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Review Article

Implication of SIRT1 on Development of Nonalcoholic Fatty Liver Disease and Impact of Carotenoid Intervention

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Abstract

Cellular senescence is a biological process that drives age-related disease development, including age-related nonalcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma (HCC). Although the growth arrest associated with senescence can prevent malignant transformation, senescence also alters the cellular microenvironment through a senescence-associated secretory phenotype (SASP), resulting in increased inflammation, disease progression, and tumorigenesis over time.

Sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase, controls many signaling pathways involved in senescence, aging, and tumorigenesis. The loss of SIRT1 activity has been shown to promote NAFLD by mobilizing free fatty acids to the liver from adipose tissue in mice. The contribution of hepatocyte senescence to NAFLD and HCC has been reported; however, whether diminished SIRT1 activity drives adipocyte senescence, facilitates lipolysis, and promotes NAFLD/HCC is currently unclear. Studies have shown that feeding tomato powder (a source of lycopene) or lycopenoids (lycopene and its metabolite, lycopenoic acid) increases SIRT1 levels in the liver and adipose tissue, preventing NAFLD and HCC. It is unknown whether loss of SIRT1 activity promotes senescence-driven lipolysis/SASP in liver and adipose tissue or if carotenoid consumption limits this senescence, thereby preventing NAFLD and HCC.

This review proposes that 1) lack of SIRT1 activity induces senescence and increases lipolysis/SASP in hepatocyte senescence and adipose tissue, the latter increasing movement of fatty acids to the liver and promoting hepatocyte senescence, NAFLD and HCC; and 2) dietary carotenoids increase SIRT1 activity and regulate hepatocyte senescence, adipocyte senescence, and lipolysis, and thus prevent NAFLD and HCC development.

ABBREVIATIONS

α-SMA: Alpha-smooth muscle actin; Akt: Protein kinase B; AMPK: AMP-activated protein kinase; AST: aspartate aminotransferase; ATM: Ataxia telangiectasia mutated; ATR: Ataxia telangiectasia and Rad3-related protein; BCO1: Beta-carotene-15,15'-oxygenase; BCO2: Beta-carotene-9',10'-oxygenase; CDK: Cyclin-dependent kinase; CDKI: Cyclin-dependent kinase inhibitor; CHK1: Checkpoint kinase 1; CHK2: Checkpoint kinase 2; CIP/KIP: Cyclin-dependent kinase interacting protein/kinase inhibitory protein; CXCL1: Chemokine (C-X-C motif) ligand 1; DDR: DNA damage response; DEN: Diethylnitrosamine; dsDNA: Double-stranded DNA; E2F: E2 transcription factor; ECM: Extracellular matrix; FOXO: Forkhead box O; GATA4: GATA binding protein 4; HCC: Hepatocellular carcinoma; HSC: Hepatic stellate cell; IL: interleukin; IHH: Immortalized human hepatocyte; INK4: Inhibitors of CDK4; LKB1: Liver kinase B1; MAPK: mitogen-activated protein kinase; MCP-1: Monocyte chemoattractant protein-1; mTOR: Mammalian target of rapamycin; NAD: Nicotinamide adenine dinucleotide; NAFL: Nonalcoholic fatty liver; NAFLD: Nonalcoholic fatty liver disease; NAM: Nicotinamide; NAMPT: Nicotinamide phosphoribosyltransferase; NASH: Nonalcoholic steatohepatitis; NF-κB: Nuclear factor kappa B; NKK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator-1 alpha; PPARα: Peroxisome proliferator-activated receptor alpha; Rb: Retinoblastoma protein; ROS: Reactive oxygen species; SA-β-gal: Senescence-associated beta-galactosidase; SASP: Senescence-associated secretory phenotype; SIRT1: Sirtuin 1; TAF: Telomere-associated DNA damage foci; TNF-α: Tumor necrosis factor-alpha

