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Review

Correlation Between High Fat Diet, Cholestasis, Hepatocyte Apoptosis and the Development of Hepatic Carcinoma

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Abstract

Several prognoses have been determined for Hepatic Carcinoma (HCC). In this paper, I will explore the mechanism by which a high-fat diet (HFD) promotes the development of HCC. I first discuss the relationship between an HFD and the development of bile acid transport issues from the liver (cholestasis). As a result of cholestasis, an over-accumulation of hydrophobic bile acids is left surrounding the liver cells (hepatocytes). The bile acids create a cytotoxic environment for the hepatocytes. Damage done to hepatocyte mitochondria is specifically noteworthy as bile acids decrease levels of ATP as well as disrupt their physical structure. I have analyzed three pathways in which bile acids instigate apoptosis in hepatocytes, following intrinsic, extrinsic, and endoplasmic reticulum stress mechanisms. Finally, I have explored the correlation between frequent apoptosis and the development of HCC through DNA mutation in the Mcl-1 gene and other tumor suppressor genes.

Introduction

Researchers have identified Hepatic Carcinoma (HCC), the most common type of liver cancer, as the fifth most frequent cancer diagnosed worldwide with the third highest mortality rate among cancers.[1] The number of HCC cases and deaths observed have steadily increased over the past several years. In 2020, it was estimated that there were about 42,000 HCC cases and around 30,000 deaths.[2] Researchers have concluded that a correlation is present between excessive fatty tissue and gastrointestinal cancers, including HCC.[3] [4] There is a strong relationship between obesity and the development of HCC.[5] Linear regression models predict obesity

"Researchers have concluded that a correlation is present between excessive fatty tissue and gastrointestinal cancers, including HCC."

rates will increase by 33% by 2030, resulting in approximately 51% of the US population suffering from obesity.[6] Researchers have hypothesized a relationship between a high-fat diet (HFD) and gastrointestinal cancers.[7] Thus, understanding the mechanism through which HCC develops from an HFD and obesity is becoming more critical. Studies have been conducted correlating an HFD and liver damage, specifically regarding bile duct function.[8] This paper will discuss and analyze how an HFD develops bile duct dysfunction, also known as cholestasis. I have also examined how cholestasis instigates several mechanisms of excessive hepatocyte apoptosis, which promotes mutations in DNA, leading to tumorigenesis of HCC.

High Fat Diet Promotes Bile Acid Synthesis and Transport Dysfunction

First, it is important to establish that an HFD promotes bile acid synthesis and transport dysfunction, resulting in its overaccumulation in the liver. An experiment confirmed a significant correlation between an HFD and an increase in bile acid retention.[9] In the investigation, newborn C57BL/6J mice, an "inbred mouse strain" commonly used in anti-tumor research,[10] were injected with STZ (Streptozotocin), a chemical toxic to pancreatic beta cells which produce insulin.[11] The purpose of which was to develop diabetic symptoms in the mice. The mice were fed a regular diet for the first four weeks of life. At week four, a subsection of mice were introduced to an HFD. At week six, symptoms of fatty liver were observed with no signs of an inflammatory reaction. At week eight, "moderate inflammatory infiltrate" was present, including "neutrophils, lymphocytes and monocytes, and ballooning degeneration of hepatocytes," indicating an innate immune response against an HFD. At week twelve, chronic fibrosis was noted, indicating the pathology of nonalcoholic steatohepatitis (NASH), a fatty liver disease. At week twenty, all STZ-HFD mice developed HCC. Additionally, elevated levels of numerous bile acids were observed in hepatic cells of STZ-HFD mice beginning at week twelve and remained high through to week twenty (Figure 1).[9]

