces oxidative stress–linked DNA damage in HCC, and that chronic HBV infection can cause insistent inflammatory response and induce oxidative DNA damage in liver cells. DSBs can be observed during hepatocyte regeneration in response to liver tissue cell death.[5,99] On the other hand, alterations in oncogenes and tumor suppres-

sor genes are very important in the development of HCC. De La Caste et al. (1998) analyzed several HCC models, both human (associated with HBV and HBC viruses) and mouse (with HCC developed in transgenic mice expressing the oncogene c-myc or H-Ras in the liver). They demonstrated that 26% of human HCC and 50% of mouse HCC exhibit β-catenin mutations.[20]

Hu et al. (2010) investigated whether HBV DNA integration occurred at sites of DSBs, which are one of the most detrimental forms of DNA damage. An 18-bp I-SceI homing endonuclease recognition site was introduced into the DNA of the HepG2 cell line by stable DNA transfection; the cells were then incubated in patients' serum with high HBV DNA copies. At the same time, DSBs were induced by transient expression of I-SceI after transfection of an I-SceI expression vector. Using nest PCR, viral DNA was detected at the sites of the break. It appeared that integration occurred between part of the HBVx gene and the I-SceI–induced breaks. The results suggest that DSBs, as a form of DNA damage, may serve as potential targets for hepadnaviral DNA insertion and that the integrants necessarily lead to widespread host genome changes.[100]

Livezey et al. (2002) investigated the effect of the HBV-X gene (HBX) on the stability of the host genome using HepG2 stable transfectants (HepG2-HBX) and vector controls (HepG2-neo). All of the HepG2-HBX clones analyzed contained the integrated HBX gene and the HBX transcript. The data showed that HepG2-HBX cells have increased chromosome alterations and more micronuclei formation compared to vector controls. Micronuclei were shown to originate from all chromosomes; however, those originating from chromosomes 2, 3, 7, 18, and 20 were found in greater numbers in cells expressing the HBX gene. Interestingly, chromosomes 2, 18, and 20 were three of the chromosomes found rearranged in HepG2-HBX clones. These data provide evidence that genomic integrity is affected in cells expressing the HBX gene. De novo cytogenetic alterations identified in HepG2-HBX clones implicate the involvement of HBX and support the hypothesis that HBX may interfere with normal cellular processes responsible for genomic integrity, increasing the risk of genetic mutations in infected hepatocytes.[101]

It has been suggested that transgenic mice overexpressing HBx exhibit an increased susceptibility to mutations if exposed to mutagens. Gehrke et al. (2004) investigated whether HBx expression increases the level of the mutational precursor 8-OH-dG in hepatocellular DNA. They found that 8-OH-dG concentrations in genomic DNA of the HBx protein expressing the HBx recombinant HepG2 cell line correlate with the factor of transactivation. The 8-OH-dG levels were reduced after incubation of HBx recombinant cell lines with 0.1 or 1 mM of the antioxidant N-acetylcysteine.[102]

Globally, 130–150 million people have chronic HCV infection. A significant number of those who are chronically infected will develop liver cirrhosis or liver cancer. Approximately 700,000 people die each year from HCV-related liver diseases, with the most affected regions being Africa and Central and East Asia. Depending on the country, HCV infection can be concentrated in certain populations (for example, among people who inject drugs) and/or in general populations. There are multiple strains (or genotypes) of the HCV virus, and their distribution varies by region.[103,104]

During chronic HCV infection, increased oxidative/nitrosative stress can cause DNA damage. Studies have shown that HCV infection affects not only hepatocytes but also immune cells; oxidative DNA damage in circulating leukocytes can be determinative of the progression the disease. Shawki et al. (2014) investigated DNA damage in peripheral blood lymphocytes from HCC patients infected with HCV and showed levels of DNA damage in the form of single- and double-strand breaks.[105] Additionally Machida et al. (2006) reported that HCV infection is mostly linked with damage and mutations that are mediated by nitric oxide (NO). NO especially damages mitochondria and thus induces DSBs.[106]

2. Human Immunodeficiency Virus 1

Human immunodeficiency virus 1 (HIV-1) has been spreading as an epidemic in Africa and America in the last thirty years. According to WHO, 36.7 mil-

lion people are infected with HIV-1 throughout the world and 18.2 million people are on antiretroviral therapy. The mother-to-child transmission rate is 7 out of 10.[107] In patients with HIV-1, HCC is becoming an important cause of mortality, probably because of coinfection with HCV or HBV. HIV-1 infection shortens the survival of patients with HCV-related cirrhosis.[108] In addition, hepatocarcinogenesis can be more rapid and aggressive in HIV/HCV coinfected patients.[109,110] For patients who have HCV and are coinfected with HIV-1, there is a twofold increase in the risk of cirrhosis and a sixfold increase in the risk of end-stage liver disease as compared to monoinfected HCV-positive patients.[111] Moreover, immunosuppression secondary to HIV infection and the direct impact of the virus on liver parenchyma are suggested to contribute to HCC outcome.

