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Chapter 4

Cellular Senescence and Their Role in Liver Metabolism in Health and Disease: Overview and Future Directions

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Abstract

Chronic liver disease has globally risen mainly due to a prevalent hepatitis C virus (HCV) infection rate and an epidemic of obesity. It is estimated by the year 2030, 2.2 billion people around the world will be overweight and 1.1 billion people will be obese. Diabetes and obesity are the main risk factors for the development of the metabolic syndrome and in the liver of non-alcoholic fatty liver disease (NAFLD) which could progress to non-alcoholic fatty steatohepatitis (NASH) related cirrhosis and liver malignancy. At present there is not effective therapy for NASH besides loss of weight and exercise. Furthermore, optimal management of HCC with curative intent includes resection or liver transplantation. Nevertheless, these therapies are limited because the degree of liver dysfunction or the medical conditions at the time of diagnosis and the scarcity of available liver grafts. The role of cellular lipid management and metabolism in human health and disease is taking a center stage. The present overview articulates the current pathophysiology of fatty liver disease under the aging processes, potential biological markers of liver disease diagnosis and progression and future therapies.

Keywords: review, senescence, cell aging, mitochondrial function, cancer, obesity, NASH, ROS, lipids, metabolism

1. Introduction

1.1. Global burden of chronic liver disease

Chronic liver disease has globally risen due to mainly a prevalent hepatitis C virus (HCV) infection rate and an epidemic of obesity [1–7]. During the last 2 decades, global viral hepatitis

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has increased by 163% (from 0.89 million to 1.45 million) and in 2013, viral hepatitis infections became the 7th leading cause of death [7]. While HBV infection is decreasing in most endemic areas due to successful vaccination policies, HCV infection lacks the benefits of a vaccine [7]. Even though HVC antiviral therapies recently introduced in clinical practice are highly successful, its implementation is limited due to access and/or financial constraints. Morphological studies in HCV showed hepatocyte lipid accumulation similar to the one that occurs in obesity.

Our group has estimated that 2.2 billion people will be overweight and 1.1 billion people will be obese globally by 2030 [8, 9]. In addition, 36.1% of adult men and 32.4% of adult women were diagnosed with the metabolic syndrome in 2010 [10]. Obesity represents the core component of the metabolic syndrome, a cluster of metabolic disarrangements including dyslipidemias, insulin resistance status, hypertension, diabetes and organ metabolic disturbances such as non-alcoholic fatty liver disease (NAFLD) and its inflammatory component non-alcoholic fatty steatohepatitis (NASH), nephropathy, cardiomyopathy and muscle dysfunction [10]. Hepatocellular carcinoma (HCC) has been reported more often in non-cirrhotic livers in the background of NASH and its risks factors include male gender, older age, cigarette smoking, obesity and insulin-resistant states [3, 4, 11]. Overweight and obesity are associated with an overall increase in liver primary cancers of 17% and 89%, respectively [2, 11, 12] and males with a BMI > 35 had a 3.5–4 increase risk of liver malignancy [12] (**Figure 1**). Optimal management of HCC with curative intent includes resection or liver transplantation. Nevertheless, these therapies are limited because the

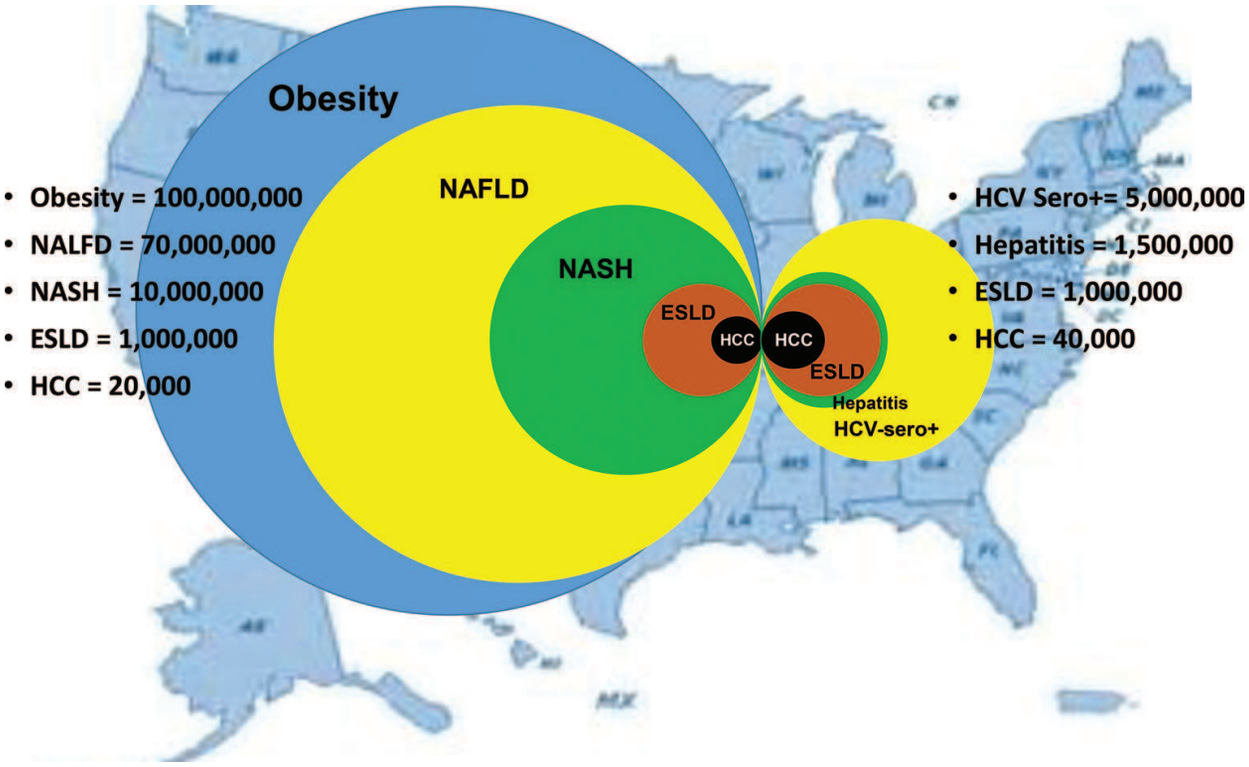

Figure 1. *Main causes of end stage liver disease (ESLD) and hepatocellular carcinoma (HCC).* Hepatitis C virus infection (HCV) and obesity are the main cause of the global and Western increase in ESLD and HCC. In USA 1 out of 3 adults is overweight and approximately 5 M people is HCV seropositive. While prevention relays in stopping virus transmission and implementing programs of healthy caloric intake and exercise programs, treatments of established ESLD and its malignant consequence are similar. Nevertheless, the most effective surgical treatment, liver transplantation is limited due to scarcity of donors and loco-regional therapies have limited survival effect due to malignant recurrence or progression of liver dysfunction.

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degree of liver dysfunction or additional medical conditions at the time of diagnosis and the scarcity of available liver grafts. The role of cellular lipid management and metabolism in human health and disease is taking a center stage [13]. Higher fat intake, lower physical activity and a progressively aging population are among the behavioral and social factors of this phenomenon that add to the genetic load [1]. An overview of the role of cell aging and senescence in liver metabolic responses to high caloric intake will be performed in the pages to follow.

1.2. Regeneration, necrosis, apoptosis and senescent: a constant changing balance

The liver is a unique organ with an innate ability to regenerate through mass compensation to satisfy portal flow and metabolic demands [14]. After injury and cell necrosis, immune recall of resting cells occurs and activation of oval-precursor cells in conjunction with platelets migration switch to a cell division renewal cycle [15, 16]. Mitosis is more prominent at the peri-portal stem cell niche site (zone 1) assuring clonal expansion until reaching zone 3 (peri-central vein) [15, 16]. In health, liver mass homeostasis is closely regulated through a delicate balance among regeneration, apoptosis (programmed cell termination), and senescence. During states of acute liver injury, the pendulum moves towards a regenerative and repair phase, however, during chronic states of liver injury collagen synthesis and deposition persists leading to organ fibrosis. In addition, natural processes of organ aging play a main role in organ response to both acute and chronic injuries. Primary cell life span is determined by a limited number of cell duplications, the so called Hayflick limit [17]. After such limited divisions, cells enter a state of cell replicative senescence which is believed to be triggered by shortening of telomere ends. Replicative cellular senescence is a stable form of cell arrest characterized by a lack of cell proliferation activity and apoptosis resistance mediated through a lack of mitogen response even though the cells remain metabolically active. On the other hand, cells can be induced to a senescence status by a variety of cellular stressors such as DNA damage, UV light, radiation, oncogene activation, increased H_2O_2 production and heat stress [17, 18]. Senescent cells undergo morphological changes as they acquire an enlarged and flattened morphology, in addition to an increase expression of the senescence associated markers ß-galactosidase (SA-ß-GAL), an accumulation of the senescence associated heterochromatic foci (SAHF) and DNA damage foci, and the expression of the senescence associated secretary phenotype (SASP) [18]. Senescent status is achieved and maintained by active signaling of p53, a tumor suppressor gene that exercises its effects through activation of p21, a potent cell cycle inhibitor, and the p16-retinoblastoma protein [18]. Cells induced into an irreversible cell cycle arrest at the G1 phase will undergo metabolic disturbances with an increase reactive oxygen species (ROS) production, decrease adenosine triphosphate (ATP) synthesis and accumulation of lipofuscin [17].

