MOLECULAR PATHWAYS IMPAIRED IN ALCHOLIC AND NON ALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT
Fatty liver disease is gaining increasing recognition as a major health issue worldwide. If left untreated, liver may become so seriously scarred that it can no longer heal itself, progresses to cirrhosis which is life threatening. Fatty liver disease (FLD), whether it is alcoholic FLD (AFLD) or nonalcoholic FLD (NAFLD), encompasses a morphological spectrum consisting of hepatic steatosis (fatty liver) and steatohepatitis. FLD may be considered a single disease with multiple etiologies. This review summarizes recent cellular and molecular advances in the understanding of the mechanisms leading to end-stage FLD. Key risk factors including overweight, insulin resistance, a sedentary life-style and an altered dietary pattern have been identified in recent years. Pathogenesis of Fatty liver disease involves the role of nuclear receptors, proinflammatory cytokines, apoptosis, mitochondrial dysfunction, oxidative stress, sphingolipids, C-Jun-N-terminal kinase (JNK) pathway, phosphatidylinositol 3-kinase (PI3K/AKT) pathway, AMP Activated Protein Kinase (AMPK) pathway, DiacylglycerolAcyltransferase (DGAT), Visceral Adipocyte Tissue (VAT), Peroxisome Proliferators-Activated Receptors (PPARs), ceramides, Tumor suppressor gene, Sterol regulatory element binding proteins (SREBPs) and NF-E2-related factor-2 (Nrf2-ARE) pathway. Prevention and therapy opposing the development of steatosis can be achieved by a multifactorial approach: control of alcohol consumption, avoidance of obesity and of excess dietary long-chain fatty acids, or their replacement with medium-chain fatty acids. Progress in the understanding of the pathogenesis of Fatty liver disease and its progression to inflammation and fibrosis will results in prospects for their better prevention and treatment.

Keywords: Fatty liver disease, hepatic steatosis, steatohepatitis, alcohol, fibrosis, apoptosis.

INTRODUCTION
Fatty liver is the accumulation of lipid within hepatocytes. Clinically, FLD is divided into two broad categories, alcoholic FLD (AFLD) and nonalcoholic FLD (NAFLD), to bring the emerging nonalcoholic causes into existence and to distinguish them from alcoholic liver disease. Fatty Liver Disease (FLD), whether it is alcoholic FLD (AFLD) or nonalcoholic FLD (NAFLD), possesses a morphological spectrum consisting of hepatic steatosis (fatty liver) and steatohepatitis. FLD, if not treated can progress toward the development of cirrhosis and hepatocellular carcinoma. FLD is a single pathological condition with multiple etiologies that include alcohol consumption, obesity associated-insulin resistance, and many metabolic disorders. When there is increased consumption of energy and decreased combustion of calories, the unburnt energy is conserved in the form of fat in adipose tissue, leading to obesity. These pathogenic events are responsible for the metabolic syndrome consisting of Type 2 diabetes mellitus, dyslipidemia, atherosclerosis, hypertension, and hepatic steatosis which leads to FLD.
CAUSES OF FATTY LIVER

It is widely known that behavioral factors are main factors in the pathophysiology of fatty liver and in this aspect, an increased energy intake and diet composition is considered to be a major player. Furthermore, studies showed that a sedentary lifestyle with decreased physical activity represents another factor for fatty liver. Although these risk factors may successfully be abolished by adopting moderate lifestyle, the existence of other risk factors may need intense treatment. Among them, a disproportionate fat distribution, particularly with high visceral adiposity releasing humoral factors regulating liver fat are relevant. Finally, abnormal hepatic lipid oxidation as well as dysregulated lipogenesis, affected by genetics, may be of pathophysiological relevance. Mitochondria play a significant role in metabolism of hepatocyte and it represents the primary site for the oxidation of fatty acids and oxidative phosphorylation. Mitochondria occupy about 18% of the liver cell volume. Thus, the above mentioned findings readily suggest that mitochondrial function is a major regulator of liver fat. In addition, when there is abnormal mitochondrial function or even when an excess of free fatty acid is available, which is a case of fatty liver, reactive oxygen species (ROS) can occur leading to oxidative stress, which is thought to be important for the progression of disease and leading to NASH and fibrosis.