Although highly active antiretroviral therapy (HAART) increases survival rates of HIV-1 patients, such drugs are suggested to have hepatotoxic effects that may also contribute to HCC occurrence after HIV-1 infection.[107] Ryom et al. (2016) observed that cumulative use of stavudine, didanosine, tenofovir, and (fos)amprenavir was independently associated with increased end-stage liver disease/HCC rates, and concluded that intensified monitoring of liver function should be considered among all individuals who are receiving HAART for longer time periods.[112]

IV. CONCLUSION

Liver cancer is the sixth most common cancer in the world, with 782,000 new cases diagnosed in 2012, and it is the third most common cause of cancer death. Liver cancers are associated with multiple risk factors such as chronic HBV and HCV infections, HIV-1 and HAART therapy, diet contaminants (AFB1, OTA), alcohol consumption, obesity, and chemical exposures (acrylamide, PFCs, PCBs, VCM).[7]

In liver cancers, many complex mechanisms are involved. HCC is caused by both epigenetic and genetic alterations, and increased understanding of these epigenetic and genetic mechanisms may aid the development of new strategies for prevention. Like many other cancer types, HCC can be caused by DNA changes that turn on oncogenes or turn off tumor suppressor genes. In addition, HCC mostly originates from chronic injury and inflammation that promote oxidative DNA damage and large-scale genomic alterations such as chromosomal aberrations. Genetic mechanisms that may lead to HCC include alterations in cellular signaling (including alterations in RB, p53, EGF, and WNT/β-catenin pathways) and changes in the expression of oncogenes (H-Ras, EGRF, Myc).[16–23]

Epigenetic mechanisms are now suggested to be as important as genetic mechanisms in the development of HCC. They include global changes in DNA methylation (hypomethylation/hypermethylation); histone modifications; and alterations in chromatin structure, in transcription factors, in microRNAs (miRNAs), and in noncoding RNAs (ncRNAs).[29–37]

HCC may also be caused by genomic instability and dysregulation of DNA damage repair and/or failing cell cycle checkpoints.[113] Studies have shown that persistent inflammatory response can induce DNA oxidative damage in liver cells and that these damages may be converted into DNA strand breaks, particularly DSBs, during hepatic regeneration.[99] DSBs are a major threat to genome integrity as they may lead to chromosomal aberrations, uncontrolled replication, and cell dysfunction or death.[114]

Occupational exposure as well as exposure by different routes (particularly ingestion and inhalation) to certain industrial chemicals or chemicals that are present in food processing can lead to HCC with different mechanisms of action. Regulatory authorities should take serious occupational health measures to reduce work exposure to such chemicals (acrylamide, vinyl chloride). In addition, cooking methods can be changed to reduce exposure to acrylamide (boiling instead of frying), to BaP (no barbecuing), or to PFCs, particularly PFOA (steel or glass instead of Teflon in pots and pans). In addition, governments should continuously check the environment to prevent PCB exposure of the general population.

In developing countries, exposure to aflatoxins and ochratoxins is much higher than it is in the de-

veloped world. Therefore, quantifying the human health impacts and the burden of disease due to aflatoxin exposure is an important issue particularly in certain parts of Asia and Africa. Governments should increase food and drink monitoring and increase surveillance of aflatoxicosis and HCC. Although aflatoxin exposure is not a new issue and is continually discussed by health organizations, developing parts of the world need new and more effective strategies to address this food insecurity. New strategies will improve public health, reduce aflatoxicosis outbreaks, and decrease the incidence of HCC.[115]

Humans are abundantly exposed to carcinogens in mixtures. Although the basics of the dose–response principle for mixtures are well known, several toxicological challenges (dose metrics, nonquantified effects of toxicity-modifying factors) complicate the interpretation of research data and restrain researchers' ability to relate the presence, nature, and extent of interactions among mixture components.[116] However, it is generally suggested that coexposure to certain chemicals with carcinogenic substances or exposure in the presence of a preexisting liver disease can increase the risk of HCC in certain populations. For instance, alcohol is a cocarcinogen and its consumption in the presence of HBV and HCV may undoubtedly lead to liver cancer. Moreover, AFB1 exposure in combination with HBV and HCV is likewise very dangerous because it can lead to different types of liver cancer as well. The combined effects of hepatic carcinogens must be considered.