Changes in the content of daily oral intake can influence life span and thus cell aging. The cell death-inducing DNA fragmentation factor α-subunit-like effector A (Cidea), is a transcriptional coactivator implicated in lipid accumulation, cell stress and cell aging. Authors showed in rodents, that a high lipid diet up-regulated Cidea with hepatic lipid accumulation, cell stress, mitochondrial dysfunction and genetic upregulation of aging [19]. Other studies, in support of this findings have shown a life span reduction up to 30% in genetically obese mice (ob/ob) and this reduction was reversed by a caloric restricted diet [19]. Lipid enriched diets are associated in humans with DM type 2, HTN and cardiovascular events all

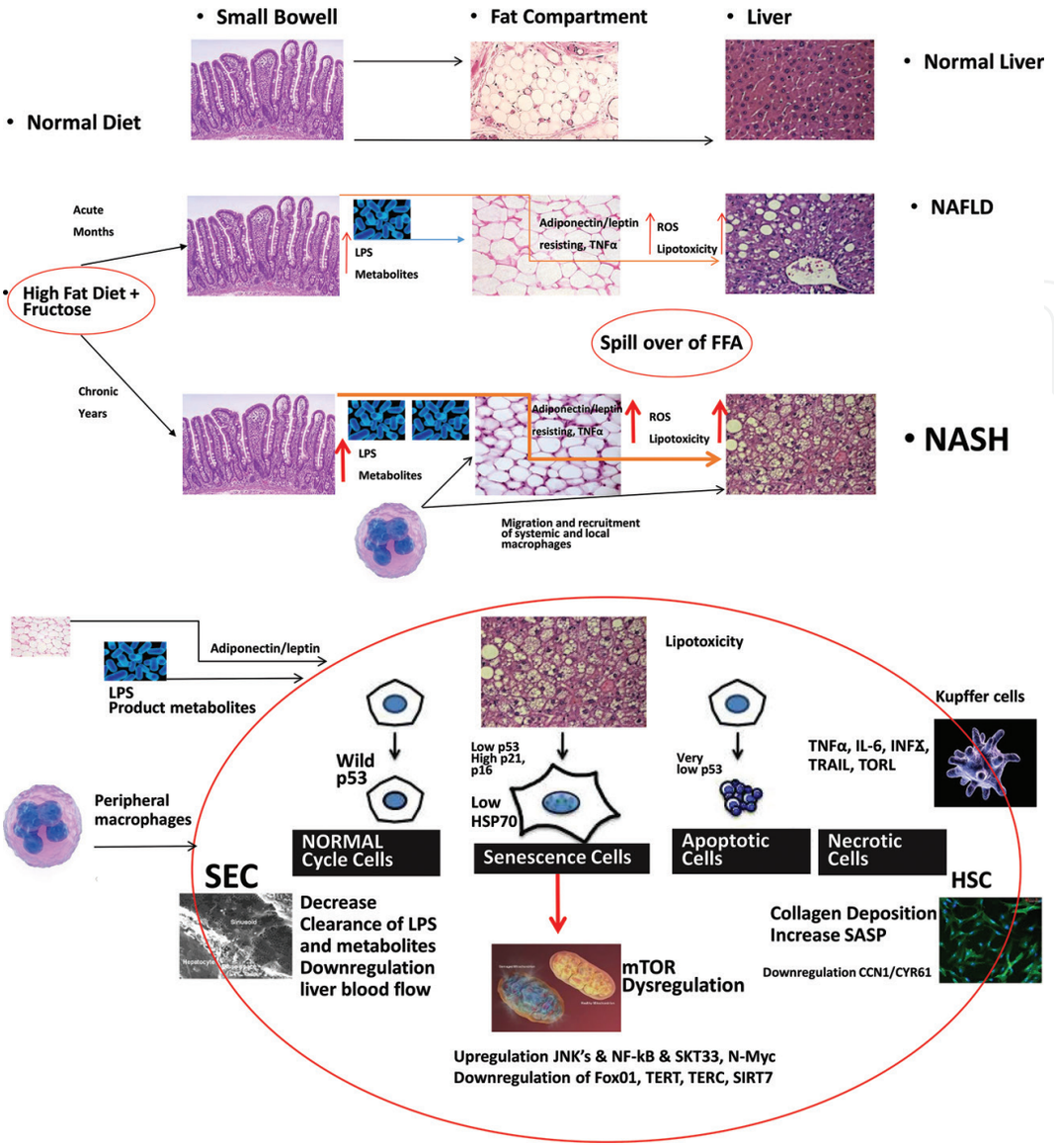

Figure 2. (A) *Morphological changes observed in the mice model of high fat diet (HFD) plus fructose (Western Diet) in the microbiota, fat content tissue and liver.* Liver cells accumulate FA in form of TG from the spill-over of lipid excess in the fat compartment and after saturated the normal processes of liver lipid metabolism. (B) *Local liver inflammatory response from lipid excess.* Lipotoxicity an addition to increase LPS activates SEC, HSC and Kupffer cells inducing more parenchymal cells into senescence and apoptosis which changes the local mielue into an inflammatory microenvironment. Continuous HFD decreased further mitochondrial function with lower ATP production and increase collagen deposition leading to progressive liver fibrosis, liver dysfunction. The state of progressive liver fibrosis due to a local and systemic inflammatory state results in an increasing insulin resistance status with the full metabolic syndrome phenotype. Its progression results in decompensated ESLD and the development of malignancy.

of which limit life span. Caloric restriction without malnutrition can extend life span while caloric excess has the opposite effect [20]. Thus, the choice of oral intake has a profound impact on life span.

The free radical intermediates hypothesis of cell aging still remains the most reasonable in the induction and maintenance of the senescent status [17, 21]. ROS, reactive nitrogen sp., and lipid peroxide are important regulators of cell signaling that provides reliable maintenance of

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cellular components, support redox-state and regulate the function of highly metabolic active cells as in hepatocyte and immune cells [20, 21]. ROS in excess from over-oxidation of lipids, proteins, nucleic acids and other macromolecules is associated with a misalignment of their functional activity, reactions that if they last through the cell cycle can lead to permanent cell dysfunction and/or accelerated aging cell process. Thus, an excess of food intake in form of continuous lipid charge will test the oxi-redox systems that keeps the fragile mitochondrial equilibrium in balance. Continuous metabolic stress changes the equilibrium towards lower levels of antioxidants (glutathione sp.) with further increase of ROS that in turn accelerates processes of apoptosis and senescence. Former processes further lead to arrest of regeneration and activation of hepatic stellate cells (HSC) and therefore fibrogenesis (**Figure 2A**).

The hormonal mielau modulates cellular response to caloric intake. Insulin and somatotropin signaling are critical not only in the control of cell aging and longevity under conditions of abundant food supply but also in mediating the effects of caloric restriction on life span. In a rodent model of thyroxine induced aging, thermogenesis was directly correlated with increased mitochondrial function, increased ROS production, decreased concentration of glutathione reduced, downregulation in the activity of antioxidants enzymes and increased senescent marker expression in the liver as well as in other organs [21]. Estrogen influences lipid metabolism through nuclear receptors which enhances apoptosis of mutated cells, improves mitochondrial function, and decreases the metabolic syndrome phenotype [10, 22]. Actions that may explain, at least in part the constant disparity of overall life expectancy by gender.

Lifestyle changes such as exercise and caffeine supplementation have shown to increase the ratio of reduced/oxidized glutathione in liver and muscle tissue in the rodent model [23]. Although liver enzymes were identical in experimental and controls groups, plasma levels of cytokines associated with inflammation (IL-1ß, IL-6, TNF-α and INF-α) and cell aging were found to be significantly decreased in the experimental group when compared to controls [23]. It was noted that although exercise increased the production of ROS, exercise also evoked a beneficial increase in levels of cell antioxidants, and lowered levels of oxidative damage when cells were exposed to a second injury, i.e. lipid charge. Thus, the concept of exercise inducing gene expression of antioxidant enzymes that may protect the cell from other insults was called 'hormesis' [23]. Although caffeine, a member of the methyl-xanthine family increased the ratio of reduced-oxidized glutathione, no other markers of cell stress were modified. Perhaps, caffeine potentiates further the beneficial effects of exercise.

2. Liver metabolism in health and disease

The reduced tri-peptide glutathione (GSH) is the major antioxidant in the body responsible for maintaining the intracellular redox balance. 90% of the GSH in plasma derives from the liver [24] and aging is associated with a progressive decline in the levels of GSH in humans and rodents [25]. Senescent liver cells in culture showed elevated ROS leading to a state of chronic oxidative stress. In addition, age associated decline in GSH has been linked to an activation of neural sphingolipid hydrolase enzyme (NSMase) and the accumulation of bioactive ceramide, a precursor of inflammation [25]. The availability of L-cysteine is the rate-limiting

factor of GSH synthesis and oral supplementation of cysteine alleviates GSH deficiencies in humans and rodents [25]. GSH deficiency can be alleviated by the oral intake of cysteine and its restoration rates appears to be age and sex dependent. Older animal models are associated with increased cellular stress and an enhanced subcellular injury after heat stress associated with an increased iron intracellular deposition [26]. These cause damages to mitochondria and lysosomes. Although a more precise mechanism of organelle damage was not enunciated, iron deposition mediated a decrease in Transferrin-receptor-1 which upregulates the iron storage protein ferritin after heat stress. Nevertheless, the synthesis of the iron exporter protein ferroportin was delayed [26]. Effect that may explain at least in part, organelle damage in the aging cell that occurs after natural oxidants depletion (**Figure 2B**).

A diet enriched in calories and lipids increases free fatty acids (FA) in plasma obligating cells to protect themselves from lipotoxicity or death by either oxidizing FA's or sequestering them as triacylglycerol (TAG) within lipid droplets (LD) [13]. PGC-1, an exercise-induced transcriptional coactivator may play an important role in coordinating intra-muscular LD-signaling with mitochondrial remodeling. TAG within lipid droplets are the major form of energy storage in the body (muscle, liver, fat tissue) and a reservoir of membrane lipid component. TAG synthesis is initiated by glycerol-3-phosphatase acetyltransferases (GPAT) at the mitochondrial and sarcoplasmic reticulum membranes and it is completed at the sarcoplasmic reticulum by the sn-1-acyl-glycerol-3-phosphatase acyltransferase (AGPAT), phosphatidic acid phosphatase (PAP) and sn-1,2-diacylglycerol acyltransferase (DGAT) [13]. Synthetized LD-TAG are localized preferentially in proximity to mitochondrial membranes named "contact zones". Once TAG's are released, they are mainly used in the mitochondria for ATP synthesis via oxidative phosphorylation from the ß-oxidation path. The "athlete paradox" states that the accumulation of TAG in the trained and insulin sensitive cells is in greater proportion than the TAG accumulation in cells from diabetic subjects with insulin resistance. This observation supports the hypothesis of mitochondrial dysfunction as a factor of TAG accumulation from a sustained lipid charge.