METABOLIC CONSEQUENCES OF FATTY LIVER

a) Dyslipidemia: An increased rate of synthesis of hepatic triglyceride and production of VLDL particle, which results in low HDL cholesterol and increased density of LDL particle, is considered to be a major factor of dyslipidemia. Furthermore, a decrease in lipoprotein lipase activity is also an underlying mechanism for dyslipidemia.

b) Dissociation of fatty liver and insulin resistance: Although hepatic fat accumulation, both in animals and in humans, is mainly associated with insulin resistance, and other parameters regulating insulin sensitivity such as overall obesity, body fat distribution, or circulating adipokines. In other words, for the identification of hepatic steatosis, subjects can be divided who have very high and very low insulin resistance suggesting that a dissociation of fatty liver and lower insulin sensitivity exists.

c) Fat accumulation in the liver induces hyperglycaemia, inflammation and the secretion of parameters that can be referred to as “hepatokines” for example, fetuin-A, thereby inducing atherosclerosis, and possibly hepatocyte dysfunction and cell death. However, the same amount of hepatic fat accumulation may be strongly associated with hepatic lipotoxicity, resulting in aggravation of high glucose level, subclinical inflammation, and an imbalance in hepatokine production as well as in their metabolic consequences. This condition may be referred to as malign fatty liver.

ALCOHOLIC FATTY LIVER DISEASE

Alcohol from a long time known to cause fatty liver by altering NADH/NAD⁺ redox potential in liver, which, in turn, inhibits fatty acid oxidation and the activity of tricarboxylic acid cycle reactions. More recent studies indicate that metabolism of ethanol impair fatty acid oxidation and stimulate lipogenesis. Ethanol interferes with DNA binding and transcription activating properties of peroxisome proliferator-activated receptor-α (PPAR-α). Ethanol-fed mice when treated with a PPAR agonist can reverse fatty liver even in case of continued ethanol consumption. Ethanol also activated sterol regulatory element binding protein 1, and stimulate lipogenic enzymes. These effects are due to inhibition of AMP-dependent protein kinase, reduction in plasma adiponectin, or increased levels of TNF-α in the liver. The understanding of these ethanol effects provides new therapeutic targets to reverse alcoholic fatty liver.

NONALCOHOLIC FATTY LIVER DISEASE

Nonalcoholic fatty liver disease (NAFLD) is a condition in which large amount of fat deposits in the liver of a patient without a history of alcohol abuse. NAFLD is categorized into simple steatosis and nonalcoholic steatohepatitis (NASH). In NASH, there is not only steatosis but also intralobular inflammation and hepatocellular ballooning is present, which often lead to progressive fibrosis. NAFLD is associated with obesity and insulin resistance; the current Western diet, high in saturated fats and fructose, plays an important role in pathogenesis of NAFLD. There are several mechanisms which
can accumulate triglycerides in hepatocytes. In NAFLD, there is formation of steatotic droplets in liver. Visceral adipose tissue dysfunction in obesity and insulin resistance results in overexpression of many cytokines which have a role in liver injury in NAFLD\textsuperscript{11}.

**MOLECULAR SIGNALING PATHWAYS INVOLVED IN FATTY LIVER DISEASE**

**COMMON PATHWAYS IN AFLD AND NAFLD**

NAFLD and AFLD both are leading causes of non-viral chronic liver diseases, and the prevalence of these two clinical disorders is gradually growing worldwide. The pathogenic difference between both the disorders is not easy to differentiate, so expertise from new evidence of clinical classification of FLD agree that similar major molecular mechanisms are shared between NAFLD and AFLD, including inflammatory pathways and fibrogenesis. Apoptosis is a common cell death process shared by NAFLD and AFLD, but AFLD is mainly associated with extrinsic pathway related to tumor necrosis family, which is derived by proapoptotic BCl\textsubscript{2} family members. In contrast, NAFLD is associated with FAS-induced apoptosis, which is related intricate network of metabolic stressors, activation of caspases, and that stimulate apoptosis, necrosis, and inflammation. It is worth mentioning that opening of mitochondrial membrane permeability transition pore and increased endoplasmic reticulum stress are central features in both AFLD and NAFLD. Hence, these data suggest that similar disease mechanisms lead to the clinical outcome of NAFLD and AFLD\textsuperscript{12}.