It is suggested that exposure to certain chemicals, toxins, and substances of abuse be reduced in order to decrease liver cancer rates. Governments should take serious measures to prevent mycotoxin exposure, and populations should be warned about biological agents (most of which, like HBV, HCV, and HIV-1, can be transmitted sexually). Lifestyle changes can reduce liver cancer risk in the general population and should therefore be recommended.

REFERENCES

1. Baba AI, Câtoi C. Chapter 9. Comparative Oncology. Tumors of the liver and gallbladder. In: Baba AI, Câtoi C. editors. Bucharest: The Publishing House of the Romanian Academy. 2007.
2. American Cancer Society. What is liver cancer? 2017. Available from: https://www.cancer.org/cancer/liver-cancer/about/what-is-liver-cancer.html Accessed 01 Mar 2017.
3. Center MM, Jemal A. International trends in liver cancer incidence rates. Cancer Epidemiol Biomarkers Prev. 2011;20:2362–2368.
4. Lee SM, Kim-Ha J, Choi WY, Lee J, Kim D, Lee J, Choi E, Kim YJ. Interplay of genetic and epigenetic alterations in hepatocellular carcinoma. Epigenomics. 2016;8:993–1005.
5. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. Gastroenterology. 2004;127:35–50.
6. Seitz HK, Stickel F. Risk factors and mechanisms of hepatocarcinogenesis with special emphasis on alcohol and oxidative stress. Biol Chem. 2006;387:349–360.
7. American Cancer Society. General information about carcinogens. 2017. Available from: https://www.cancer.org/cancer/cancer-causes/general-info.html Accessed 01 Mar 2017.
8. Chappell G, Kutanzi K, Uehara T, Tryndyak V, Hong HH, Hoenerhoff M, Beland FA, Rusyn I, Pogribny IP. Genetic and epigenetic changes in fibrosis-associated hepatocarcinogenesis in mice. Int J Cancer. 2014;134:2778–2788.
9. Muriel P. Role of free radicals in liver diseases. Hepatol Int. 2009;3:526–536.
10. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer. 2003;3:276–285.
11. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem. 2004;266:37–56.
12. Kulkarni A, Wilson DM 3rd. The involvement of DNA-damage and -repair defects in neurological dysfunction. Am J Hum Genet. 2008;82:539–566.
13. Wang Z, Li Z, Ye Y, Xie L, Li W. Oxidative Stress and Liver Cancer: Etiology and Therapeutic Targets. Oxid Med Cell Longev. 2016;2016:7891574.
14. De Bont R, van Larebeke N. Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis. 2004;19:169–185.
15. Sedelnikova OA, Redon CE, Dickey JS, Nakamura AJ, Georgakilas AG, Bonner WM. Role of oxidatively induced DNA lesions in human pathogenesis. Mutat Res. 2010;704:152–159.
16. Aparicio T, Baer R, Gautier J. DNA double-strand break repair pathway choice and cancer. DNA Repair (Amst). 2014;19:169–175.
17. White RR, Milholland B, de Bruin A, Curran S, Laberge RM, van Steeg H, Campisi J, Maslov AY, Vijg J. Controlled induction of DNA double-strand breaks in the mouse liver induces features of tissue ageing. Nat Commun.2015;6:6790.