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is rapidly becoming an increasing global public health problem [1,2], as it is the primary cause of chronic liver disease and affects approximately 25% of adults worldwide [1,3]. NAFLD represents chronic, progressive liver disease encompassing a range of liver conditions with the presence of ≥ 5% steatosis in the absence of alcohol consumption [1,4-6]. NAFLD is mainly manifested as nonalcoholic fatty liver (NAFL), which is characterized hepatically by steatosis only, without inflammation or hepatic ballooning [1-3]. Although NAFL is mostly a benign condition, with a relatively low risk of developing into hepatocellular carcinoma (HCC) (an annual incidence of 0.08-0.63 per 1,000 person-years) [4,7], it can progress to the more dangerous subtype of NAFLD known as nonalcoholic steatohepatitis (NASH), with approximately 7-30% of NAFL case progressing to NASH [2,8]. Along with hepatic steatosis, NASH has the presence of inflammation and hepatocyte ballooning, which is hepatocyte injury [1], and has a prevalence between 12-14% [1]. It is worth noting that the terms NAFL and NAFLD are often used interchangeably, as NAFL encompasses NAFLD's basic definition, whereas NASH has additional disease features. NASH can progress to NASH with hepatic fibrosis (30-37% of NASH) [9,10], then cirrhosis (10-20% of NASH or 4-14% of all NAFLD) [8-10], and lead to a higher risk of developing HCC [11], with an HCC annual incidence of 5.29 per 1,000 person-years [4]. Notably, NASH is becoming one of the main causes of HCC, since there are now vaccinations and improved treatments for the leading causes of HCC, hepatitis B and C viruses, and the prevalence of NASH is increasing alongside obesity, type 2 diabetes, and metabolic syndrome [7,12]. For HCC patients in the US, approximately 30-40% has NAFLD [13]. Globally, HCC is the most common type of primary liver cancer [14,15] and is the second most common cause of cancer-related deaths behind lung cancer [15]. Although treatments for tumor resection, liver transplant, and locoregional therapies are potentially curative treatments for early to intermediate stage HCC, most HCC cases are not diagnosed until late stage [16].

Considerable evidence supports the initial pathogenesis of NAFLD being mainly due to lipid accumulation and insulin resistance. Although hepatic fat accumulation alone is not necessarily detrimental to the liver, the hepatocytes can become overwhelmed by energy substrates in lipid metabolism, which can lead to lipotoxicity and be influenced by genetic susceptibility, obesity, and diet [2,8,12]. Therefore, conditions like obesity, with excess dietary intake, can negatively affect NAFLD progression. Similarly, insulin resistance, associated with conditions like metabolic syndrome and type 2 diabetes, indirectly promotes NAFLD by increasing fat accumulation in the liver. The suppression of lipolysis, which is dependent on insulin, is reduced in the adipose tissue, thereby increasing free fatty acids in blood circulation [2,17]. Hepatocytes take in the excess free fatty acids and store them as triglycerides and lipid droplets. Insulin resistance also directly contributes to NAFLD because the high insulin level promotes de novo lipogenesis [2,17]. Consequently, NAFLD is strongly associated with obesity and diabetes, and metabolic syndrome is the greatest risk factor for NAFLD [12]. Studies have reported that of people with type 2 diabetes, approximately 55-70% have NAFLD [1,18] and 30-40% have NASH [1,4,18]. Similarly, of those who are obese, more than 80% have NAFLD [2] and 25-30% has NASH [1]. Although a considerable part of the global adult population is affected by NAFLD, there is still relatively little mechanistic research looking to explore its causation [3]. Currently, there are no FDA-approved drugs for the treatment of NAFLD, and no standard therapy exists [1,12,19].

Cellular senescence, a state of permanent cell cycle arrest, contributes to many progressive disease types. Research now shows, particularly in hepatocyte senescence, that senescence is not only associated with fat accumulation and inflammation in NAFLD stages [20], but also initiates the development NAFLD [2,21]. As more research points to cell senescence as the driver of NAFLD development and progression, the exact mechanism has not been well-defined. Specifically, sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase, plays a vital role in multiple activating and inhibitory cell senescence pathways [22-24]. It is also affected by several triggers of cell senescence, particularly oxidative stress [22]. Diminished levels of SIRT1, brought on by factors such as age, obesity, and diabetes, may lead to hepatic and adipocytic cell senescence and the development and progression of NAFLD.