The protein family of perilipins (Plin) is associated with LD's and their scaffolding may affect the interaction between TAG and the mitochondria [13]. The Plin family consists of Plin1 to 5; the most common PAT (perilipin/ADRP/TIP47) interacts with LD in different proportions. In the liver, down-regulation of Plin2 promotes a reduction of hepatic steatosis and increases insulin sensitivity, albeit a reduction in both Plin2 and Plin3 is associated with insulin resistance [13]. In the heart, a Plin5 deficiency causes increased lipid oxidation, increased ROS production and decreased cardiac function. In heart and skeletal muscle TAG and FA are the main metabolic source of energy through the ß-oxidation pathway, suggesting a very tightly regulated process from cell storage to mitochondrial metabolic use. While TAG may come from LD, FA's are mainly transported in plasma as albumin-bound or as part of the very low density lipid-protein (VLDL) complex. Different transmembrane transporter systems are involved in their translocation to the inner cell compartment where the long chain fatty acid (LCFA) forms thioesters with coenzyme A (CoA). LCFA-CoA can form TAG for storage as LD, or can enter the outer mitochondrial membrane where CPT1 catalyzes the reaction of LCFA-CoA to LC-acylcarnitine. The former compound can actively cross the inner mitochondrial membrane with the exchange of carnitine for acylcarnitine. CACT is highly expressed in tissues with predominant ß-oxidation metabolism.

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2.1. In Health

The metabolism of FA in the mitochondrial matrix is sequentially catalyzed through a ß-oxidation process by four enzyme families: acyl-CoA dehydrogenase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase. While acyl-carnitine is converted back to acyl-CoA to enter the TCA cycle, dehydrogenases activity shows different affinity for short, medium, long and very long FA's [13]. Every cycle of ß-oxidation renders acyl-CoA and shortens the FA chain by 2 carbons providing the equivalents of electron donors NADH and $FADH_2$ which is the driving force for the synthesis of ATP. Although the ß-oxidation pathway is an effective way of ATP production, an overload of FA may play the role of un-coupler that exercises an inhibitory effect on the respiratory chain through a proton-phoric effect on the inner mitochondrial membrane. This effect results from the implicit effect of FA on mitochondrial membrane porosity by opening of the permeability transition pores, which results in the subsequent loss of electrical gradient and arrest of the respiratory chain [13]. The above concept favors the hypotheses of mitochondrial dysfunction from FA overload as the primary step of the insulin resistance state in obese patients. Other authors have found no mitochondrial respiratory changes in the steatotic liver but in mitochondria from skeletal muscle from a rodent model of high fat diet induced obesity [27]. Authors have proposed mitochondrial changes are due to an adaptation of the mitochondria to the high lipid charge rather than a defect per se in its function. No explanation was provided by the authors regarding the increase in ROS and inflammatory changes observed. Others suggested a protected effect from caloric lipid surplus against the development of metabolic dysfunction, as long as cells maintained functional adipocyte storage with low levels of tissue inflammation [28]. Once adipose accumulation saturates cell capacity, fatty excess spills over into other tissues leading to LD accumulation with subsequent lipid oxidation and an inflammatory response which precedes the metabolic syndrome manifestations [28]. The Delete in Breast Cancer-1 (DBC-1) protein is an important regulator of fat accumulation and storage in fat tissue which exercises its action by inhibition of SIRT1. In the DBC-/KO mice exposed to HFD, it was observed high plasma levels of FA with no liver steatosis, lower expression of senescence cells and increased storage of FA in the adipocytes with no development of insulin resistance [28]. Thus, DBC-1 protected liver and adipocytes from senescence by preservation of the fat compartment function with liver sparing and insulin sensitivity. Interestingly, a comparison has been made between this rodent model and the so called "healthy obese subjects" where there is fat accumulation but no signs of metabolic syndrome or systemic inflammation.

2.1.1. Metabolism and inflammation

Obesity depresses the anti-inflammatory effects of the heat shock proteins (HSP70) pathway, an inhibition that may contribute to the progression from NAFLD to NASH [29]. Excess of lipids and fuels trigger a low grade inflammatory response in both fat and liver tissues that correlates with the impaired insulin responsiveness. TBARS, a simple but fair estimate of lipoperoxidation/malondialdehyde (systemic oxidative stress) produced throughout the body was shown to be elevated in plasma from NASH patients when compared to normal subjects and its levels were correlated with insulin-resistance status [29]. The former response involved activation of the c-Jun NH2-terminal kinases (JNK's), endoplasmic reticulum (ER)

stress, unfolded proteins response (UPR), and the ceramide pathway by blocking nuclear factor κB (NF-κB) expression at different levels [29]. In liver tissue, HSP70 downregulates TNF-α and inducible nitric oxide synthase (NOS2), genes that increase the inflammatory response in rodents. In addition, HSP70 in humans induces apoptosis and increases the concentration of cyclopentenone prostaglandins, a potent local inhibitor of inflammation. In human liver and fat tissues, the suppression of HSP70 was strongly correlated with the upregulation of JNK1 and JNK2 [29]. The authors hypothesized that the senescence-like state in fat cells have evolved in obese individuals as an adaptation to the metabolic overutilization of fat cells, supporting the observation that hepatocyte senescence predicts NAFLD progression to NASH and to cirrhosis. Patients with different grades of ESLD from NASH or HCV had significantly decreased levels of glutathione reduced and increased levels of glutathione oxidized in plasma when compared to healthy controls [30]. Therefore, a continuous increase in the cell oxidative stress consumes the antioxidant protective mechanisms and increases the spillage of oxidative molecules. Increased oxidative compounds accumulation may induce a progressive larger number of liver cells into senescence which in turn will enlarge the SASP component worsening the inflammatory environment with further increase of stressors into the liver mieleu by triggering an activation of local and systemic immune-regulators (**Figure 3**).

In old mice, hyperglycemia increased chromatin remodeling and polyploidy levels; changes observed as well in non-obese diabetic mice [31]. Genes involved in glycemic control and metabolism are also involved in inflammation such as Ppargc1a (PGC-1α). It acts on the histone deacetylase SIRT1 as a metabolic sensor in hepatocytes and increases gene activation involved in the gluconeogenesis pathway [31]. Furthermore, PGC-1α also plays a role in lipid metabolism [31]. Through the thyroid receptor pathway, it induces the expression of Srb1 (Scavenger receptor B member 1), enhances the uptake of cholesterol esters from high density lipoproteins (HDL) in the liver and inhibits the expression of Srebp-1 (sterol regulatory element-binding transcription factor-1) down-regulating fatty acid synthesis [31]. As SASP builds up due to an increasing number of cells entering senescence, an increasing insulin resistance state starts to develop with its manifestation, hyperglycemia which further favors replicative cell arrest.

The fat compartment has emerged not only as an energy reservoir but as an endocrine organ capable of modulating metabolic states where the adiponectin/leptin ratio determines an anti or pro-inflammatory response. Leptin is a 167-aminoacid hormone expressed predominantly in adipocytes. Its signaling is an important determinant of food intake, adiposity and energy expenditure [32]. In the *ob*/*ob* mouse, a homozygous mutation in the gene that encodes leptin is associated with increased appetite, obesity and an insulin-resistant state. When leptin was provided to the ob/ob animal, there was a dramatic improvement in glucose homeostasis and energy metabolism. Although leptin related glucose homeostasis is largely conserved in rodents and humans, most subjects with insulin-resistant diabetes have a hyperleptinemic state with a central resistance to leptin [32]. Adiponectin is a protein hormone of 244 amino acids synthesized as a monomer of 28–30 kDa and assembled in various molecular weights: low, medium and high molecular weight (LMW, MMW and HMW) oligomers [33, 34]. HMW oligomers are the major relevant forms in terms of physiological activities of adiponectin while, low amounts of HMW oligomers represent an independent risk factor for several metabolic pathologies such as obesity-related diseases. Adiponectin plays a pivotal role in energy metabolism being an insulin-sensitizing hormone and it is involved in a wide variety of physiological cellular processes including inflammation, immunity and vascular

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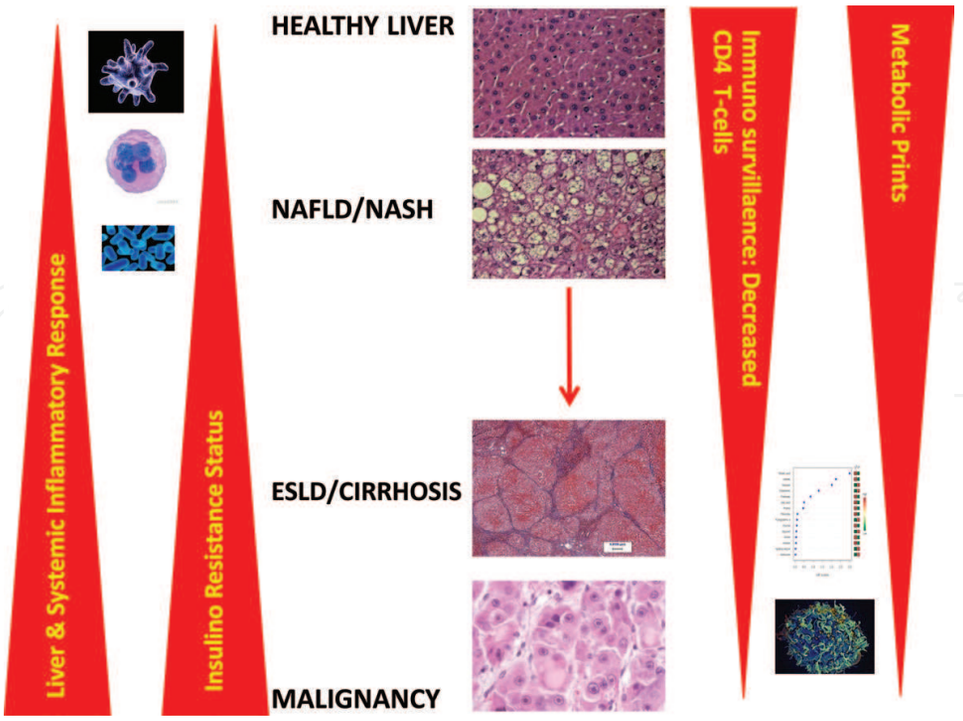