18. Hsieh YH, Chang WS, Tsai CW, Tsai JP, Hsu CM, Jeng LB, Bau DT. DNA double-strand break repair gene XRCC7 genotypes were associated with hepatocellular carcinoma risk in Taiwanese males and alcohol drinkers. Tumour Biol. 2015;36:4101–4106.
19. Tang Z, Qin L, Wang X, Zhou G, Liao Y, Weng Y, Jiang X, Lin Z, Liu K, Ye S. Alterations of oncogenes, tumor suppressor genes and growth factors in hepatocellular carcinoma: with relation to tumor size and invasiveness. Chin Med J (Engl). 1998;111:313–318.
20. de La Coste A, Romagnolo B, Billuart P, Renard CA, Buendia MA, Soubrane O, Fabre M, Chelly J, Beldjord C, Kahn A, Perret C. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. Proc Natl Acad Sci USA. 1998; 95:8847–51.
21. Xue KX. Molecular genetic and epigenetic mechanisms of hepatocarcinogenesis. Ai Zheng. 2005;24:757–68.
22. Zender L, Villanueva A, Tovar V, Sia D, Chiang DY, Llovet JM. Cancer gene discovery in hepatocellular carcinoma. J Hepatol. 2010;52:921–29.
23. Tanabe KK, Lemoine A, Finkelstein DM, Finkelstein DM, Kawasaki H, Fujii T, Chung RT, Lauwers GY, Kulu Y, Muzikansky A, Kuruppu D, Lanuti M, Goodwin JM, Azoulay D, Fuchs BC. Epidermal growth factor gene functional polymorphism and the risk of hepatocellular carcinoma in patients with cirrhosis. JAMA. 2008;299:53–60.
24. Mann DA. Epigenetics in liver disease. Hepatology. 2014;60:1418–25.
25. Pogribny IP, Tryndyak VP, Boureiko A, Melnyk S, Bagnyukova TV, Montgomery B, Rusyn I. Mechanisms of peroxisome proliferator-induced DNA hypomethylation in rat liver. Mutat Res. 2008;644:17–23.
26. Yamada Y, Jackson-Grusby L, Linhart H, Meissner A, Eden A, Lin H, Jaenisch R. Opposing effects of DNA hypomethylation on intestinal and liver carcinogenesis. Proc Natl Acad Sci USA. 2005;102:13580–5.
27. Shen J, Wang S, Zhang YJ, Kappil MA, Chen Wu H, Kibriya MG, Wang Q, Jasmine F, Ahsan H, Lee PH, Yu MW, Chen CJ, Santella RM. (2012). Genome-wide aberrant DNA methylation profiles in hepatocellular carcinoma. Hepatology. 2012;55:1799–1808.
28. Song MA, Tiirikainen M, Kwee S, Okimoto G, Yu H, Wong LL. Elucidating the landscape of aberrant DNA methylation in hepatocellular carcinoma. PLoS One. 2013;85:5761.
29. Nishida N, Kudo M, Nagasaka T, Ikai I, Goel A. Characteristic patterns of altered DNA methylation predict emergence of human hepatocellular carcinoma. Hepatology. 2012;56:994–1003.
30. Cai MY, Hou JH, Rao HL, Luo RZ, Li M, Pei XQ, Lin MC, Guan XY, Kung HF, Zeng YX, Xie D. High expression of H3K27me3 in human hepatocellular carcinomas correlates closely with vascular invasion and predicts worse prognosis in patients. Mol Med 2011;17:12–20.
31. He C, Xu J, Zhang J, Xie D, Ye H, Xiao Z, Cai M, Xu K, Zeng Y, Li H, Wang J. High expression of trimethylated histone H3 lysine 4 is associated with poor prognosis in hepatocellular carcinoma. Hum Pathol. 2012;43:1425–35.
32. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. Nature. 2011;469:343–9.
33. Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, Ghosh D, Sewalt RG, Otte AP, Hayes DF, Sabel MS, Livant D, Weiss SJ, Rubin MA, Chinnaivan AM. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. Proc Natl Acad Sci USA. 2003;100:11606–11.
34. Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RG, Otte AP, Rubin MA, Chinnaiyan AM. The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature. 2002;419:624–9.
35. Au SL, Wong CC, Lee JM, Fan DN, Tsang FH, Ng IO, Wong CM. Enhancer of zeste homolog 2 epigenetically silences multiple tumor suppressor microRNAs to promote liver cancer metastasis. Hepatology. 2012;56:622–31.
36. Aoki R, Chiba T, Miyagi S, Negishi M, Konuma T, Taniguchi H, Ogawa M, Yokosuka O, Iwama A. The polycomb group gene product Ezh2 regulates proliferation and differentiation of murine hepatic stem/progenitor cells. J Hepatol. 2010;52:854–63.
37. Wang L, Zhang X, Jia LT, Hu SJ, Zhao J, Yang JD, Wen WH, Wang Z, Wang T, Zhao J, Wang RA, Meng YL, Nie YZ, Dou KF, Chen SY, Yao LB, Fan DM, Zhang R, Yang AG. c-Myc-mediated epigenetic silencing of microRNA-101 contributes to dysregulation of multiple pathways in hepatocellular carcinoma. Hepatology. 2013;59:1850–63.
38. Jiang L, Cao J, An Y, Geng C, Qu S, Jiang L, Zhong L. Genotoxicity of acrylamide in human hepatoma G2 (HepG2) cells. Toxicol in Vitro. 2007;21:1486–92.
39. Yao X, Zhong L. Genotoxic risk and oxidative DNA damage in HepG2 cells exposed to perfluorooctanoic acid. Mutat Res. 2005;587:38–44.
40. Lamy E, Völkel Y, Roos PH, Kassie F, Mersch-Sundermann V. Ethanol enhanced the genotoxicity of acrylamide in human, metabolically competent HepG2 cells by CYP2E1 induction and glutathione depletion. Int J Hyg Environ Health. 2008;211:74–81.
41. Mottram DS, Wedzicha BL, Dodson AT. Acrylamide is formed in the Maillard reaction. Nature. 2002;419:448–9.
42. Erkekoglu P, Baydar T. Acrylamide neurotoxicity. Nutr Neurosci. 2014;17:49–57.
43. Zyzak DV, Sanders RA, Stojanovic M, Tallmadge DH, Loye Eberhart B, Ewald DK, Gruber DC, Morsch TR,