Carotenoids, a class of natural bioactive pigments, have garnered attention across different medical fields for their antioxidative properties and beneficial role in preventing certain cancers and other pathological conditions. Specifically, researchers have taken an interest in the non-provitamin A carotenoid, lycopene, as evidence points to its benefits in NAFLD [25-28] and other conditions [29]. Lycopene's anti-inflammatory and antioxidant properties may have the potential to target specific aspects of cellular senescence, which in turn may treat or prevent NAFLD and HCC.

This review aims to assess how diminished SIRT1 levels can lead to cell senescence, which in turn promotes NAFLD and can progress into liver cancer. Additionally, the possibility of molecular targeting SIRT1 for therapeutic intervention and the potential for carotenoid intervention to increase SIRT1 levels and play a vital role in diminishing senescent cells and preventing or reversing NAFLD progression will be discussed.

CELLULAR SENESCENCE

Cellular senescence is characterized as a state of permanent cell cycle arrest that is marked by the cell's inability to divide and proliferate, yet still has metabolic activity [2,8]. Various factors are associated with triggering cell senescence, including DNA damage, telomere shortening, oncogene expression, reactive oxygen species (ROS), and other factors that lead to cell injury, including genotoxic stress and inflammatory cytokines [2,8,30]. This review will focus on ROS, the major trigger of hepatocytic senescence (Figure 1) [8,21,31,32]. The central source of ROS is the mitochondria, and with increased production of ROS,