Figure 3. *Local and systemic responses that occur in the progression of NAFLD to NASH and ESLD associated HCC and their metabolomic print.* The liver local progression from NAFLD to NASH is associated with a local inflammatory response that eventually involves several other organs evolving into a systemic reaction. The systemic inflammatory response is associated with the development of an insulin resistance status and the metabolic syndrome phenotype: HTN, central obesity, DMType II and NASH. The continuous liver lipotoxicity decreases mitochondria function and decreases ATP production as well as enhances the secretion of lipid intermediates which are toxic to CD4 T lymphocytes. All together enhances a regenerative stimulus of senescence cells with mitochondrial dysfunction generating at some point a metabolic swap of ATP production to the cytosol, which may be associated with a mitochondrial generated apoptotic switch and in an environment of progressive fibrosis and therefore low oxygen and nutrients delivery favoring the survival of the already highly mutated cells which in turn have escaped physiological cell cycle control and immuno-recognition assuring cell clone growth. Metabolic disturbances precede variations in cell cycle and genetic expression creating metabolic signatures of liver status in health and disease.

physiology. Adiponectin acts through three major physiologically different and distinctly expressed receptors: AdipoR1, AdipoR2 and T-cadherin. Adipo receptors mediate pleiotropic adiponectin actions through signaling mechanisms involving AMPK, ERK1/2, AKT and P38. In addition, Polymerase I and transcription release factor (PTRF) regulates adipocyte differentiation, perhaps fat cell senescence and thus may determine fat compartment expandability, condition that under continuous HFD exposure increase the spill-over of FFA to the liver in combination to a pro-inflammatory adipokine repertoire [35].

2.1.2. Signaling of energy expenditure and metabolism

Rapamycin has an effect in cell life span with significant changes in the liver transcriptome, effects that are more pronounced when animals are exposed to caloric restriction [36, 37]. In a rodent model, rapamycin prevented senescent changes with significant differences by gender but with some common genetic pathways, mainly in the preservation of mitochondrial function. Those pathways included protein ubiquitination, NRF2-mediated oxidative stress response and glucocorticoid and OGF-1 signaling [36, 37]. Cell culture studies, indicated that treatment with rapamycin decreased mitochondrial membrane potential, decreased O_2

consumption, and increased ATP production [36, 37]. Other effects from transcriptome pathways include a decrease in proteasome activity in parallel with an increase in cell autophagy, suggesting protein quality improvement processes and increased resistance to oxidative cell stress effects associated with reduced cell aging.

A highly conserved signaling pathway in all eukaryotic cells is the Target of Rapamycin (TOR), which plays a central role as regulator of cell growth and metabolism. The mammalian TOR complex (mTOR) encompasses two structurally and distinct proteins. While the mTORC1 is associated with anabolic processes such as protein synthesis, lipid synthesis, nutrient uptake and inhibition of catabolic processes including autophagy, mTORC2 is an insensitive to rapamycin regulation protein. mTOR2 becomes activated by a family of kinases like the serum/glucocorticoid kinase (SGK) and protein kinase C (PKC). mTORC1 is upregulated by growth factors, cellular energy status and is inhibited by the macrolide rapamycin [36–38]. Protein synthesis is one of the most energy demanding cell functions; a favorable redox status activates mTORC1 which in turn exercises its actions down-stream through the ribosomal protein 6 kinase (S6K) and the eukaryotic translation initiation factor 4E binding protein (4R-BP) [36–38]. Due to the high demand of ATP, mitochondrial function regulation is of paramount importance on mTOR signaling pathways. Moreover, mitochondrial dysregulation and continuous mTOR activation may play a metabolic central role in the transformation and survival of cancer cells. Mutated cells with malignant potential may shift their bioenergetic state from ATP mitochondrial production to cytosol ATP production through the tricarboxylic acid (TCA) cycle. A connection between mTORC2 and mitochondrial function and cancer appears to be dependent through the HK2 pathway [38]. Nevertheless, recently mTORC2 has been linked to cytoskeleton regulation through the actin remodeling pathway, which has been suggested to have an effect on insulin sensitivity/resistance balance [36, 37]. Whole energy expenditure was affected by the mTORC1 signaling pathway, as demonstrated in the tissue specific knockout mice where a down-regulation of signaling pathways on adipose tissue also impacts on thermogenesis and systemic sensitivity to insulin [38]. In addition, mTORC2 signaling pathway, in the same animal model was a crucial regulator of liver and pancreas metabolism affecting animal growth and insulin homeostasis. mTORC 1 and 2 signaling in the liver affects systemic glucose and insulin homeostasis mainly due to their effects on Akt and hepatic glucose uptake. Interestingly, liver tumors in the tissue specific raptor knockout mice, showed a shift from glucose to glutamine as the main fuel source, making tumor cells glutamine addictive with high expression of mTORC1 and FGF-21. Rapamycin treatment may be beneficial as it may inhibit growth on glutamine addictive tumors. Some liver transplant programs switch their immunosuppression protocol from tacrolimus to rapamycin in patients with high risk for HCC recurrence after transplantation. However and on retrospective studies, its effect on long term overall survival on patients after liver transplantation for HCC, have had conflicting results [39, 40].

The functional relationship between poly-unsaturated lipid metabolism, inflammation and cancer development has been discussed in multiple avenues. Cyclooxygenases (COX's) and lipoxygenases (LOX's) are enzymatic families that metabolize poly-unsaturated fatty acids. COX is present in two isoforms (COX-1 and COX-2) that produce prostaglandins (PG's) and thromboxanes, respectively [41]. LOXs constitute a family of dioxygenases that insert O_2 into poly-unsaturated fatty acids with regional specificity [41]. These metabolites are biologically active hydroperoxyeicosatetraenoic acids that upon reduction forms hydro-eicosatetranoic

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acids (HETE's), while the metabolism of linoleic acids preferentially results in hydroxyl-octadecadienoic acids (HODE's), metabolites known to modulate inflammation and carcinogenesis [41]. An excess of poly-unsaturated fatty acids could enhance a higher production of HETE's and/or HODE's with an override of pathways that enhances cancer development. Hepatic COX-2 overexpression induces spontaneous HCC formation in vitro and in mice through Akt, SKT33 and mTOR signaling cascades [42]. In the healthy liver, the inhibitor of the prostaglandin degrading enzyme 15-PGDH potentiates liver regeneration after partial hepatectomy when compared to control and sham animals [43]. Thus, prostaglandin active derivatives have the potential not only to modulate local inflammatory responses but to promote cell regeneration in the healthy cell and potentially reversal of cell arrest in the senescent cell.

2.2. Metabolism in the liver graft

Evidence seems to indicate a peculiar aging pattern for liver grafts after transplantation. Biological age of the graft does not correspond to its behavior when transplanted to a different environment of a younger recipient [44]. One of the most important intracellular protease systems is represented by the proteasome, the central catalytic unit of the ubiquitin-proteasome system (UPS). No difference in the accumulation of oxidized proteins and polyubiquitin conjugates with maintenance of their proteolytic activity was found in liver grafts after transplantation from younger donors to older recipient when compared to liver grafts from older donors placed into younger recipients. Furthermore, there was an increase of the ß5i/α4 ratio, suggesting a shift towards proteasomes containing immune-subunits [44]. Thus, it appears older liver grafts transplanted in younger recipients switched their biological metabolism to resemble the recipient's metabolic age. However, the pattern of liver cell senescence may differ. Liver biopsies, as judged by the senescent markers telomerase and SMP-30 from older transplanted livers showed histological damage in asymptomatic patients with up to 43% and 64% at 5 and 10 years, respectively [45].

In the warm ischemic/reperfusion liver model, glycogen synthase kinase 3 (GSK-3) inhibition ameliorated liver injury upon reperfusion through an energy-dependent mitochondrial mechanism [46]. GSK-3 is a serine/threonine kinase regulated by inactivation through serine phosphorylation. GSK-3 inhibition down regulates the opening of mitochondrial permeability transition pore (MPTP) site, preventing leakage of mitochondrial respiratory chain proteins; a key step in the activation of caspase dependent apoptosis and therefore mitochondrial-dependent cell termination. This effect was present in young animals but abrogated in old animals, and a partial response was re-established in the older group by glucose infusion with hepatic glycogen build up storage [46]. Authors speculated that during reperfusion glycogen degradation provides mitochondrial fuel in forms of glutamate and α-ketoglutarate maintaining enough energy levels that preserve mitochondrial membrane integrity or mitohormesis lowering ROS production, factors needed to decrease MPTP susceptibility. Former approach in the human was entertained, where liver graft glycogen replenishment was performed during the donor phase and evaluated upon reperfusion [47, 48]. Metabolic benefit with improved organ graft function was observed only in borderline grafts and the ones with high fat content. Nevertheless, the concept of metabolic replenishment with further graft function improvement may be refined by strategies of ex-vivo euthermic graft perfusion prior implantation [49–56].

2.3. The chronically diseased liver

Hepatocyte senescence expression has been shown to be present in up to 80% of the cells in advanced liver disease [57]. The effects of insulin in the liver cell are mediated through two main cellular pathways: the phosphatidylinositol 3-kinase (PI3K)-Akt and the Ras-MAP kinase (MAPK) pathways. While both pathways are active in the regulation of cellular growth, proliferation and differentiation the PI3K-Akt mediates the metabolic actions of insulin. Those actions include activation of mTOR1 and its S6 kinase and the inactivation of glycogen synthase kinase-3 (GSK3) as well as its AS160 with nuclear exclusion of the Forkhead box protein (Fox01) [57]. In culture, HepG2 cell lines showed a signaling defect downstream of the Akt pathway with an impact upon insulin mediated Fox01 cytosol sequestration and AS160 phosphorylation; a cascade that translated into insulin resistance of older cells when compared to younger cells. Nevertheless, maintenance of the senescent state requires an active role in the transcriptional activity of Fox01 as cell cycle inhibitor, even in the presence of growth factors. Thus, it appears gluconeogenesis and insulin resistance are unwanted but unavoidable effects of Fox01 gene, which is involved in cell cycle arrest, detoxification of oxygen species, DNA repair and gluconeogenesis [57].