### 1. Tumor suppressor gene

The p53 gene was identified as the first tumor-suppressor gene. The p53 protein prevents multiplication of stressed cells or causes them to undergo programmed cell death (apoptosis). The stresses that activate p53 are vast, ranging from DNA damage to oxidative stress, hypoxia, and heat shock\textsuperscript{16}. In ob/ob mice p53 is activated in adipocytes and it is also involved in the mechanisms of dysregulated gene expression in adipose tissue of obese animals. These mice have excessive fat deposition in adipocytes and liver cells thereby activating the p53 pathway, the same mechanism could be followed in the steatotic hepatocytes of obese animals, possibly leading to hepatocellular injury\textsuperscript{14}. Steatotic hepatocytes are under various stresses like oxidative stress and DNA damage that induce p53, which leads to liver cell damage and plays an important role in the development of fatty liver disease\textsuperscript{15}. Excess fat accumulation in the hepatocytes leads to hepatocellular injury, and that this is possibly caused by the cellular toxicity of excess free fatty acids, oxidative stress, and lipid peroxidation\textsuperscript{16}. P53 has been shown to maintain balance between oxidative and glycolytic metabolism in cancer cells. Hence it is well known to be a key regulator of cellular metabolism\textsuperscript{17}. Recently, p53 has also been involved in NAFLD because of its role in regulating fatty acid oxidation. Similar to its role in alcoholic fatty liver disease, p53 has been shown to be upregulated in the livers of mice with NAFLD\textsuperscript{18} and has been linked to hepatocytes apoptosis in NAFLD\textsuperscript{19}. To test the preventive effect of p53 as a therapeutic target in NAFLD, Derdak et al. looked at the effect of a p53 inhibitor, pifithrin-α-nitro (PFT) on various parameters of NAFLD in a murine model\textsuperscript{19}. In HFD fed C57BL/6 mice, PFT treatment was found to decrease hepatic steatosis, apoptosis, and oxidative stress. These effects are believed to be secondary as reduction in the upregulation of miRNA34a was also found. Another recent study confirmed miR-34a upregulation and involvement of SIRT1 and p53 in liver steatosis and observed that treatment with ursodeoxycholic acid reversed p53 upregulation and restored SIRT1 expression\textsuperscript{20}.

### 2. Nuclear receptors (NRs)

NRs are ligand-activated transcription factors. They play various metabolic and regulatory functions. Levels of many natural and synthetic ligands including hormones, biomolecules (lipids), vitamins, bile acids, metabolites, drugs, and xenobiotic toxins have great influence on activity of NRs. In liver, NRs control hepatic inflammation, regeneration, fibrosis, and tumor formation\textsuperscript{21}. Body’s metabolic needs, including the synthesis and control of the pathways involved in the metabolism of cholesterol, fatty acids, carbohydrates, aminoacids, serum proteins, and bile acids, and the detoxification of drugs and xenobiotics are dependent on hepatocytes. Some members of the NR superfamily provide hepatic mechanisms for self-regulation in hepatocytes\textsuperscript{22}.

#### 2.1 Liver X Receptor

The transcriptional factor liver X receptor (LXR) is involved in metabolism of cholesterol. The LXR gene encodes two different units, LXR\textalpha and LXR\textbeta, both have diverse patterns of expression but have similar target DNA binding elements
and ligands. On activation, LXR induces the expression of a bunch of genes which helps in lipid metabolism; LXR helps in cholesterol absorption, efflux, transport, and excretion. Besides its metabolic role, LXRs are also involved in immune and inflammatory responses in macrophages. LXR forms heterodimers with the retinoid X receptor (RXR) within the nucleus. Binding of the RXR to LXR results in complex formation along with corepressors. Corepressors involved in this complex are silencing mediator of retinoic acid, thyroid hormone receptor, and nuclear corepressor. LXR positively regulates several enzymes involved in lipoprotein metabolism including lipoprotein lipase (LPL), human cholesteryl ester transport protein, and the phospholipid transfer protein. Proinflammatory molecules are involved in NAFLD whose expression are repressed by LXR. These include inducible nitric oxide synthase (iNOS), cyclooxygenase 2, interleukin-6 (IL-6), IL-1β, chemokine monocyte chemoattractant protein-1, and chemokine monocyte chemoattractant protein-3.