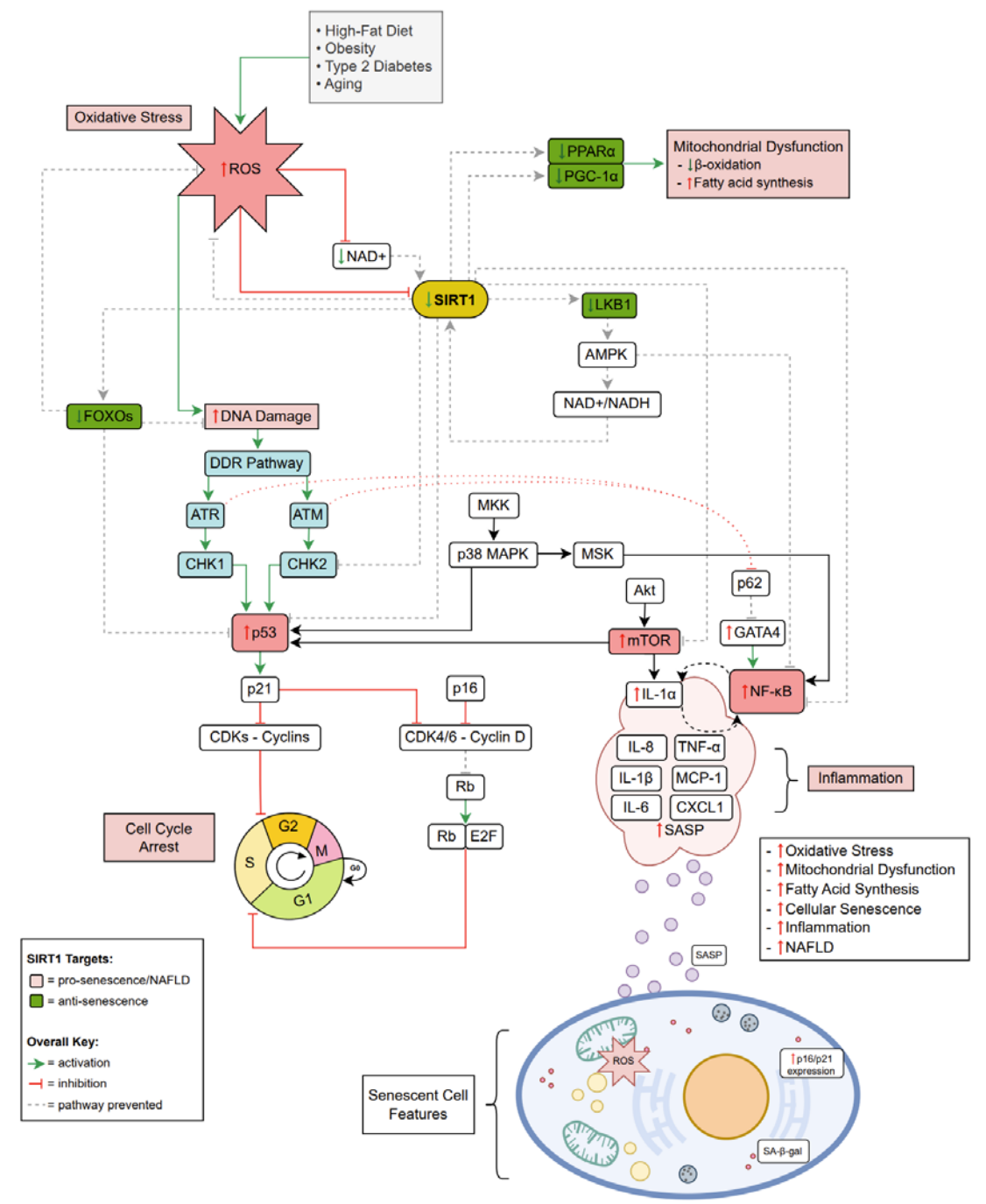

Figure 1 Proposed pathway of diminished sirtuin 1 (SIRT1) expression and activity leading to cellular senescence. Green lines with arrowheads represent activation/enhancement. Red lines with perpendicular heads represent inhibition/suppression. Grey dotted lines represent pathways prevented. Green up/down arrows next to labels represent factors related to anti-senescence that are increasing/decreasing. Red up/down arrows next to labels represent factors related to pro-senescence that are increasing/decreasing.

Abbreviations: Akt: Protein kinase B; AMPK: AMP-activated protein kinase; ATM: Ataxia telangiectasia mutated; ATR: Ataxia telangiectasia and Rad3-related protein; CDK: Cyclin-dependent kinase; CHK1: Checkpoint kinase 1; CHK2: Checkpoint kinase 2; CXCL1: Chemokine (C-X-C motif) ligand 1; DDR: DNA damage response; E2F: E2 transcription factor; FOXO: Forkhead box O; G0: Resting phase (of the Cell Cycle); G1: Gap 1 (of the Cell Cycle); G2: Gap 2 (of the Cell Cycle); GATA4: GATA binding protein 4; IL: interleukin; LKB1: Liver kinase B1; M: Mitosis (of the Cell Cycle); MAPK: mitogen-activated protein kinase; MCP-1: Monocyte chemoattractant protein-1; mTOR: Mammalian target of rapamycin; NAD: Nicotinamide adenine dinucleotide; NAFLD: Nonalcoholic fatty liver disease; NAMPT: Nicotinamide phosphoribosyltransferase; NF-κB: Nuclear factor kappa B; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator-1 alpha; PPARα: Peroxisome proliferator-activated receptor alpha; Rb: Retinoblastoma protein; ROS: Reactive oxygen species; S: Synthesis (of the Cell Cycle); SA-β-gal: Senescence-associated beta-galactosidase; SASP: Senescence-associated secretory phenotype; SIRT1: Sirtuin 1; TNF-α: Tumor necrosis factor-alpha.