Strothers MA, Rizzi GP, Villagran MD. Acrylamide formation mechanism in heated foods. J Agric Food Chem. 2003;51:4782–7.
44. IARC (International Agency for Research on Cancer). 1994. Acrylamide. Available from: https://monographs.iarc.fr/ENG/Monographs/vol60/mono60-16.pdf Accessed 01 Mar 2017.
45. Puppel N, Tjaden Z, Fueller F, Marko D. DNA strand breaking capacity of acrylamide and glycidamide in mammalian cells. Mutat Res. 2005;580:71–80.
46. Porta M, Zumeta E. Implementing the Stockholm Treaty on Persistent Organic Pollutants. Occup Environ Med. 2002;10: 651–52.
47. Shain W, Bush B, Seegal R. Neurotoxicity of polychlorinated biphenyls: structure-activity relationship of individual congeners. Toxicol Appl Pharmacol. 1991;111: 33–42.
48. Dong H, Su C, Xia X, Li L, Song E, Song Y. Polychlorinated biphenyl quinone-induced genotoxicity, oxidative DNA damage and γ-H2AX formation in HepG2 cells. Chem Biol Interact. 2014;212:47–55.
49. IARC (International Agency for Research on Cancer). Polychlorinated biphenyls. 2016. Available from: http://monographs.iarc.fr/ENG/Monographs/vol107/mono107.pdf Accessed 01 Mar 2017.
50. EPA (Environmental Protection Agency). Polychlorinated biphenyls (PCBs). Available from: https://www.epa.gov/pcbs/learn-about-polychlorinated-biphenyls-pcbs. Accessed 01 Mar 2017.
51. Rocha de Oliveira C, Ceolin J, Rocha de Oliveira R, Gonçalves Schemitt E, Raskopf Colares J, De Freitas Bauermann L, Hilda Costabeber I, Morgan-Martins MI, Mauriz JL, Da Silva J, Reyes JM, Possa Marroni N. Effects of quercetin on polychlorinated biphenyls-induced liver injury in rats. Nutr Hosp. 2014;29:1141–8.
52. Al-Anati L, Viluksela M, Strid A, Bergman Å, Andersson PL, Stenius U, Högberg J. Hydroxyl metabolite of PCB 180 induces DNA damage signaling and enhances the DNA damaging effect of benzoapyrene. Chem Biol Interact. 2015;239:164–73.
53. Song X, Li L, Shi Q, Lehmler HJ, Fu J, Su C, Xia X, Song E, Song Y. Polychlorinated biphenyl quinone metabolite promotes p53-dependent DNA damage checkpoint activation, S-Phase cycle arrest and extrinsic apoptosis in human liver hepatocellular carcinoma HepG2 cells. Chem Res Toxicol. 2015; 28:2160–9.
54. Waldman JM, Lioy PJ, Greenberg A, Butler JP. Analysis of human exposure to benzo(a)pyrene via inhalation and food ingestion in the Total Human Environmental Exposure Study (THEES). J Expo Anal Environ Epidemiol. 1991;1:193–225.
55. IARC (International Agency for Research on Cancer). Benzo(a)pyrene. 2010. Available from: http://monographs.iarc.fr/ENG/Monographs/vol110/mono110.pdf Accessed 01 Mar 2017.