FA overload can damage the respiratory chain in the mitochondrion through a dual role: as an un-coupler and as an inhibitor [13]. Impairment of the key respiratory state 4 → 3 can occur via inhibition of ATP-synthase thereby producing an increase production of ROS irrespective of ADP concentration. The concept of redox-optimized ROS balance (R-ORB) postulates that ROS efflux from the mitochondrion will attain a minimum at intermediate values of oxidation, when VO_2 reaches a maximum following ADP stimulation. Under state 3 respiration, GSH and thioredoxin systems are essential for minimizing ROS release from the mitochondria [13]. Moreover, mitochondria from cells with chronic liver disease under oxidant challenge displayed a two-fold increase in H_2O_2 emission when compared to controls along with a 50% decrease in GSH [13]. Since 90% of GSH in plasma is excreted by the liver, glutathione sp. could serve as a surrogate of cell/mitochondrial stress and their ratio in plasma may reflect overall liver redox balance [24]. In animal models of liver malignancy, with or without cirrhosis glutathione sp. (glutathione reduced-GSH, glutathione oxidized-GSSG and ophthalmate) predicted the growth of malignant cells on normal livers as early as 14 days after malignant cells implantation and differentiated animals with cirrhosis by tumor status (HCC+ vs. HCC−) [58, 59]. Furthermore, glutathione sp. in plasma were part of the metabolic signature that discriminated healthy controls and subjects after liver transplantation with normal graft function from subjects with chronic liver disease (**Figure 3**). In addition, metabolic prints graded patient's degree of end stage liver disease which correlated with the MELD score, and they were able to separate patients with cirrhosis by tumor status, i.e. HCC+ vs. HCC− [30].

Others argued mitochondrial dysfunction by FA's respiratory chain uncoupling is incompatible with thermo-regulatory principles that governs mitochondrial respiratory chain through energy demand: intracellular lipids will accumulate whenever FA's supply exceeds the energy needs of the cell [13]. While TAG-LD in cells from a trained individual increases as the source of energy, in the diabetic obese subject TAG-LD are the result of accumulation with the subsequent potential overproduction of lipid derived toxins in the form of LCFA-CoA, diacylglycerides (DAG) and ceramide, metabolites responsible, at least in part for the development of insulin cell resistance [13]. The former theory is attractive in the heart and skeletal muscle. In contractile

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cells, optimal excitation-contraction coupling requires an optimal energy and O_2 supply which in turn affects the Ca^{2+} handling at the sarcoplasm reticulum (SR) release channels (ryanodine receptors), the SR Ca^{2+} pumps and the sarcolemmal Na^{+}/Ca^{++} exchanger. The heart at rest beats in average 100,000 times per day catalyzing about 6 kg of ATP to ADP. The mitochondrion provides the ATP needed for contraction (≈66%) and the ATP needed for ion transporting (≈33%) essential for the cardiac electrical activity. Thus, the link among lipid supply and mitochondrial function, insulin sensitivity/resistance and ion pump exchange is established for optimal cardiac function or dysfunction in the obese individual [13]. Perhaps, there is no argument lipid oxidation confers a metabolic advantage during starvation and exercise, but its role as the fuel of election during food abundance against metabolic disease deserves further studies.

In liver, cellular senescence is associated with a pro-fibrogenic environment and the relation between advanced liver fibrosis and shortening of the cell telomere appears to be consistent [11, 60]. Telomeres are repetitive DNA sequences (TTAGGG) associated with the specialized protein shelterin. They are located at the chromosomal end acting as a cap that stabilizes and protect the chromosome from erosion and miss-identification as DNA breaks. During normal cell division, telomeres shorten due to the "end replicating problem": the inability of DNA polymerases to fully replicate the 3′ end of chromosomes [61]. Germline cells overcome this problem by expressing telomerase, a reverse transcriptase that maintains telomere length by synthetizing new DNA sequences at the end of the chromosome [60]. The telomerase complex includes a reverse transcriptase (TERT) and the RNA component (TERC) [61]. In other somatic cells, continuous cell division results in telomere shortening which in turn start signaling cell arrest mechanisms, i.e. senescence or apoptosis. Failure of cell arrest signaling, as in a silence p53 state sparks further cell proliferation with chromosomal end-to-end fusions and instability. In addition, exhaustion of liver regenerative paths and invested mechanisms of telomere repair could be overcome under continuous and chronic cell injury with subsequent acceleration of cell senescence and aging. Some studies had shown that telomere biology is involved in HCC initiation and its progression [60]. Therefore, telomere shortening is a physiological marker of cell aging signaling and/or cell arrest preventing further cell division; failure of cell arrest may end in chromosomal instability and subsequent mutations favoring tumor development [11, 61]. In fact, the strength of the DNA damage response (DDR) in the normal cell depends ultimately to the degree of p53 gene regulation: a higher p53 response is associated with apoptosis, a lower response is associated with cell senescence and a silence p53 response may favor tumor development and growth [62]. In addition, a sirtuin (SIRT7) showed an in vivo hyperacetylation of p53 and the SIRT7 knockout mice suffered among other maladies steatotic liver disease. Sirtuins were initially identified in yeast as the Silent Information Regulator (SIR). In mammals, SIRT protein family comprises seven distinct members involved in cellular survival, senescence and tumorigenesis [62]. The SIRT7 knockout mice showed a 2.5 fold increase in the liver triglyceride content and an increased accumulation of hepatocyte inflammatory markers [62]. Findings that were associated with liver cells mitochondrial dysfunction through a deacetylate GABPß1 mitochondrial protein pathway and with the development of HCC through maintaining a deacetylated state of H3K18 at promoters sites of many tumor suppressor genes [62].

Lipodystrophic syndromes are rare and heterogeneous diseases, genetic or acquired, where partial atrophy is associated with a phenotype consistent with insulin-resistant diabetes, dyslipidemia and NAFLD. Although the genetic cause of these syndromes are largely

unknown, most of the monogenic diseases have in common primary alterations in the fat tissue consistent with disturbances of the adipogenesis process or defects in the formation, maintenance and/or regulation of the lipid droplet [63]. Acquired syndromes are seen mainly after HIV therapy with anti-retroviral agents as zidovudine and stavudine (tNRTI's). Agents known to render mitochondrial toxicity with metabolic disturbances similar to the metabolic syndrome seen in obesity. This metabolic adverse effects include premature aging associated with impaired prelamin-A maturation [63]. Lamin-A alterations could produce fragile nuclear envelopes, alter chromatin organization, increase oxidative stress and promote premature senescence at the cellular level. The metabolic disturbances observed in genetic or acquired lipodystrophic syndromes support the hypothesis of a primary fat compartment dysfunction as the source of metabolic disturbances, similar to the ones detected in obesity.

Chronic liver disease is associated with an increased translocation of intestinal bacteria contributory to the liver inflammatory response and may promote the development of HCC [12]. Liposaccharide (LPS) produced by Gram (−) bacteria hosted in intestines from obese humans and rodents was associated with the transition of NAFLD to NASH and consequently to its progression to cirrhosis and HCC. LPS is recognized by the Toll like receptor 4 (TLR4) which is expressed upon cell activation on migrating and local macrophages (Kupffer cells). TLR4 is central for the secretion of TNF-ß and IL-6, cytokines present in the chronic inflammatory environment that precedes the detection of malignancy [12]. Further support to the role of LPS was found by interventions such as gut sterilization, removal of LPS or inactivation of TLR4; maneuvers that diminished tumor growth in chronically injured livers [12]. In experimental models, dietary or genetic obesity alterations on the gut microbiota increased levels of metabolites like deoxycholic acid (DCA) that in turn damages DNA. The enterohepatic circulation may further enhance the concentration of such metabolites by both encouraging the senescent-associated secretory phenotype response and favoring a tumor-promoting environment.

3. Cellular senescence

3.1. Hepatocytes

Historical views of liver cell replication supports the physiological properties of the hepatocytes to restore function as response to parenchymal loss [64]. However, massive or unending injury may overcome regenerative processes or may promote a dysfunctional repair process leading to progressive liver fibrosis, development of portal hypertension and eventually liver failure. Senescent status was induced in HepG2 cells by exposure to H_2O_2. Its consequences and metabolic activity were interrogated [18] and morphological changes were noted with respect to SA-ß-GAL and SAF's expression, cell cycle arrest as well as the upregulation of p53, p21 and p16 genes. Regarding cytokine expression, IL-8 was upregulated while IL-6 was downregulated. Disturbances in glucose and lipid metabolism were evident with upregulation of growth hormone/IGF1 (SOCS2) and glycolysis (PGM2LT). Nonetheless, the downregulation of glucogenolysis and gluconeogenesis (G6PC) were more prominent. The unsaturation of fatty acids was hyperactive (FADS3) with parallel hypo activity of lipoprotein and hepatic lipase activity through the Apo-lipoprotein (APC3) system. APC3 also limits the uptake of chylomicrons by the liver

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cell. Other fatty acid downregulated proteins included SORL1 (involved in the uptake of LDL), ACSM2B (a medium-chain fatty-acid-CoA ligase) and PHGDH indirectly involved in amino-acid synthesis [18]. In addition, senescent cells secreted a variety of bioactive molecules including pro-inflammatory cytokines and chemokines that may influence extracellular matrix and the micro-environment but as well modulate the immune response with the promotion of macrophage migration leading to further increase in the inflammatory mielue [65]. Monocyte chemotactic protein (MCP-1) could provide a signal for monocyte recruitment into the liver followed by activation of Kupffer cells with the upregulation of death ligands. The expression of Fas ligand, TNF-α, and TNF-related apoptosis inducing ligand (TRAIL) further aggravates lipo-apoptosis [66]. In addition the FFA palmitate increases the expression of TRAIL and abrogation of the TRAIL receptor expression suppresses the inflammation induced by nutrient excess in mice [66].