oxidative stress and mitochondrial dysfunction can ensue. These triggers induce the DNA damage response (DDR) pathway, which can stop the cell cycle, with persistent DDR pathway activation establishing senescence in the cell [21]. The DDR pathway starts with the formation of a complex of DDR factors. Within the complex are the serine/threonine protein kinases, which are important for activating the p53 pathway, ataxia telangiectasia mutated (ATM), activated primarily upon persistent double-stranded DNA (dsDNA) damage [33], and ataxia telangiectasia and Rad3-related protein (ATR), activated by a broad spectrum of DNA damage, including dsDNA breaks and single-stranded DNA gaps [32,33]. ATM's downstream target, checkpoint kinase 2 (CHK2), becomes phosphorylated and activated, then CHK2 activates p53. Similarly, activated ATR can phosphorylate checkpoint kinase 1 (CHK1), which also activates p53 [8,32,34]. Activated p53 is one of the key players in transforming normal cells into senescent cells [32]. Once p53 is activated, it can then activate p21, a member of the cyclin-dependent kinase (CDK) interacting protein/kinase inhibitory protein (CIP/KIP) CDK inhibitor (CDKI) family. It is the main inhibitor of the cyclin E (protein required for CDK activation) and CDK2 complex [32]; however, p21 can inhibit all CDKs and their respective cyclins and lead to the G1/S phase cell cycle arrest [20]. Another important CDKI and player in cell senescence is p16, a member of the inhibitors of CDK4 (INK4) CDKI family [32,35]. This protein can inhibit both CDK4 and CDK6, which are bound to cyclin D. Inhibiting CDK4/CDK6 inhibits the phosphorylation of the retinoblastoma protein (Rb), allowing the active dephosphorylated Rb to remain bound to the E2 transcription factor (E2F) preventing cell cycle progression from the G1 to S phase [21,32]. There are key indicators that a cell is senescent, including high expression of the CDKIs p16 or p21, active senescence-associated beta-galactosidase (SA-β-gal), which is characterized by having enzymatic activity at a different pH than more common isoforms and express senescence-associated secretory phenotype (SASP) [2,8,34]. SASP reflects the secretory activity of senescent cells that contributes to pro-inflammatory processes and stimulates the immune system. The secretion includes a variety of factors, like cytokines, chemokines, proteases, and growth factors [36,37]. Some common ones present in hepatic senescence are interleukin (IL) 1, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-alpha (TNF-α) [2,32,35]. The paracrine functions of SASP depend on the upstream factors of persistent DDR signaling, including ATM/ATR and CHK2/CHK1. Additionally, this phenotype depends on factors like the protein complex nuclear factor kappa B (NF-κB), a major inducer of SASP that facilitates cell senescence, initiates and maintains SASP, and promotes inflammation [2,32]. Furthermore, the regulation of SASP has been strongly linked to factors from various response pathways, namely p38 mitogen-activated protein kinase (MAPK), mammalian target of rapamycin (mTOR), and GATA binding protein 4 (GATA4) [2].

The transcription factor p38 MAPK aids in the regulation of NF-κB expression [2,19] and can induce ROS production and further modulate p53-mediated cell cycle arrest in human hepatocytes when at increased levels [38]. Additionally, mTOR can increase the cell surface-bound levels of IL-1α for IL-1α to induce and maintain SASP through its feedback loop with NF-κB [39], which stimulates gene transcription for certain inflammatory cytokines, such as IL-6 and IL-8 [40]. Furthermore, when ATM and ATR kinases are activated in the DDR pathway, the degradation of GATA4 by selective autophagy-mediated through p62 is suppressed, and GATA4 is able to activate NF-κB. Although ATM and ATR kinases are associated with the DDR pathway, the GATA4 pathway is independent of p53 [41]. Importantly, as senescence continues, SASP components affect neighboring cells and cause them to be senescent as well and can promote chronic inflammation and tissue dysfunction [8].

Cellular Senescence in the Development of NAFLD

Recently, evidence has pointed to cell senescence having a causal role in the development of NAFLD versus just an association or putative inducer [2,8,21,31]. Ogrodnik et al. showed that hepatocyte senescence leads to fat accumulation and the elimination of senescent hepatocytes reduces hepatic steatosis in mice [21]. One of the ways they demonstrated that eliminating senescent cells decreases hepatic fat accumulation was by looking at INK-ATTAC mice. These mice were genetically altered to have specific elimination of p16-expressing senescent cells when induced. The mice were fed either normal chow or a high-fat diet, and the high-fat diet mice displayed increased senescent markers in hepatocytes, including p16 mRNA expression and SA-β-gal activity. The mice were then administered a small molecule that dimerizes a relevant fusion protein, FKBP-CASP8, and induces the elimination of p16-expressing cells, which resulted in a significant reduction of all analyzed senescent markers and reduced liver fat deposition. Next, the researchers demonstrated that hepatocyte-specific senescence stimulates hepatic fat accumulation by studying mice that had induced hepatic senescence via X-ray irradiation. They found that senescent hepatocytes had increased fat droplet intensity compared to non-senescence hepatocytes [21]. Furthermore, they wanted to determine if impaired fatty acid β-oxidation lead to fat accumulation. As previously mentioned, cell senescence and increased ROS can lead to mitochondrial dysfunction [2,8,17]. With mitochondria capacity decreased, there will be impaired fatty acid β-oxidation hindering fatty acid elimination and leading to increased fat accumulation in the liver [2]. By demonstrating that senescent hepatocytes had decreased cellular oxygen consumption compared to control hepatocytes, Ogrodnik et al. showed that hepatic-specific senescence led to fat accumulation in the liver via decreased fatty acid β-oxidation [21].