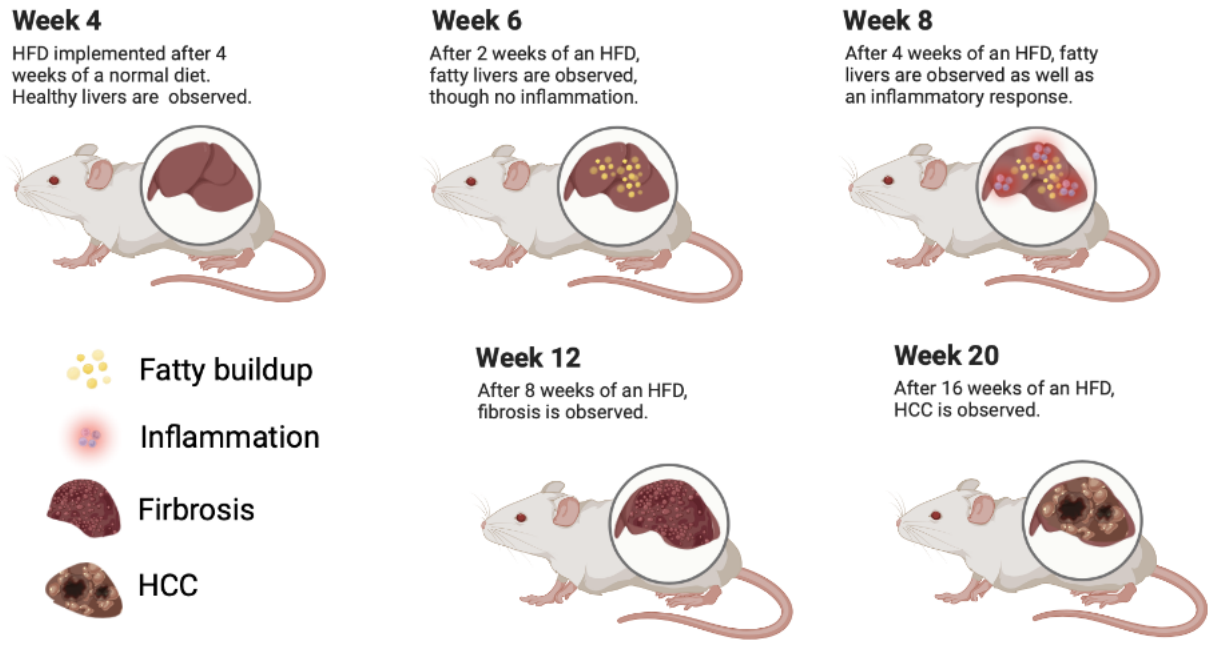

Figure 1 Adapted by Isaac Silverman from "Mouse Panels (Layout 3x2)", by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates

However, the International Agency for Research on Cancer had previously labeled STZ as a possible carcinogen, so these results prompted the independent studies of STZ and an HFD on the mice. This would allow firmer deduction if an HFD could be responsible for HCC. Indeed, HCC did result from an HFD alone. In fact, in all-male mice treated with STZ, HCC did not develop.[12] Thus, a relationship can be formed between an HFD and HCC. Furthermore, of the mice that developed HCC after being given an HFD, the plasma and liver bile acids such as TCA, GCA, and TCDCA showed the most "statistical significance" compared to untreated mice.[9]

In addition, raised TCDA was discovered to increase hepatocyte regeneration and decrease regulation of CEBPα, a tumor suppressor gene, indicating a carcinogenic correlation.[9]

Toxicity of Bile Acids

Hepatocytes are responsible for synthesizing bile acids directly from cholesterol by adding hydroxyl groups and oxidizing the molecules' side chain, making them less hydrophobic. The hydrophobicity of different bile acids depends on the number

of hydroxyl groups added.[13] In addition, some of these hydrocycholestorols undergo further biotransformations, making them less hydrophobic.[14] The stronger the hydrophobicity of the bile acids that accumulate in the hepatocytes, the more efficient it is in solubilizing membrane lipids[15] and having higher cytotoxicity.[16]

Numerous studies have concluded a correlation between an overabundance of bile acids and hepatocyte death. One study observed the cytotoxicity of the increased presence of the bile salts chenodeoxycholate (CDC), glycochenodeoxycholate (GCDC), and taurochenodeoxycholate (TCDC) (formed from their acid CDCA, GCDCA, and TCDCA, respectively) in rat hepatocytes. It was determined that the salt GCDC is the most toxic to hepatocytes. In an experiment, after four hours, approximately >10% of hepatocytes suspended in GCDC were viable, while approximately 40% of CDC and 30% of TCDC exposed cells were left viable. A control group of hepatocytes was also observed with approximately 70% viability. Another experiment proved that GCDC toxicity is dependent on its concentration.[17]

Bile Acid Induced Hepatocyte Mitochondrial Damage

The previously mentioned study also demonstrated that GDCDA develops mitochondrial malfunction and is a mechanism of hepatocyte death. They observed that 86% of cellular ATP was depleted after only 30 minutes following the addition of GCDCA, compared to cells provided fructose and GCDCA, which only lost 50% of ATP. In the first experiment, the presence of ATP depletion without a "glycolytic substrate," such as fructose, indicated to researchers that an issue developed within the cell's mitochondria leading to their death via anoxia, an absence of oxygen.[17] (Figure 2)