2.2 Farnesoid X Receptor
The farnesoid X receptor (FXR) is also a member of the NR superfamily. It has a typical NR structure and contains a hydrophobic pocket that possesses lipophilic molecules such as bile acids. Bile acids bind to FXR and activate this NR. Activation of the FXR decreases both hepatic lipogenesis and plasma triglyceride and cholesterol levels, induces the genes involved in lipoprotein metabolism/clearance, and represses hepatic genes involved in the synthesis of triglycerides. FXR-mediated suppression of hepatic lipase expression increases the hepatic uptake of HDL and also promotes reverse transport of cholesterol. Hepatic lipase is an enzyme which decreases HDL particle size by hydrolyzing its triglycerides and phospholipids in hepatic sinusoids, which facilitates hepatic uptake of HDL cholesterol. Activation of the hepatic FXR regulates carbohydrate metabolism by regulating gluconeogenesis, glycogen synthesis, and insulin sensitivity.

2.3 The pregnane X receptor (PXR) and constitutive androstane receptor (CAR)
PXR induces lipogenesis in a SREBP-independent manner. In PXR-transgenic mice, lipid accumulation and marked hepatic steatosis are associated with increased expression of the fatty acid translocase CD36.

CD36 is a multiligand scavenger receptor which is present on the surface of a number of cell types. It may contribute to hepatic steatosis by facilitating the high-affinity uptake of fatty acids from the circulation. PXR-mediated activation of PPARy may also promote hepatic steatosis by increasing the expression of CD36 directly or indirectly. PXR activation is also suppresses several genes which are involved in fatty acid β-oxidation, such as PPARα and thiols. Activation of the CAR might suppress lipid metabolism and lower serum triglyceride levels by reducing the level of SREBP-1. SREBP-1 is a master regulator of lipid metabolism. The inhibitory effects of the CAR on lipid metabolism might also be attributed to induction of Insig-1. Insig-1 is a protein with anti lipogenic properties. During fasting, CAR interacts with PPARα and interferes with fatty acid metabolism by binding to DNA elements overlapping with the PPARα-binding site in the promoter region of 3-hydroxyacyl CoA dehydrogenase. 3-hydroxyacyl CoA dehydrogenase is an important enzyme in peroxisomal fatty acid β-oxidation.

3. PI3K/AKT/PTEN Pathway
Inflammation is found to be the major reason behind the diseases and may lead to fibrosis and subsequent cirrhosis. In metabolic dysfunctions including obesity, metabolic syndrome, and the FLD, deregulation of the phosphatidylinositol 3-kinase (PI3K)/AKT pathway in hepatocytes is a common molecular event. PTEN is a tumor suppressor which negatively regulates the PI3K/AKT pathways through its lipid phosphatase activity. PTEN plays an important role in hepatic insulin sensitivity and the development of steatosis, steatohepatitis, and fibrosis. Phosphatidylinositol-3 kinase (PI3K) and serine-threonine protein kinase AKT (also known as protein kinase B) activates immune cells by regulating key inflammatory cytokines, and modulates PI3K/AKT signaling pathway which may contribute to specific therapeutic effects for the NAFLD. The second messengers generated by the activation of PI3K is dephosphorylated by PTEN which is its physiological function, thereby suppressing insulin signaling downstream of PI3K. In PTEN-deficient mice, synthesis and storage of triglyceride in hepatocytes is found to be increased because of the upregulation of PI3K/AKT activity. As a result of the lack of PTEN activity, there may be increased hepatocyte fatty acid uptake and increased fatty acid synthesis.
4. JNK Pathway

C-Jun-N-terminal kinase (JNK) is a mitogen-activated protein kinase (MAPK) family member. There are 3 isoforms of JNK in mammals: JNK1, JNK2, and JNK3. JNK1 and JNK2 are expressed in almost all cells, including liver parenchymal cells, whereas JNK3 is mainly expressed in brain, heart, and testis. In hepatocytes, JNK signaling is associated with cell death, survival, differentiation, proliferation, and tumorigenesis. In nonparenchymal liver cells, such as hepatic macrophages (Kupffer cells) and hepatic stellate cells (HSCs), JNK is involved in inflammation and fibrosis. TNF activates NF-B via the IKK complex and induces expression of antiapoptotic genes that are regulated by NF-B and block caspase-8 – dependent cell death and prolonged activation of JNK. Long term JNK activation increases ROS accumulation, which progressively leads to oxidative inhibition of JNK dual-specificity phosphatases. Movement of activated JNK towards the outer membrane of mitochondria is another important step in induction of JNK-mediated hepatocyte death. Mitochondrial Bcl-XL, Mcl-1, and Sab are substrates for JNK. TNF helps in movement of phosphorylated JNK and MKK4 and theBcl-2 family memberBax to the mitochondrial outer membrane where they promotes generation of mitochondrial ROS and sustained activation of JNK, inducing hepatocyte death.