Similarly, Bonnet et al. injected senescence-inducing drugs nutlin-3a (an activator of p53), etoposide (a DNA damaging agent), and doxorubicin (another DNA damaging agent) into two different human hepatocyte cell lines, the immortalized human hepatocyte (IHH), a hepatocyte cell line with close resemblance to primary hepatocytes, and HepG2, a human liver carcinoma cell line [42]. They found that the drugs induced the common senescence markers for both human cell lines, including p16,

p21, p53, and SA-β-gal, and these were associated with hepatic steatosis development. Interestingly, they found that the cell senescence increased insulin-stimulated phosphorylation of the insulin receptor, protein kinase B (Akt), and p38 MAPK. Phosphorylation of the insulin receptor can lead to the activation of Akt and/or p38 MAPK pathways [43], which can lead to the activation of transcription factor mTOR and promotion of NF-κB and/or p53 and NF-κB activation, respectively. Activation of these pathways suggests the stimulation of NF-κB and, therefore SASP [42]. Overall, Bonnet et al. demonstrated inducing hepatocyte senescence promotes hepatic fat accumulation and has a causal role in NAFLD development.

Adipocytic Senescence and Hepatic Fat Accumulation: Although hepatocyte senescence is critical for NAFLD development, adipocyte senescence must also be considered. Similar to hepatocyte senescence, factors such as aging, a high-fatty diet, and obesity lead to increased senescence in adipocytes. However, for reasons still not fully understood, adipose tissue seems to be affected first by senescence compared to other organs and tissues [44]. For senescent adipocytes, like senescent hepatocytes, hypoxia-induced over-secretion of cytokines and chemokines will lead to inflammation. Pro-inflammatory factors, such as IL-6, TNF-α, IL-1β and chemokines, will accumulate in the adipose tissue and eventually secrete into the circulation, impacting the inflammatory pathways of the liver and triggering inflammatory cell accumulation [2,45]. Donnelly et al. found that peripheral fats stored in the adipose tissue can contribute to NAFLD by flowing into the liver through the plasma non-esterified fatty acid (NEFA) pool [46]. They discovered that in human subjects, 59.0% ± 9.9% of hepatic triglycerides originate from the NEFA pool in the plasma. Therefore, dysregulation of adipocyte lipolysis may contribute to the progression of NAFLD. Triglyceride accumulation makes up the majority of fat accumulation in the hepatocyte, which is necessary for NAFLD development [46]. Furthermore, Duval et al. found that mice exhibiting NASH showed adipose tissue dysfunction. They discovered a tight relationship between adipose tissue dysfunction and NASH pathogenesis, as opposed to mice that did not develop NASH [47]. Additionally, as mentioned in the introduction, NAFLD is strongly associated with obesity, with more than 80% of obese people having NAFLD [2], and type 2 diabetes, with approximately 55-70% of people with type 2 diabetes [1,18]. With the connection between NAFLD and obesity in mind, increased fat mass directly leads to greater fatty acid release from adipose tissue and dysfunction [47,48]. Similarly, peripheral insulin resistance, associated with type 2 diabetes, in people with NAFLD leads to adipose tissue releasing fatty acids at increased levels [48]. Further, Jaeger et al. examined mice with a defect in adipose tissue-derived fatty acid supply to the liver to determine the role of adipose tissue-derived fatty acids in regulating hepatic gene expression. They found that adipose tissue-derived fatty acids are a primary regulator of hepatic fasting gene expression, which is necessary for the liver process of controlling hepatic triglyceride breakdown [49]. Therefore, suppressing or inhibiting adipocyte cell senescence mediating lipolysis could be protective against hepatic steatosis.