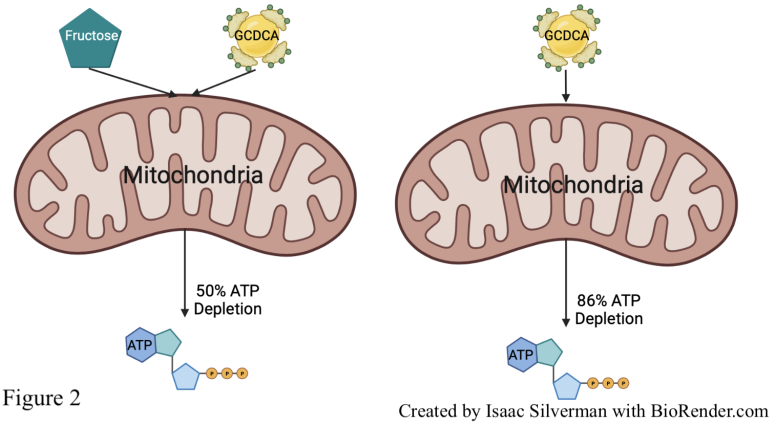

Figure 2

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Other studies have indicated similar abnormal mitochondrial behavior due to high bile acid

concentrations, including "swelling, pleomorphism, and abnormal cristae."[18] Hydrophobic bile acids have been found to inhibit several enzymes involved in cellular respiration, specifically in complexes I, III, and IV of the electron transport chain.[19] Additionally, hydrophobic bile acids are proven to disrupt the membrane potential of cristae[20] as well as serve as protonophores, resulting in an increased membrane solubility of H+ ions.[21] Researchers have hypothesized that non-parenchymal cell inflammation and fibrogenic responses may be attributed to such mitochondrial issues in parenchymal liver cells that emerge with cholestasis. Modified hepatocytes may emit immune signals such as cytokines, chemokines, and lipid peroxide products and signaling growth molecules, which would further an immune response. This would further lead to hepatic cell fibrogenesis, surrounding cell damage, and ultimately cell death.[22] In some cases of cholestasis, hepatocyte necrosis, as well as aforementioned mitochondrial impairments resulting in ATP depletion,[17] leading to oncolysis and cell death.