56. Dybing E, O'Brien J, Renwick AG, Sanner T. Risk assessment of dietary exposures to compounds that are genotoxic and carcinogenic—an overview. Toxicol Lett. 2008;180:110–17.
57. Tung EW, Philbrook NA, Belanger CL, Ansari S, Winn LM. Benzoapyrene increases DNA double strand break repair in vitro and in vivo: a possible mechanism for benzoapyrene-induced toxicity. Mutat Res Genet Toxicol Environ Mutagen. 2014;760:64–9.
58. Eriksen KT, Raaschou-Nielsen O, Sørensen M, Roursgaard M, Loft S, Møller P. Genotoxic potential of the perfluorinated chemicals PFOA, PFOS, PFBS, PFNA and PFHxA in human HepG2 cells. Mutat Res. 2010;700:39–43.
59. Dimitrov S, Kamenska V, Walker JD, Windle W, Purdy R, Lewis M, Mekenyan O. Predicting the biodegradation products of perfluorinated chemicals using CATABOL. SAR QSAR. Environ Res. 2004;15:69–82.
60. IARC (International Agency for Research on Cancer). 2016. Perfluorooctanoic acid. Available from: https://monographs.iarc.fr/ENG/Monographs/vol110/mono110-07.pdf. Accessed 01 Mar 2017.
61. American Cancer Society. Teflon and perfluorooctanoic acid (PFOA). 2017. Available from: https://www.cancer.org/cancer/cancer-causes/teflon-and-perfluorooctanoic-acid-pfoa.html Accessed 01 Mar 2017.
62. EPA (Environmental Protection Agency). 2006. National Service Center for Environmental Publications (NSCEP. SAB Review of EPA's Draft Risk assessment of Potential Human Health Effects Associated with PFOA and its Salts. Available from: https://nepis.epa.gov/Exe/ZyNET.exe/901S0J00.TXT Accessed 01 Mar 2017.
63. Fernández Freire P, Pérez Martin JM, Herrero O, Peropadre A, de la Peña E, Hazen MJ. In vitro assessment of the cytotoxic and mutagenic potential of perfluorooctanoic acid. Toxicol in Vitro. 2008;22:1228–33.
64. Panaretakis T, Shabalina IG, Grandér D, Shoshan MC, DePierre JW. Reactive oxygen species and mitochondria mediate the induction of apoptosis in human hepatoma HepG2 cells by the rodent peroxisome proliferator and hepatocarcinogen, perfluorooctanoic acid Toxicol Appl Pharmacol. 2001;173:56–64.
65. Shabalina IG, Panaretakis T, Bergstrand A, DePierre JW. Effects of the rodent peroxisome proliferator and hepatocarcinogen, perfluorooctanoic acid, on apoptosis in human hepatoma HepG2 cells. Carcinogenesis. 1999;20:2237–46.
66. Florentin A, Deblonde T, Diguio N, Hautemaniere A, Hartemann P. Impacts of two perfluorinated compounds (PFOS and PFOA) on human hepatoma cells: cytotoxicity but no genotoxicity? Int J Hyg Environ Health. 2011;214:493–9.
67. Sherman M. Vinyl chloride and the liver. J Hepatol. 2009;51:1074–81.