Cellular Senescence and NAFLD Progression: NAFLD progression from fatty liver is further driven by inflammation and fibrosis. With consistent ROS and SASP, pro-inflammatory markers will draw in macrophages, neutrophils, and other immune cells, leading to inflammation [2,8,17,19]. Also, steatosis with NAFLD makes the liver more susceptible to injury, including hepatocyte ballooning, through lipid peroxidation-induced oxidative stress and inflammatory response, promoting the transition into NASH [26]. Continuous inflammation will induce the activation and proliferation of hepatic stellate cells (HSCs), the main contributor to extracellular matrix (ECM) formation, and can lead to NASH with fibrosis (Figure 2) [8,50].

Ogrodnik et al. found a correlation between hepatocyte senescence and NAFLD severity [21]. They examined liver biopsies of human patients with simple steatosis (NAFL) or steatosis with non-specific inflammation and steatohepatitis (NASH). They evaluated telomere-associated DNA damage foci (TAF) and p21 as senescent markers. They chose TAF as a marker based on the evidence that decreased telomere length and increased DNA damage are associated with steatosis grade. Along with confirming that TAF and p21 are significantly increased in patients with high liver fat content compared to controls, they discovered that the percentage of TAF-positive and p21-positive hepatocytes correlated with NAFLD activity score and that TAF and p21 were positively related with steatosis grade [21]. However, they also found the expression of p21 to be restricted to hepatocytes and not found in other liver cell types, like HSCs. Perhaps that indicates that other cell types are active, for example, macrophages or endothelial cells.

However, cell senescence becomes more complicated as it can be pathogenic, but it can also lead to a resolution in certain cells and stages of disease. It is protective against certain cancers and diseases with mutating potential as it prevents mutated or malignant cells from proliferating and progressing [8,32]. This is important to consider when assessing the role of cell senescence and the cell type and location affected by senescence in NAFLD progression to NASH, NASH with fibrosis, cirrhosis, and HCC. Evidence shows hepatocyte senescence leads to NAFLD development and is associated with NAFLD severity and fibrosis progression [21,51]. However, cell senescence in other liver cell types, like HSCs, may be protective and help resolve disease severity. For instance, HSC senescence can help favor their immune clearance, assuming normal immune response and resolution of fibrosis [30,52]. Likewise, senescent HSCs produce less ECM parts than activated HSCs, helping prevent fibrosis progression. On the other hand, Yoshimoto et al. demonstrated in mice that gut deoxycholic acid (DCA), a microbial metabolite known to cause DNA damage through ROS production and a liver cancer promoter increases SASP in HSCs [53].

Wijayasiri et al. looked at the effect of hepatocyte senescence on HSC activation in liver fibrosis progression. They assessed human liver specimens from patients with NAFLD and fibrosis at different stages and cultured primary human HSCs in cell-free conditioned media that had been exposed to either senescent or control human liver HepG2 cells [51]. The NAFLD patient biopsies