Methods of Bile Acid Induced Hepatocyte Apoptosis

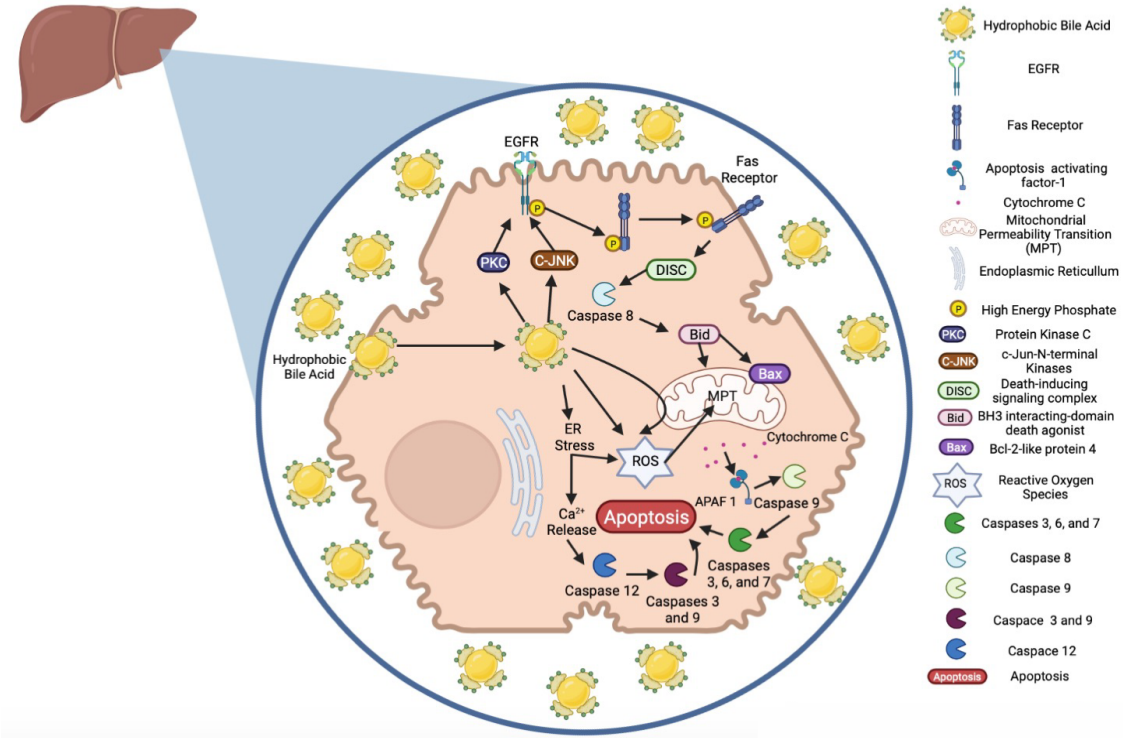

Figure 3

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Studies have indicated that hepatic apoptosis can result through several different protease cascades triggered by bile acids.[23] The first is an "intrinsic pathway," (Figure 3) which results from the mitochondrial release of apoptotic molecules due to bile-acid-induced oxidative stress.[24] Researchers discovered that "bile-acid-induced oxidative stress" may significantly impact the development of liver diseases.[25] Bile acids induce the creation of reactive oxygen species (ROS), which "oxidatively modify lipids, proteins, and nucleic acids" and ultimately end in hepatocyte apoptosis.[21] Additionally, researchers have proven that the bile acid GCDCA induces mitochondrial permeability transition (MPT).[26] Correlation has been made between MPT and ROS (Reactive Oxygen Species) generation and several other mitochondrial malfunctions, including decreased levels of oxidative phosphorylation,[27] leading to liver toxicity.[28] MPT results in the release of cytochrome c, a protein that initiates apoptosis when oxidized by ROS.[29] Cytochrome c stimulates the Bax protein (bcl-2-like protein 4) to move towards the mitochondria and release additional cytochrome c.[21] The Bax protein also interacts with IRE1α, a protein associated with creating endoplasmic reticulum stress,[30] by disrupting the homeostasis of its protein folding.[31] It also is responsible for upholding the activation of the protein-coding gene STAT3, which supports hepatocyte regeneration.[32] Furthermore, researchers have proven ROS is accountable for decreasing the number of antioxidant defenses in the cell. Among those are ubiquinone-9 and ubiquinone-10 which are antioxidants involved in the electron transport chain and prevent lipid peroxidation, the decreasing amount thus disrupts the metabolism and allows lipid peroxidation to occur.[21] Lipid peroxidation breaks down the mitochondrial membrane, which effects are previously stated, and the cell membrane.[33]

Another pathway of cholestasis-induced apoptosis is the pathway triggered by hydrophobic bile acids stressing the endoplasmic reticulum (Figure 3) by a mechanism previously mentioned. An excess amount of Ca2+ ions are released from the endoplasmic reticulum into the cytosol due to the presence of GCDCA.[17] The overabundance of Ca2+ ions stimulates an intracellular protease cascade of caspase enzymes (caspase 12 followed by caspases 3 and 9), leading to apoptosis.[34] Researchers

have discovered that the presence of C/EBP homologous protein (CHOP), a transcriptional regulator,[35] is an essential factor in hepatic cell death via cholestasis.[36] Furthermore, they observed a correlation between CHOP and the emergence of liver fibrosis due to hepatic cell damage caused by cholestasis.[36]

A third "extrinsic" pathway (Figure 3) of cholestatic derived apoptosis results from the activation of the death Fas receptor.[37] ROS instigated by hydrophobic bice acids has also proven in vitro to activate protein kinase c (PKC) and c-Jun-N-terminal kinases (JNK),[21] each of which manages cellular processes which promote tumor development.[38] [39] These molecules then stimulate the epidermal growth factor receptor (EGFR), which phosphorylates Fas and translocates it to the plasma membrane. The overabundance of Fas receptors on the membrane leaves the hepatocyte susceptible to apoptotic substrates.[37] In the event of Fas stimulation, a protease reaction is used as the apoptotic mechanism. Fas first forms a death-inducing signaling complex (DISC). As a result, caspase 8 is activated, which increases levels of cathepsin B[37], a cysteine protease, which several studies have found to be associated with tumor cell development and metastasis.[40] The activation of Caspase 8 separates and transports the pro-apoptotic Bid protein (BH3 interacting-domain death agonist) to the mitochondria, which opens the MPT pores. As previously discussed, MPT triggers the Bax protein as part of "intrinsic apoptosis." Additionally, in this pathway, as a result of MPT, the apoptotic molecule cytochrome c is released, which connects procaspase 9 with apoptosis activating factor-1(APAF-1) to activate caspase 9, which marks the point of no return in the hepatocyte death as it activates caspase 3.[41] In hepatocytes that are Fas deficient, researchers hypothesize that the death TRAIL-R2 receptor is involved with apoptosis, specifically with GDCDA.[42]

"Researchers have determined a correlation between increased levels of hepatocyte apoptosis and hepatocarcinogenesis."