Prior assumptions on cellular senescence determined that cell cycle arrest was a mechanism to protect the cell towards tumorigenesis. Nevertheless, it has been shown that the cell in cycle arrest can produce pro-inflammatory mediators, the senescence-associated secretory phenotype that promotes tumor growth [67]. During chronic liver disease, senescent machinery becomes "hijacked" perhaps triggering proliferation and transformation of hepatocytes, thus, promoting metabolic adaptation which may enhance tumor grafting and growth [68, 69]. The above metabolic paths could at least in part, be mediated by the over expression of the phosphatase and tensin homolog (PTEN) described in T-leukemia but later shown in liver tumors to inhibit the pentose phosphatase pathway (PPP) by binding to glucose-6-phophodiesterase (G6PD). With no active G6DP dimer, cells favor glycolysis with the production of lactate even in the presence of oxygen [70].

Aging and senescent liver cells have different genetic paths that may converge to similar metabolic traits. Aging liver cells have a proliferative response after injury associated with the repression of C/EBPα, Farnesoid X Receptor (FXR), telomere reverse transcriptase (TERT), and a decrease in the Wnt signaling pathway [71, 72]. A physiological Wnt signaling pathway involves a soluble ligand that binds to the Frizzled receptor (Fzd) and the LRP5/6 co-receptor on the plasma membrane; this interaction activates the cytoplasmic Disheveled protein which inhibits the ß-catenin (Ctnnb1) destruction complex (APC, GSK3ß, and Axin) by preventing Ctnnb1 phosphorylation and its subsequent destruction. Stable ß-catenin (intact Wnt signaling) translocates to the nucleus to form a complex with Lef and Tcf transcription factors that target genes as c-Myc and Cyclin D1. In cell culture and a mice model of HCC, tumor growth was ablated by the suppression of N-Myc downregulated gene 1 (NDRG1) expression; it promoted HCC cells to go into cell arrest [73]. The induction of senescence on malignant cells was accomplished by upregulation of the tumor suppressor genes p53, p21 and p16 in addition to decreased phosphorylated Rb. Senescent liver cells response to injury included transcription of Nf-kB, Myb, Nkx2-1, Nr5a2 and Ep300 factors; proteins known to be involved in inflammation, cell differentiation, lipid metabolism and chromatin remodeling. In addition, the chronic inflammatory phenotype of senescent cells induces telomere dysfunction and accelerates liver cell aging [74]. Thus, decreased physiological cell signaling that occurs with aging plus stress induced cell senescence may add to the lipid toxic microenvironment by promoting a vicious circle that overrules redundant mechanisms that prevent uncontrolled cell division. Mechanisms that imply an apoptosis "switch" from a pro-apoptotic to an anti-apoptotic status. Nonetheless, it is no clear the role of mitochondrial Bcl-2 proteins family and their expression may determine cellular fate [75].

Cellular events that follows are the activation/repression of factors involved in cell proliferation. In the liver cell, the known transcriptional shift includes activation of FOXO3, FOXII, E2F1, c-jun, C/EBPß, Myb, USF and neutralization of inhibitors of cell proliferation such as Rb family and C/EBP family of proteins [76]. In C/EBP-S193A mice, failure to stop liver regeneration after surgery correlated with the epigenetic repression of C/EBPß, p53, FXR, SIRT1, PGC1α and TERT. The repression was performed by a protein formed by C/EBPß-HDCAC1 complex which also inhibit the promoters of enzymes for glucose synthesis PEPCK and G6P [76]. The response of cell cycle engaged hepatocytes and cell cycle arrested hepatocytes (senescent cell) to injury is different and it may awake an unregulated cell growth on quiescent stem liver cells [76, 77]. Oval shaped liver cells may differentiate into cholangiocytes with a distinct metabolism and perhaps pathway towards malignancy [15, 16]. Although cholangiocytes are metabolically very active cells involved in the secretion and resorption of water and soluble bile components, they are not directly involved in the metabolism and/or regulation of biliary lipid species (cholesterol, bile acids and phosphatidyl-choline vesicles) [78, 79].

3.2. Hepatic stellate cells (HSC) & portal myofibroblasts (MF)

HSC are quiescent cells that express typical markers of both neural cells and adipocytes (glial fibrillary acid protein-GFAP, peroxisome proliferator-activated receptor gamma-PPAϫ, and adiponectin receptors). They are activated by cytokines, growth factors, ROS, damaged cells and apoptotic bodies [64]. In health, MF are located adjacent to bile duct epithelia and are the first responder to biliary injuries. Upon activation HSC's acquire a MF phenotype, cells that upon phagocytosis of LD and/or apoptotic bodies from damaged cells get additional energy and became Fas-ligand and TNF-α unresponsive to apoptosis; mechanism in use for increase collagen synthesis and deposition [64]. Furthermore, activation of the adenosine receptor A_{2A} increases HSC proliferation and inhibits death and senescence by down regulation of p53 and Rb through the cAMP-PKA/Rac1/p38 MAPK pathway [80]. Activated MF's express CCN1/CYR61, an important regulator of inflammation and wound healing. Cystein-rich 61-protein (CCN1/CYR61) is a matrix-cellular protein that induces senescence at later stages of wound healing by promoting tissue remodeling through fibrogenic cell apoptosis and attenuation of TGF-ß signaling [81]. HSC and MF senescent fibrogenic cells no longer proliferate, thereby reducing the load of ECM deposition. In addition, senescent fibrogenic cells express an increase in the secretion of metalloproteinases (MTP's) leading to matrix degradation. Apoptotic fragments from HSC and MF are cleared by natural killer cells promoting wound healing, the best characterized mechanism of fibrogenesis resolution [64, 81]. NF-κB is a key regulator for HSC survival and proliferation by maintaining the expression of Mcl-2. Inhibition of NF-κB increases HSC apoptosis by up-regulation of the JNK pathway. Thus, the activation as well as the induction of senescence/apoptosis of HSC/MF are normal wound healing mechanisms that promote the establishment of normal organ architecture and function with clear paths of initiation and resolution.

During chronic cell injury, such as in a state of high caloric intake enriched with lipids, an increase and progressive pool of biologically active HSC's may become prominent [11]. An incremental chronic state of fibrogenesis alters hepatic architecture leading to a concomitant increase in portal flow resistance, portal hypertension and the development of collateral circulation. In addition, HSC's produce a microenvironment with altered extracellular matrix (ECM) that provides biochemical and mechanical cues to the growth and establishment of tumor cells [67]. Nevertheless,

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since 90% of the HCC's flourish in a highly progressive fibrotic ECM, the question raises if it is the changes on the microenvironment that further promotes metabolic transformation with an "apoptotic switch" and tumor development. Interestingly, progressive liver fibrogenic ECM becomes enriched with vascular growth factor (VGF) receptor promoting angiogenesis, paving the way for the much needed arterial high O_2 supply for HCC expansion [67].

The different components of the ECM, cellular and non-cellular interact directly and indirectly with malignant cells therefore changing the phenotype of the evolving cells that in turn produces feedback signals to further adapt the microenvironment to the needs of the malignant cell. The link between the actin cytoskeleton and the microenvironment provides an input of intracellular contractile forces capable of regulating signaling pathways fundamental to the definition of cell phenotype, mechanism that constitute the ECM "out-side-in" code to the cell. In response, the anchored cells expressed adhesions molecules and secreted proteins that signals HSC and other ECM regulators increasing anchoring sites in response to the "in-side-out" signaling [67]. Therefore, the metabolic transformation of the already stressed parenchymal cells help to choose a path different to senescence and necrosis but to a path of unregulated regeneration, thus escaping apoptosis. A path that needs an ECM differentiation to assure cell survival in a non-efficient energy redox status.

3.3. Sinusoidal endothelial cells (SEC)

SEC's are specialized endothelial cells that lie flat in the liver sinusoids along and in direct contact with the hepatocytes. Through their membranes and specialized pores or fenestra passes high concentrations of metabolites, proteins and other blood compounds, traffic which is regulated by the size of the fenestra. SEC's play a critical role in immune-activation, rolling of T cells, macrophages and PMN migration. Liver sinusoidal endothelial cells may be affected with age and obesity. SEC from old individuals have impaired and reduced expression of VEGF likely due to impaired nuclear transport of P-STST3 and P-CREB transcription factors [82, 83]. In a rodent model of sepsis, endothelial nitrogen oxide synthase (eNOS) deficient mice and aging mice had the same mortality and mitochondrial dysfunction upon the isolation of SEC mitochondrion [84]. In obesity and during early fibrogenesis, SEC lose their fenestra, decreasing the exchange of metabolites and increase the secretion of several basement membrane components (type IV collagen, perlecan, entactin and laminin) [64]. Authors concluded that an endothelial base-line dysfunction in the aging animal is manifested by a weakened antioxidant response and inappropriate energy production from mitochondrial dysfunction due to a tipped-balance of the SEC oxi-redox systems when exposed to additional stress. This is seen in the obese towards a state of energy depletion and cellular death, apoptosis or activation of a pro-coagulant/pro-fibrogenic phase. The changes of SEC's with aging may limit O_2 delivery and availability to liver cells with its potential effects on mitochondrial function, a pro-fibrogenesis state and the promotion of insulin resistance status. Changes exaggerated in obesity, implying obesity may promote accelerated SEC aging processes. Interestingly, endothelial cellular senescence was inhibited in vitro and in the rodent by the activation of the liver x receptor (LXR), a nuclear receptor involved in the control of hepatic lipid and cholesterol metabolism [85]. Furthermore, LXR has been shown to play an important role in glucose metabolism, cytokine production and anti-inflammatory response.