5. Role of Visceral Adipose Tissue (VAT)

VAT has greater lipolytic potential than subcutaneous adipose tissue, and the release of FFA from visceral fat directly into the portal circulation creates a first-pass effect. Serum FFA derived from VAT by lipolysis is the main source of hepatic TG in NAFLD. VAT supplies fat to the liver via portal vein hence plays an important role in the pathogenesis of hepatic steatosis. Visceral adiposity has also been shown, together with insulin resistance, to be an independent predictor of the presence of a steatosis-associated increase in portal pressure. The mechanisms linking VAT and the liver are currently poorly understood. The drainage of the venous blood of the gastrointestinal system, including the VAT, via the portal system to the liver represents a unique anatomical link between the two. VAT can directly influence the liver via portal vein. NAFLD-induced changes in liverhaemodynamics might inversely influence VAT.

6. Nrf2-ARE Pathway

A series of antioxidant genes are induced by elevated ROS and electrophiles through which there is activation of antioxidant response element (ARE) which ultimately protect cells against oxidative stress. NF-E2-related factor-2 (Nrf2) is a member of the cap’n’collar family of bZIP transcription factors which primarily regulates ARE containing gene expression. Oxidative stress and electrophiles leads to activation of Nrf2 in a variety of tissues and cells and plays a role as a multiorgan protector through target gene induction. In chronic alcohol-fed mice increased levels of Nrf2 protein and mRNA levels were observed in liver tissues or hepatocytes. HepG2 cells overexpressing CYP2E1 (E47 cells) showed increased Nrf2 mRNA and protein expression compared with control HepG2 (C34 cells). Nrf2 is activated in E47 cells as shown by an increase in nuclear translocation of Nrf2 and Nrf2-ARE binding activity and upregulation of Nrf2-regulated genes such as GCLC and HO-1. Nrf2 knockout mice have reduced ability to detoxify acetaldehyde, leading to accumulation of the toxic metabolite. Due to loss of Nrf2 a marked steatosis and inflammatory response was observed in Kupffer cells in ethanol-fed mice. Furthermore, chronic ethanol consumption led to a progressive depletion of total and mitochondrial reduced GSH, which was associated with more pronounced structural and functional changes to mitochondria of Nrf2-knockout mice. Yates et al. carried out a global analysis of mouse hepatic gene expression and revealed that both genetic and pharmacologic activation of Nrf2 induce a larger cluster of genes associated with lipid metabolism.

7. Diacylglycerolacyltransferase (DGAT)

Hepatic TG production is result of uptake of free fatty acids (FAs) to the liver and de novo synthesis both. Hepatic TG removal is result of FA oxidation and formation of very low density lipoprotein (VLDL) particles. The final step and rate-limiting reaction in TG synthesis is catalyzed by acyl CoA:diacylglycerolacyltransferase (DGAT), which covalently joins a fatty acyl-CoA and a diacylglycerol (DG) molecule to form TG. In mammals, DGAT occurs in two isoforms, DGAT1 and DGAT2, from distinct gene families. Both isoforms of DGAT are widely expressed in white adipose tissue and are present in high levels. DGAT1 is most highly expressed in the small intestine, whereas
DGAT2 is primarily expressed in the liver. DGAT1 is associated with VLDL synthesis and DGAT2 is associated with steatosis thus both the isoforms play different roles in TG metabolism. Overexpression of liver-specific DGAT2 in mice results in hepatic steatosis. Chronic alcohol exposure contributes to upregulation of hepatic DGAT2 which ultimately leads to the development of ALD. Abnormal hepatic metabolism of methionine, specifically the suppression of transmethylation reactions, plays a mechanistic role in alcohol-induced DGAT2 upregulation by suppressing MEK/ERK1/2 activation. Therefore, inhibition of ERK1/2 activation represents a critical link between methionine metabolism and fatty liver development via upregulation of hepatic DGAT2 expression.