Apoptosis Induced HCC

Researchers have determined a correlation between increased levels of hepatocyte apoptosis and hepatocarcinogenesis. In addition, they have discovered frequent hepatocyte regeneration with DNA damage

in human common fragile sites, areas on chromosomes determined to have frequent mutations, after large amounts of hepatocyte death.[43] Notably damaged genomic regions were FHIT, WWOX, and PARK2, all previously determined to serve as tumor suppressors.[44] These results indicated genetic dysfunction is present far before abnormal cell growth.[43]

Researchers also experimented with newborn mice mutated without the "anti-apoptotic Bcl2-family member myeloid cell leukemia 1 (Mcl-1) gene."[43] This instigated hepatocyte apoptosis and HCC tumorigenesis similar to the pathology caused by an HFD bile acid overaccumulation. Elevated levels of aspartate transaminase and alanine transaminase (AST and ALT), two liver enzymes associated with liver damage, were hypothesized to be present in these mice. Indeed, over one year of the experiment, Mcl-1 mutated mice (Mcl-1Δhep) demonstrated high ALT and AST. Over the year the ALT and AST levels decreased, being most statistically significant at 2 and 4 months when hepatocyte apoptosis and regeneration were the highest noted in mice that contracted HCC. Additionally, ALT levels in Mcl-1Δhep/HCC always remained higher than mice that had not developed HCC (Mcl-1Δhep/No HCC). In comparison, wild type mice always remained lower than all Mcl-1Δhep mice. 2-month-old mice also demonstrated a statistically significantly increased level of caspase 3 activations, indicating apoptosis, and DNA damage. Researchers determined that Mcl-1Δhep mice livers contained a significant number of genes enriched for HCC and hepatocyte apoptosis and regeneration.[43]

However, further experiments were conducted to determine if the loss of the Mcl-1 gene was responsible for the hepatocyte apoptosis and HCC or if tumor necrosis factor receptor 1 (TNFR1), a death receptor, was responsible. Crossbred Mcl-1Δhep and TNFR1 deficient mice were used to prevent "TNFR1-dependent apoptosis." At 2 months, Mcl-1Δhep/TNFR1 mice exhibited slightly lower ALT levels compared to Mcl-1Δhep mice. Additionally, both types of mice which developed HCC had significantly higher ALT levels compared to their non-HCC counterparts. Also, a larger amount of Fas receptors, activated in extrinsic apoptosis, were present in Mcl-1Δhep mice. Significantly higher levels of caspase 8 activation were also discovered

in Mcl-1Δhep mice, which researchers deduced to be the dependent variable for hepatocyte apoptosis, via an extrinsic pathway, and high ALT and AST. After 1 year, 28% of Mcl-1Δhep/TNFR1 mice displayed HCC, compared to 50% of Mcl-1Δhep, which did. Like the Mcl-1Δhep mice at 2 months, the Mcl-1Δhep/TNFR1 mice also showed statistically significant high levels of ALT and AST. Additional observations of the Mcl-1Δhep mice livers displayed an increased production of the inflammatory cytokines IL6, IL33, and IFNγ, which signal for inflammation, in contrast to Mcl-1Δhep/TNFR1 mice in which they were reduced. This experiment demonstrating the correlation between hepatocyte apoptosis and HCC tumorigenesis in mice resembles patients' similarly observed HCC development. Thus, it provides evidence for hepatocarcinogenesis due to increased hepatocyte apoptosis, which creates rapid hepatocyte regeneration with DNA mutations.[43] Other studies similarly prove that mutated gene replications have carcinogenic consequences.[45] They explain how the risk of HCC is determined by the activity of a patient's liver disease and its perpetuation,[46] including cholestasis. [17]

Conclusion

A relationship between an HFD and the development of HCC has been determined. As a result of an HFD, cholestasis promotes an accumulation of bile acids, such as GCDCA, which damage hepatocyte mitochondria and lead to apoptosis. Rapid hepatocyte apoptosis has been discovered to mutate anti-tumor genes, such as Mcl-1, which allows for HCC tumorigenesis. Numerous past and ongoing studies are focused on discovering inhibitors of bile acids over-accumulation and inhibitors of apoptotic mechanism components. Understanding the processes discussed in this article are essential to developing such inhibitors. Additionally, continuous research is in effect regarding regulating obesity and determining a healthy non-HFD which will prevent bile acid build up. HFD are especially prevalent in the United States, and these discoveries will hopefully prevent the development of HCC and possibly provide treatment to the millions already affected.

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