Three types of SEC's co-exist in the normal liver sinusoid: mature SEC, SEC progenitors and bone marrow-derived SEC progenitors [86]. Mature SEC are gatekeepers of fibrogenesis by maintaining HSC in their inactivated state. SEC's regulate sinusoidal blood flow through their action on HSC and thus keep a low portal pressure [86]. In addition, mature SEC's have the largest endocytic capacity in the body fulfilling their dual cell clearance capacity (from the arterial/systemic and portal/gut systems). The liver endocytic function has been implicated in a liver-renal axis where the lack of SEC-stabilin-2 receptors inhibit the clearance of toxic molecules that manifest with mild liver fibrosis without liver dysfunction but with renal glomerular fibrosis. Not only do SEC's have many glycoproteins that serve as receptors for bacterial epitopes but as receptors for immune-modulation and pro-coagulant activity. The above mentioned SEC functions are at least partially lost at the time of sinusoid capillarization [86]. SEC capillarization is characterized by the disappearance of the fenestrae, development of a basement membrane and the appearance of characteristic markers. This phenomenon happens in chronic liver injury and it precedes activation of HSC and sequestration of macrophages. The angiogenesis process that follows is mediated by VEGF, an angiocrine response that drives neo-vessel formation in direct proportion to the degree of the sinusoidal pressure gradient. Furthermore, SEC pseudo-capillarization refers to changes that occur in endothelial cells associated with aging and senescence. It is manifested by a decrease of up to 50% of their fenestrae, development of a patchy basement membrane and partial SEC dysfunction [86]. Chronic exposure of high fat diet may accelerate aging/senescence of SEC, endothelial dysfunction with recruitment of systemic immune cells and activation of Kupffer cells inducing HSC into a fibrogenic state followed by an angiocrine response that decreases hepatic blood flow, O_2 delivery, and clearance of toxic molecules. As metabolic stress of neighbor hepatic cells already in mitochondrial distress due to fat accumulation progresses, a constant and growing inflammatory mielue enhances tumor development, immune-recognition failure and malignant cell expansion.

Interestingly, aging endothelial cells from the fat compartment of mice was associated with adipose dysfunction manifested by ectopic (liver) fat deposition and adipose tissue fibrosis, increased adipose mitochondrial oxygen flux, altered lipid utilization, increased tissue oxidative stress and lower gene expression in visceral fat [87]. Nevertheless, and most important, these findings were associated with reduce fat tissue vascularity, reduced angiogenic capacity and endothelial dependent dilation with reduced nitric oxide (NO) bioavailability [87]. Limited oxygen mitochondrial availability contributes to the pro-oxidative older adipose tissue phenotype that can further impair both insulin action and vascular function, a key element in local and systemic insulin-resistant related metabolic syndromes. Changes that are exaggerated in obesity, implying obesity may promote accelerated aging processes in many organs.

3.4. Resident liver immunocells

The anatomical location of the liver and its dual blood supply ensures an optimal exposure of antigens to the hepatic resident immune cells not only from nutrients and GI microbiota but from systemic compartments, such as the adipose compartment. Kupffer cells in concert with NK, CD4+ T-cells, and local antigen presenting cells modulates the liver immune status. Kupffer cells constitutes 80% of the tissue fixed macrophages and 20% of the non-parenchymal cell population of a normal liver [9]. Their characteristic macrophage activity is polarized mainly in portal tracts where the antigen dynamics is higher from food and bacteria. Innate macrophages have the potential to initiate an inflammatory response of different proportions by upregulating

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adhesion molecules such as ICAM-1, and cytokines as TNF-α, IL-1, IL-6, MIP1α, TGF-ß and RANTES. Activation that can only lead to antigen presenting, cell to cell communication and amplification and enrichment of the microenvironment with ROS promoting subsequent parenchymal cell apoptosis/necrosis. Natural Killer (NK) and CD8+ T cells developed a specific signature in livers with NASH from mice under HFD [88]. The depletion of CD8+ T cells protected murine from NASH progression but not from weight gain. In addition, NK T-cells in the liver expresses markers that recognize lipid antigen CD1d [9]. Liver NK cells undergo Thymus clonal double deletion but are positive for CD3 and CD56 and they were thought to be CD1d independent. Nevertheless, hepatic antigen-presenting cells may introduce microbial glycolipid antigens to NK cells, stimulating secretion of Th1 or Th2 cytokines which subsequently initiates an adaptive response. Hepatic NK cells have as well the ability to secrete osteopontin and sonic hedgehog, molecules known to promote the transition from NAFLD to NASH [9].

The most accepted hypothesis, continuous cell parenchymal damage and necrosis adds to a chronic inflammatory environment a dysregulation of the cell cycle regenerative process rendering tandem mutations and thus malignant cells was challenged [89]. On the NEMO knockout mouse, authors were able to develop HCC through a death receptor-independent FADD signaling pathway. Nevertheless, it wasn't until recently that the link between a metabolic hostile microenvironment, immune-recognition failure and HCC presence was established [90]. The enrichment of linoleic acid in the cirrhotic microenvironment of NASH patients promotes disruption of mitochondrial function in a greater proportion than other fatty acids as palmitic acid. Since CD4(+) T lymphocytes have a larger mitochondrial load than CD8(+) T lymphocytes, they not only generate more mitochondrial derived ROS but CD4(+) cells may undergo larger selective loss of mitochondrial function and viability. Therefore, disruption of mitochondrial function by linoleic acid mediates selective loss of intrahepatic CD4(+) T lymphocytes, status associated with HCC presence. Local metabolic changes could alter the immune response to a one that favors malignant cell expansion.

In the obesogenic environment, aberrant activation of immune cells has emerged as key features of the metabolic syndrome. The interaction between the adipose compartment and the liver tissue has been hypothesized as a critical interface for nutrient sensing and metabolic control [9]. In the rodent model, neutrophils infiltrate the adipose compartment as early as 3 days after starting a high fat diet, however its role as well as the role of basophils and eosinophil cells has not yet been clarified. Mast cells, which has been observed in increasing number have been implicated in the secretion of IL-6 and IFN-γ [9]. Moreover, leptin, a hormone secreted specifically by adipocytes has been found to be increased during high fat diets and upregulated the expression of leptin receptors on NK T-cells. This regulation is time sensitive, and chronic leptin stimulation change NK cells from an inflammatory like response to a damped one, favoring at long term, in the liver and perhaps in other organs a susceptibility to low recognition of no self-cells, impaired anti-tumor surveillance and a flourishing nest of cancer. The former hypothesis finds support in the obese mice, where it was observed a switch from the normal Th1 immunoresponse to the Th17 immunorepertoire, phenotype that deteriorates autoimmunity [9].

3.5. Extracellular matrix (ECM)

The extracellular matrix (ECM) is formed by a non-cellular component in tissues and organs composed primarily of water, proteins and proteoglycans. Components created an intricate

scaffold where organ cells get structural support with a dynamic and continuous traffic of water, ions, metabolites, proteins and cells on passant to maintain organ physiology. As such, ECM interactions with organ cell components regulate cell differentiation, adhesion, proliferation, migration and survival [64]. The collagen family is the major fibrillar proteins of the ECM and the body (approx. 30% of the total protein contain) [64]. There are three main classes of collagen, fibril-forming which include types I, II, III, XI, XXIV and XXVII the most common varieties and their role is mainly mechanical by conferring tensile strength to both tissue and organs. Fibril-associated collagens with interrupted triple helix (FACIT's) includes type IX, XII, XIV, XIX, XX, XXI and XXII; this subclass of proteins do not form fibrils themselves but bind to the surface of pre-existing collagen favoring fibril enlargement. Finally, type III collagen serves as anchoring collagen between the epithelial cells and the lamina reticularis constituting the basement membrane where type IV collagen is most abundant. Non-collagenous proteins include fibronectin, tenascin, laminins, fibrillins and matrix-cellular proteins. While the former peptides play a major role in cell differentiation, cell growth, adhesion and migration, matrix-cellular proteins, i.e. thrombospondin-1 and 2, osteonectin, osteopontin and cyr-61/connective tissue growth factor (CTGF) serve mainly as a vehicle for cell signaling. Proteoglycans are carbohydrate enriched proteins which retain large quantities of water regulating the smooth trafficking of molecules to and from the cell with numerous signaling active sites for growth factors.

The ECM continuous remodeling is a complex process that integrates proteins and cellular components from local and distal environments [64]. The degradation of ECM proteins are closely controlled by matrix metalloproteinases (MMP's), a superfamily of zinc-dependent endopeptidases highly regulated by specific inhibitors such as the tissue inhibitor of metalloproteinases (TIMP's). In the liver, cellular component involved in collagen synthesis and deposition included HSC, MF and vascular smooth muscle cells [64]. In chronic liver injury, an override mechanism of collagen deposition regulation promotes massive ECM expansion. The characteristic features of abnormal liver fibrogenesis as a consequence of continuous liver injury and activation of collagen secreting cells include damage to the epithelial/endothelial barrier, recruitment of inflammatory cells, secretion of cytokines and other inflammatory mediators, further generation of ROS, progressive deposition of collagen with expansion of ECM and worsening organ fibrosis and subsequent metabolic changes of portal hypertension.

4. Mitochondrial senescence

The mitochondria, a double membrane cell organelle varies in number and its presence is linearly associated with the metabolic activity of the organ and its required energy requirements in form of ATP. Within the mitochondrial matrix a series of biochemical reactions occur. Acetyl-choline primer is reduced through the tricarboxylic acid cycle converting glycolysis-derived pyruvate into NADH and succinate. The former compounds couple another set of reactions at the inner membrane border where the electron transport chain (ETC) is present to boil an oxidative phosphorylation process. The ETC is composed of five enzymatic complexes (I to V; NADH-CoQ, succinate-CoQ, CoQ-cytochrome reductases, cytochrome c oxidase and ATP synthase, respectively) where NADH is the substrate of ETC-C1 and succinate the substrate of ETC-CII [10]. After oxidation, electrons are transferred from Complex I to CII to CIII and finally to Complex

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IV where oxygen is reduced to form H_2O. The electron transport process is coupled to a proton pumping process creating a proton gradient between the mitochondrial membranes, gradient that is dissipated by Complex V (ATP synthase) through ATP synthesis. A control mechanism is created by the "proton leak", mechanism that generates heat instead of ATP [10]. Much of the leak is a catalytic reaction generated by the uncoupling proteins (UCP's) which play an important role in reducing proton gradient, heat and ROS [10]. Mitochondrial aging and senescence are linked to reduced ATP production and increase ROS production, i.e. superoxide (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) which are mostly produced because electron leakage at the level of CI and CIII [17]. Mitochondria function benefit from the role of estrogen in plasma through its binding to the nuclear estrogen receptor that enhances a signaling to prevent oxidant stress and also inhibits the renin-angiotensin-aldosterone system [10]. Thus, sex differences in mitochondrial function may explain the disparity in overall survival between men and women, differences that may be taken into account during animal models studies.