8. AMP Activated Protein Kinase (AMPK)
AMP Activated Protein Kinase (AMPK) possesses to inhibit lipid synthesis by the acute inhibition of glycerol-3-phosphate acyltransferase (GPAT) activity and transcripational regulation via sterol regulatory element binding protein-1c (SREBP-1c). AMPK helps to regulate energy metabolism, fatty acid metabolism, protein synthesis, glucose uptake, increase lipid oxidation and inhibit lipid synthesis. AMPK helps in acute inhibition of glycerol-3-phosphate acyltransferase (GPAT) which is an integral enzyme in triglyceride accumulation. GPAT is the rate-limiting enzyme catalyzing the first committed step in triglyceride synthesis. An AMP-analog aminimidazolecarboxamideribonucleotide (AICAR) chemically activates AMPK and results in reduced fat accumulation in the hepatocyte by decreasing GPAT1 activity by 30 to 40 percent. AMPK inhibits acetyl-CoA carboxylase (ACC), an enzyme that catalyzes the formation of malonyl-CoA. Malonyl-CoA inhibits carnitinepalmitoyltransferase 1 (CPT1) resulting in decreased betaoxidation and increased fat synthesis. Decreasing malonyl-CoA production results in an increase in CPT1 activity. Therefore, through AMPK’s acute role of inhibiting GPAT1 and increasing CPT1, there is an overall increase in oxidation relative to triglyceride synthesis.

9. PPARs.
The ligand-activated transcription factors belonging to the peroxisome proliferators-activated receptors (PPARs) are a subfamily of the steroid/thyroid/retinoid receptors superfamily. PPARs act as fatty acid sensors to control many metabolic programs including adipocyte differentiation, inflammation and energy homeostasis, lipoprotein metabolism, and FA oxidation that are essential for systematic energy homeostasis and represent an important target for NAFLD. In steatosis development, PPAR-γ involves activation of lipogenic genes and de novo lipogenesis and attributes as a causal role of steatosis. Hence, increased PPAR-γexpression is a feature of the steatotic liver. The specific PPAR subtype PPAR-γ is mainly expressed in the white and brown adipose tissue, where it controls the expression of genes related to lipogenesis. PPAR helps in promoting cell differentiation, FA uptake, and TAG accumulation, which reduces FA delivery to the liver. In addition, PPAR-odownregulation may play a significant role in enhancing the DNa binding capacity of proinflammatory factors NF-xB and AP-1 in the liver of obese patients, thus constituting one of themajor mechanisms for the progression of simple steatosis tosteatohepatitis.

10. Role of Cytokines
Cytokines are classified into two subtypes as T helper 1 (Th1) and T helper 2 (Th2). The Th1 cytokines are main proinflammatoryand it includes tumor necrosis factor-alpha (TNF-a), interleukin (IL)-1, IL-6 and interferon; whereas the Th2 cytokines are main anti-inflammatory and it includes IL-4 and IL-10. In general, Th1 cytokines induce Th1 cytokines and inhibit Th2 cytokine production and vice versa. TNF-a helps to activate intracellular signaling molecules which includes stress related kinases such as Jun N-terminal kinase and inhibitor kappa beta kinase beta. TNF-a helps to make cells resistant to the actions of insulin. TNF-αactivates harmful proatherogenic pathways partially by reducing HDL cholesterol, elevating expression of cholesterologenic genes and accompanied by raising potentially harmful precholesterol metabolites, and suppressing cholesterol elimination. TNF-aon activation can induce both hepatocyte cell death and hepatocyte proliferation. Both TNF-aand IL-6 stimulate hepatic lipogenesis. The inhibition of IL-10 promotes the expression of cytokines which are involved in inflammation, deterioration of insulin signal and activation of gluconeogenic and lipidogenic pathways.
11. Role of Endoplasmic Reticulum (ER) Stress

The mechanisms by which saturated fatty acids contribute to liver injury are due to disruption of endoplasmic reticulum (ER) homeostasis, or ER stress, which is a proximal event. ER stress may lead to activation of various intracellular stress pathways that can progress inflammation and lead to hepatocyte cell death and liver damage. ER is responsible for posttranslational modification of proteins destined for intracellular organelles and the cell surface. Highly saturated fat diets induce hepatic ER stress and liver damage in male Wistar rats progresses to development of liver dysfunction. In ER, during oxidative protein folding each disulfide bond formed is responsible for production of single ROS and this process is responsible for ~25% of all ROS generated in a cell. As such, loss of ER calcium homeostasis via inhibition of the sarcoplasmic reticulum ATPase (SERCA) uptake pump will reduce the folding capacity of the ER and induces ER stress and ultimately this situation will lead to ER-associated apoptosis. ER calcium stores disruption may be the reason of saturated fatty acids induces cell death in H4IIE liver cells and primary rat hepatocytes. Chronic ER stress induces numerous intracellular pathways that, if left unabated, can lead to systemic inflammation, hepatic fibrosis and hepatocyte cell death.