68. IARC (International Agency for Research on Cancer). 2012. Vinyl chloride. Available from: http://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-31.pdf Accessed 01 Mar 2017.
69. Sparrow GP. A connective tissue disorder similar to vinyl chloride disease in a patient exposed to perchlorethylene. Clin Exp Dermatol 1977;2:17–22.
70. Ward AM, Udnoon S, Watkins J, Walker AE, Darke CS. Immunological mechanisms in the pathogenesis of vinyl chloride disease. Br Med J. 1976;1:936–8.
71. Walles SA, Holmberg B. Induction of single-strand breaks in DNA of mice after inhalation of vinyl chloride. Cancer Lett. 1984;25:13–18.
72. Hong CB, Winston JM, Thornburg LP, Lee CC, Woods JS. Follow-up study on the carcinogenicity of vinyl chloride and vinylidene chloride in rats and mice: tumor incidence and mortality subsequent to exposure. J Toxicol Environ Health. 1981;7:909–24.
73. Maltoni C, Lefemine G, Ciliberti A, Cotti G, Carretti D. Carcinogenicity bioassays of vinyl chloride monomer: a model of risk assessment on an experimental basis. Environ Health Perspect. 1981;41:3–29.
74. Lee CC, Bhandari JC, Winston JM, House WB, Dixon RL, Woods JS. Carcinogenicity of vinyl chloride and vinylidene chloride. J Toxicol Environ Health. 1978;4:15–30.
75. Wang W, Qiu YL, Jiao J, Liu J, Ji F, Miao WB, Zhu Y, Xia ZL. Genotoxicity in vinyl chloride-exposed workers and its implication for occupational exposure limit. Am J Ind Med. 2011;54:800–10.
76. Pirastu R, Baccini M, Biggeri A, Comba P. Epidemiologic study of workers exposed to vinyl chloride in Porto Marghera: mortality update. Epidemiol Prev. 2003;27:161–72.
77. Wong RH, Chen PC, Du CL, Wang JD, Cheng TJ. An increased standardised mortality ratio for liver cancer among polyvinyl chloride workers in Taiwan. Occup Environ Med. 2002;59:405–9.
78. Wang JS, Groopman JD. DNA damage by mycotoxins. Mutat Res. 1999;424:167–81.
79. Wild CP, Gong YY. Mycotoxins and human disease: a largely ignored global health issue. Carcinogenesis. 2010;31:71–82.
80. IARC (International Agency for Research on Cancer). 2012. Aflatoxins. Available from: https://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-23.pdf Accessed 01 Mar 2017.
81. Yang X, Lv Y, Huang K, Luo Y, Xu W. Zinc inhibits aflatoxin B1-induced cytotoxicity and genotoxicity in human hepatocytes (HepG2 cells). Food Chem Toxicol. 2016;92:17–25.
82. Gradelet S, Astorg P, Le Bon AM, Bergès R, Suschetet M. Modulation of aflatoxin B1 carcinogenicity, genotoxicity and metabolism in rat liver by dietary carotenoids: evidence for a protective effect of CYP1A inducers. Cancer Lett. 1997;114:221–3.
83. Gursoy-Yuzugullu O, Yuzugullu H, Yilmaz M, Ozturk M. Aflatoxin genotoxicity is associated with a defective DNA damage response bypassing p53 activation. Liver Int. 2011;31:561–71.
84. IARC (International Agency for Research on Cancer). 1993. Ochratoxin A. Available from: https://monographs.iarc.fr/ENG/Monographs/vol56/mono56-18.pdf Accessed 01 Mar 2017.
85. Aydin S, Palabiyik SS, Erkekoglu P, Sahin G, Başaran N, Giray BK. The carotenoid lycopene protects rats against DNA damage induced by Ochratoxin A. Toxicon. 2013;73:96–103.
86. Palabıyık SŞ, P. Erkekoglu, Zeybek ND, Kızılgun M, Sahin G, Kocer Giray B. (2012). Ochratoxin A causes oxidative stress and cell death in rat liver. World Mycotoxin J. 2012;5:377–84.
87. Castañeda F, Kinne RK. Cytotoxicity of millimolar concentrations of ethanol on HepG2 human tumor cell line compared to normal rat hepatocytes in vitro. J Cancer Res Clin Oncol. 2000;126:503–10.
88. Cahill A, Stabley GJ, Wang X, Hoek JB. Chronic ethanol consumption causes alterations in the structural integrity of mitochondrial DNA in aged rats. Hepatology. 1999;30:881–8.
89. Venook AP, Papandreou C, Furuse J, de Guevara LL. The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. Oncologist. 2010;15:5–13.
90. Guerrieri F, Belloni L, Pediconi N, Levrero M. Molecular mechanisms of HBV-associated hepatocarcinogenesis. Semin Liver Dis. 2013;33:147–56.
91. GBD (Global Burden of Disease Study). 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;385(9963):117–71.
92. WHO (World Health Organisation). 2017. Hepatitis B. fact sheet. Available from: http://www.who.int/mediacentre/factsheets/fs204/en Accessed 01 Mar 2017.
93. Yu MW, Lien JP, Liaw YF, Chen CJ. Effects of multiple risk factors for hepatocellular carcinoma on formation of aflatoxin B1-DNA adducts. Cancer Epidemiol Biomarkers Prev 1996;5:613–9.
94. Schuppan D, Afdhal NH. Liver cirrhosis. Lancet 2008;371:838–51.
95. Wogan GN, Kensler TW, Groopman JD. Present and future directions of translational research on aflatoxin and hepatocellular carcinoma. A review. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2012;29:249–57.
96. Groopman JD, Kensler TW, Wild CP. Protective interventions to prevent aflatoxin-induced carcinogenesis in