The reasons why the mitochondrion conserves a cell independent genome are not clear, but it is intuitive to imply self-energy regulatory processes are united through a fine tune mechanism between energy expenditure (ATP use) and energy production (ATP synthesis) at every organelle level. It may provide an overall advantage for survival of the cell, the organ and entire biological living system. The gradual ROS response theory of aging argues a protective role of ROS in early life, when cell oxidative damage and ROS production are low; however, later in life ROS reaches a level where its beneficial effects (as the one observed in dietary restriction and/or exercise) are overcome by its detrimental effects elicited by a higher cell oxidative stress (as the one observed in high fat diet and sedentary habits) [17]. The effects that are amplified include loss of genomic controls (p53), microRNA dysregulation, loss of function of telomerase reverse transcriptase (TERT) and a lower immune-surveillance status. Although the role of p53 in the mitochondrion is not completely clear, p53 binds to the Peroxisome proliferator-activated receptor Gamma-Coactivator 1 alpha and Beta (PGC-1α and ß) fomenting their inhibition of expression and therefore downregulated oxidative function. In addition, p53 target p16 and p21, factors that triggers G1-phase cycle arrest by inhibiting cell cycle regulatory kinases Cdk4 and Cdk2 [17]. The third known effect of p53 at the mitochondrion level is to promote cell apoptosis by increasing mitochondrial membrane permeability with leakage of cytochrome proteins, a direct activator of the caspase cascade. The function of TERT is highly affected by levels of ROS production and its protective patterns are only observed with low ROS levels. The role of microRNA in the mitochondrial environment remains to be elucidated.

The Mitochondrial Free Radicals Theory of Aging (MFRTA) has been the most popular theory to explain the cell aging process where increasing production of mitochondrial ROS with lower ATP production are the main factors responsible for cell aging and corresponding mitochondrial ultrastructure changes [17, 91]. As mentioned, leakage of electrons at the level of CI and CIII transfer are larger with age and the higher potential for DNA damage. 8-oxo-7.8-dihydro-2'deoxyguanosine (8-oxodG) is one of the most abundant DNA mutations caused by oxidative conversion to guanosine. Furthermore, its accumulation follows an inverse and exponential curve against life expectancy in several mammals [17]. Recently, it was described that humans with longer longevity have a higher content of mitochondrial DNA (mtDNA) per cell in different organs, and support the notion of ethnic background on mtDNA influence and life span. The frequency of mtDNA mutations occurs at different rate depending on the organ. Skeletal and

cardiac muscles, liver and kidney are more affected by somatic mtDNA mutations compared to other organs such as the skin and lung [17]. Furthermore, the clonal expansion of mtDNA mutations occurs via a phenomenon called genetic drift, a random propagation and expansion of DNA mutations occurring at each DNA replication. The drift of mutations may be more important in metabolically more active organs that require more energy expenditure and therefore more ATP synthesis. The expansion of mtDNA mutations may be enhanced not only by its duplication and drift but also by a lower state of DNA damage repair mechanisms [17]. The Base Excision Repair (BER) process is impaired in senescence and aging due to a loss of function to BER associated proteins CSA and CSB. Thus, the increase production of ROS creates a vicious loop of mtDNA mutations than in turn favor an increase production of ROS perpetuating and enhanced organelle dysfunction by defective reparative mechanisms. A naturally occurring thymidine to cytidine mutation in the mitochondrial stressors $tRNA^{ILE}$ gene is associated with phenotypes of hypertension, hypercholesterolemia and hypomagnesemia [10]. Furthermore, the DNA A3243G mutation causes impaired insulin secretion and polymorphisms in the promoter of the UCP2 protein, alterations associated with increased incidence of obesity, reduced insulin secretion and DMII [10].

Mitochondrial function may be impaired in chronic high fat diet challenge as a result of a decrease in ß-lipid oxidation. Indirect evidence showed an accumulation of diacylglycerol and fatty-Acyl-CoA which in turn activates stress-related serine/threonine kinase activity and inhibit glucose transport [10]. Oxidative stress contributes further to impaired insulin signaling increasing UCP2 activity which in turn enhances "proton leak" with uncoupling of the glucose metabolism pathway and decreased ATP production. A progressive higher lipid peroxidation may favor further oxidative stress with DNA damage and low DNA repair by affecting members of the Bcl-2 family, triggering an influx of Ca^{2+} with subsequent opening of the mitochondrial permeability transition pore, cytochrome-c leakage to the cytosol and activation of the caspace-3 complex. Cell self-digestion and nuclear DNA fragmentation overcomes with the typical cell fragments morphology [10]. Alternatively, DNA damage and telomerase shortening results in mutations that may affect mitochondrial function to a level of organelle survival but inefficient ATP production assuring the "apoptosis switch" and diverting biochemical reactions to a cytosolic site for ATP production. The later assumption may find some support in the observations that tumor development and early growth is favored in low O_2 delivery zones and that tumor development is associate with increase lactate production, the Warburg effect [30, 69].

5. Future directions

Prevention of metabolic syndrome and its health consequences is primordial. A healthy diet that is balanced not only in calories but also in its components, specially fats and carbohydrates would avoid fat storage spillage from a saturated fat body compartment. In addition, a substantial use of lean mass through directed exercise will decrease further cell, organ and body aging. In the brain, the melacortin forms a network of neural food sensing connecting signals of metabolic rate with neurological sites that regulates food intake behaviors and energy expenditure homeostasis. Central administration of α-MSH reduces food intake and may also increase energy expenditure resulting in weight loss [92]. Metabolic disturbances in the liver renders liver cell changes that progress from NAFLD to NASH to cirrhosis and malignancy. A non-invasive

plasma based monitoring of such changes on disease progression and treatment response as well as for tumor screening may be possible by metabolomic liver prints in the near future [30].

There have been a myriad or reports on compounds that not only prevent but reverse cell aging and some even malignant development in the animal model [25, 93–102]. Curcumin, the major bioactive compound of turmeric spice, through its antioxidant and anti-inflammatory properties has been claimed to retard tumorigenesis and diabetes and to modulate lipid metabolism [103]. Furthermore, curcumin prevents the development of atherosclerosis and NASH, perhaps by the upregulation of a fatty acid binding protein present in adipocytes (aP2) but also found in macrophages (FABP-4). This protein is a cytosolic protein present in adipocytes and macrophages which modulates the trafficking of lipids/cholesterol processes and activation of inflammatory mechanisms through CD36 upregulation and reduced expression of NF-kß thus, decreasing cytokine secretion [103]. Prior studies showed that high fat diet and obesity promoted liver tumorigenesis by inducing chronic inflammation through the IL6/STAT3 pathway and, STAT3 activated tumors has been showed to be more aggressive in humans. Lycopene attenuated HCC occurrences in the animal model through downregulation of the STAT3 signaling [95]. The aqueous extract of *Ligustrum lucidum* fruit induced apoptosis through the activation of the caspase cascade and cellular senescence by upregulation of p21 and downregulation of RB phosphorylation [102].

Other molecules with promising cell aging and tumor repression properties included the COX-2 and a Na/K/ATP signaling mechanisms. Inhibition of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), a prostaglandin-degrading enzyme, potentiates tissue regeneration in multiple organs in mice [43]. During a chemical screen, a low molecular compound was identified capable of selectively inhibiting 15-PGDH with the subsequent increase of PGE2 levels in bone marrow and other organs, accelerating hematopoietic recovery in mice receiving bone marrow transplant and tissue regeneration in the colon and liver. It also promoted tissue regeneration in mouse models of colon and liver injury. Selective COX-2 products may have rescued telomere dysfunction, cell senescence and tissue regenerative potential [74]. However, its mechanism and signal transduction remains to be determined. pNaKtide is a synthetic peptide that conserves the active sequence for the ligand-binding capacity to the ß-subunit of the transmembrane Na/K-ATPase. Although the Na/K-ATPase mainly exercise its function as an ion exchanger pump vital for cell survival, recently it was shown to elicit nuclear signaling that regulates mitochondrial function and cell energy production through a Src/ERK pathway [104–112]. Furthermore, pNaKtide prevents the development of atherosclerosis and fatty liver disease in the HFD mice model with significant amelioration of ROS. In addition, it downregulates collagen synthesis and inhibit growth of human cancer cells in vitro. Translation of promising compounds to the treatment of patients with NAFLD/NASH is expected in the near future to further prevent the consequences of advanced liver fibrosis and HCC development.

Abbreviations

4R-BP	Factor 4E binding protein
ADP	Adenosine diphosphate
AGPAT	sn-1-acyl-glycerol-3-phosphatase acyltransferase

ATP	Adenosine triphosphate
CACT	Carnitine-acylcarnitine transferase
DBC-1	Delete in Breast Cancer-1
DGAT	sn-1,2-diacylglycerol acyltransferase
DM	Diabetes mellitus
ECM	Extracellular matrix
ER	Endoplasmic reticulum
FA	Fatty acids
Fox01	Fork head box protein
GBD	Global Burden of Disease
GPAT	Glycerol-3-phosphatase acetyltransferases
GSH	Reduced glutathione
GSK-3	Glycogen synthase kinase 3
HBV	Hepatitis B virus infection
HCV	Hepatitis C virus infection
HSC	Hepatic stellate cells
HSP70	Heat shock proteins
HTN	Hypertension
IL-1ß	Interleukin-1 beta
IL-6	Interleukin-6
INF-α	Interferon alpha
JNK's	c-Jan NH2-terminal kinases
LD	Lipid droplet
MAPK	Ras-MAP kinase
MF	Portal myofibroblast
MMP	Matrix metalloproteinases
MPTP	Mitochondrial permeability transition pore
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic fatty steatohepatitis
NF-κB	Nuclear factor κB
NO	Nitric oxide
PAP	Phosphatidic acid phosphatase

PI3K	Phosphatidylinositol 3-kinase
PKC	Protein kinase C
ROS	Radical oxygen species
SA-ß-GAL	ß-galactosidase
SAH	Senescence associated heterochromatic foci
SASP	Senescence associated secretary phenotype
SEC	Sinusoidal endothelial cells
SGK	Serum/glucocorticoid kinase
SMase	Neural Smase
TAG	Triacylglycerol
TCA	Tricarboxylic cycle
TIMP	Tissue inhibitor of metalloproteinases
TNF-α	Tumor necrosis alpha
TOR	Target of Rapamycin
UPR	Unfolded proteins response

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