12. Sterol regulatory element binding proteins (SREBPs)

The sterol regulatory element binding proteins (SREBPs) is a family of transcription factors which regulates lipid homeostasis in animal cells. SREBPs belong to the basic helix-loop-helix-leucine zipper family. Till date three SREBPs have been identified: SREBP-1a and SREBP-1c. These are produced from the same gene through the use of different promoters. SREBP-2 is encoded by a separate gene. The synthesis and uptake of cholesterol, fatty acids and phospholipids is regulated by SREBPs which activate the expression of genes which are involved in synthesis. SREBP-1c preferentially activates genes involved with free fatty acid. LXRs directly regulate SREBP-1c which is the master transcriptional regulator of fatty acid synthesis and fatty acid synthase (FAS) which is a key enzyme in the de novo biosynthesis of fatty acids. SREBP-1c remains bound to the ER and the nuclear envelope in the presence of sufficient sterol concentrations. It is synthesized as a precursor protein. This precursor protein undergoes a series of cleavage process upon sterol deprivation and results in release of NH2-terminal portion. For activation of transcription of genes involved in fatty acid synthesis these mature SREBP-1c then enters the nucleus by binding to a sterol regulatory element (SRE) or to palindromic sequences (E boxes), within promoter regions of target genes, to activate them.

13. Role of ceramides/sphingolipids

Ceramides are members of the sphingolipid family of lipids. They are integral to the structure of the lipid bilayer that makes up cell membranes. However, ceramides also have cell signaling properties. They accumulate in the liver especially during periods of increased hepatic influx of free fatty acids (FFA). They may contribute to insulin resistance. Ceramides are produced by three different pathways; de novo synthesis, a sphingomyelinase (SMase) pathway, and a salvage pathway. LDL. An antioxidant, glutathione (GSH) inhibits dihydroceramide desaturase and regulates de novo ceramide synthesis and cellular redox status. High saturated fat diet can also stimulate ceramide synthesis. Ceramide activate transactivation factor known as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB). It can also regulate pro-inflammatory gene expression. Ceramides reduce GLUT4 translocation and glucose uptake. It has been shown to impair insulin sensitivity in skeletal muscle cells. Interaction of TNF-α and ceramides causes insulin resistance and also increases mitochondrial generation of ROS. Ceramides promote apoptosis and further translocation of inflammatory cells to the liver which ultimately leads to a worsening of hepatic inflammation. Accumulation of sphingolipids in the cytoplasm, production of de novo ceramide within the mitochondria and Ceramide-derived sphingolipids, ganglioside GD3 is required by TNF-α induced mitochondrial damage. Ceramides could act as a fuel for the cellular damage caused by inflammation and worsening insulin resistance. It promotes mitochondrial dysfunction and oxidative stress, thus facilitates the progression of NASH.
CONCLUSION
Fatty liver disease is a very complex and multifactorial disorder. Sedentary lifestyle and high intake of dietary fats and alcohol exerts its effects at many levels; individual signaling molecules, cells and finally the entire organ. Integrative approaches providing a comprehensive picture of how alcohol and high fat diet affects intracellular signaling pathways in tissues at different levels is needed. A multidimensional analysis of inflammation and death signaling pathways in immune and non-immune cells of the liver to identify molecular targets in the host leading to systemic and organ inflammation will enhance our understanding of the pathogenesis of Fatty liver disease. Until now various key signaling pathways involved in pathogenesis are nuclear receptors, cytokines, various proinflammatory mediators, AMPK, JNK, VAT, Nrf2/ARE, SREBP, P53, ceramides, stress pathways such as ROS mediated activation of transcription factors, mitochondrial damage and ER stress, have been viewed as separate entities rather than an integrated network of molecular interactions in Fatty liver injury. Future approaches to enable comprehensive analysis of these interactions could offer a powerful tool to understand diagnosis, prognosis, and treatment of FLD.

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