developing countries. Annu Rev Public Health. 2008;29: 187–203.
97. Liu Y, Wu F. Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. Environ Health Perspect. 2010;118: 818–24.
98. Kumar M, Kumar R, Hissar SS, Saraswat MK, Sharma BC, Sakhuja P, Sarin SK. Risk factors analysis for hepatocellular carcinoma in patients with and without cirrhosis: a case-control study of 213 hepatocellular carcinoma patients from India. J Gastroenterol Hepatol. 2007;22:1104–11.
99. Hsieh YH, Chang YY, Su IJ, Yen CJ, Liu YR, Liu RJ, Hsieh WC, Tsai HW, Wang LH, Huang W. Hepatitis B virus pre-S2 mutant large surface protein inhibits DNA double-strand break repair and leads to genome instability in hepatocarcinogenesis. J Pathol. 2015;236:337–47.
100. Abel S, De Kock M, van Schalkwyk DJ, Swanevelder S, Kew MC, Gelderblom WC. Altered lipid profile, oxidative status and hepatitis B virus interactions in human hepatocellular carcinoma. Prostaglandins Leukot Essent Fatty Acids. 2009;81:391–399.
101. Hu X, Lin J, Xie Q, Ren J, Chang Y, Wu W, Xia Y. DNA double-strand breaks, potential targets for HBV integration. J Huazhong Univ Sci Technolog Med Sci. 2010;30(3):265–70.
102. Livezey KW, Negorev D, Simon D. Increased chromosomal alterations and micronuclei formation in human hepatoma HepG2 cells transfected with the hepatitis B virus HBX gene. Mutat Res. 2002;505:63–74.
103. Gehrke R, Brauchle MA, Reifenberg K, Hildt E, Gruetzner U, Schmitz V, Schlicht HJ, Hofschneider PH, Caselmann WH, Rabe C. Accumulation of 8-hydroxy-2′-deoxyguanosine adducts in HBx recombinant HepG2 cells and HBx transgenic mice. Digestion. 2004;70:117–26.
104. Ma Q, Li Y, Fan Y, Zhao L, Wei H, Ji C, Zhang J. Molecular mechanisms of lipoic acid protection against aflatoxin B_1-induced liver oxidative damage and inflammatory responses in broilers. Toxins (Basel) 2015;7:5435–47.
105. WHO (World Health Organisation). 2017. Hepatitis C. fact sheet. Available from: http://www.who.int/mediacentre/factsheets/fs164/en/ Accessed 01 Mar 2017.
106. Shawki SM, Meshaal SS, El Dash AS, Zayed NA, Hanna MO. Increased DNA damage in hepatitis C virus-related hepatocellular carcinoma. DNA Cell Biol 2014;33:884–90.
107. Machida K, Cheng KT, Lai CK, Jeng KS, Sung VM, Lai MM. Hepatitis C virüs triggers mitochondrial permeability transition with production of reactive oxygen species, leading to DNA damage and STAT3 activation. J Virol. 2006;80:7199–207.
108. World Health Organization (WHO). 2017. WHO at IAS 2017. Available from: http://www.who.int/hiv/en/. Last accessed: July 17th, 2017.
109. Dika IE, Harding JJ, Abou-Alfa GK. Hepatocellular carcinoma in patients with HIV. Curr Opin HIV AIDS. 2017;12:20–5.
110. Pineda JA, Romero-Gómez M, Díaz-García F, Girón-González JA, Montero JL, Torre-Cisneros J, Andrade RJ, González-Serrano M, Aguilar J, Aguilar-Guisado M, Navarro JM, Salmerón J, Caballero-Granado FJ, García-García JA; Grupo Andaluz para el Estudio de las Enfermedades Infecciosas; Grupo Andaluz para el Estudio del Hígado. HIV coinfection shortens the survival of patients with hepatitis C virus-related decompensated cirrhosis. Hepatology 2005;41:779–89.
111. Puoti M, Bruno R, Soriano V, Donato F, Gaeta GB, Quinzan GP, Precone D, Gelatti U, Asensi V, Vaccher E. HIV HCC Cooperative Italian-Spanish Group. Hepatocellular carcinoma in HIV-infected patients: Epidemiological features, clinical presentation and outcome. AIDS 2004;18:2285–93.
112. Graham CS, Baden LR, Yu E, Mrus JM, Carnie J, Heeren T, Koziel MJ. Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. Clin Infect Dis 2001;33:562–9.
113. Ryom L, Lundgren JD, De Wit S, Kovari H, Reiss P, Law M, El-Sadr W, Monforte AD, Mocroft A, Smith C, Fontas E, Dabis F, Phillips A, Sabin C. Use of antiretroviral therapy and risk of end-stage liver disease and hepatocellular carcinoma in HIV-positive persons. AIDS. 2016;30:1731–43.
114. Yang SF, Chang CW, Wei RJ, Shiue YL, Wang SN, Yeh YT. Involvement of DNA damage response pathways in hepatocellular carcinoma. Biomed Res Int. 2014;2014: 153867.
115. Barash H, R Gross E, Edrei Y, Ella E, Israel A, Cohen I, Corchia N, Ben-Moshe T, Pappo O, Pikarsky E, Goldenberg D, Shiloh Y, Galun E, Abramovitch R. Accelerated carcinogenesis following liver regeneration is associated with chronic inflammation-induced double-strand DNA breaks. Proc Natl Acad Sci USA. 2010;107:2207–12.
116. Strosnider H, Azziz-Baumgartner E, Banziger M, Bhat RV, Breiman R, Brune MN, DeCock K, Dilley A, Groopman J, Hell K, Henry SH, Jeffers D, Jolly C, Jolly P, Kibata GN, Lewis L, Liu X, Luber G, McCoy L, Mensah P, Miraglia M, Misore A, Njapau H, Ong CN, Onsongo MT, Page SW, Park D, Patel M, Phillips T, Pineiro M, Pronczuk J, Rogers HS, Rubin C, Sabino M, Schaafsma A, Shephard G, Stroka J, Wild C, Williams JT, Wilson D. Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries. Environ Health Perspect. 2006;114:1898–1903.
117. Erkekoglu P, Kocer-Giray B. Unpredictable effects of chemical mixtures on liver in health and disease. J Liver: Dis Transplant. 2012;1:1.