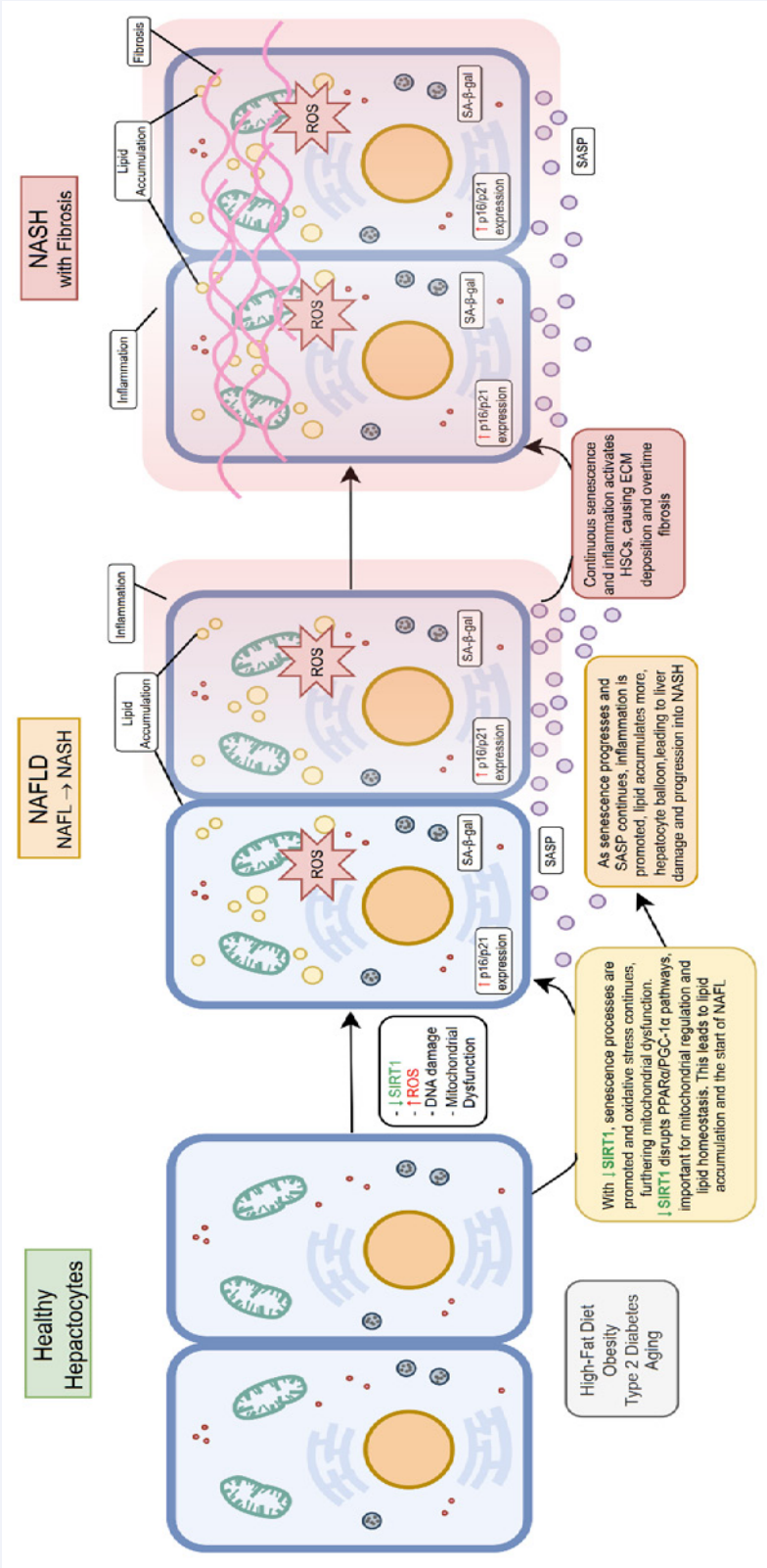

Figure 2 Proposed development and pathogenesis of nonalcoholic fatty liver disease (NAFLD).
Abbreviations: ECM: Extracellular matrix; HSC: Hepatic stellate cell; NAFL: Nonalcoholic fatty liver; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator-1 alpha; PPARα: Peroxisome proliferator-activated receptor alpha; ROS: Reactive oxygen species; SA-β-gal: Senescence-associated beta-galactosidase; SIRT1: Sirtuin 1.

showed a significant positive correlation between p16 expression in hepatocytes and alpha-smooth muscle actin (α-SMA), the most reliable marker of HSC activation. Additionally, they discovered a significant positive correlation between the fibrosis stage and p16 expression in hepatocytes. For HSCs placed in media derived from HepG2 cells, they found gene expression changes taking place. Specifically, genes involved in inflammation, including TNF-α and IL-1β, and fibrogenesis, including α-SMA and pro-collagen, were significantly upregulated compared to control HepG2 cells, suggesting HSC activation and implying that SASP influenced the HSCs. They found a strong association between the proportion of senescent hepatocytes and HSC activation, along with each of these factors having a close association with the fibrosis stage individually [51]. Therefore, cell senescence is a double agent.

Cellular Senescence and HCC

There is mixed research on the progression of NAFLD/NASH to HCC. However, a balance may exist between whether hepatic senescence is protective against HCC or a trigger for HCC. As a protective mechanism, inducing senescence in HSCs at pre-fibrotic stages may be able to prevent hepatic fibrosis, a strong HCC risk factor [31]. Additionally, the SASP phenotype of senescent hepatocytes may recruit immune cells to remove senescent cells and prevent carcinogenesis as well as prevent further growth of malignant hepatocytes [20]. With the proper immune response, macrophages and other immune cells can respond to and clear premalignant senescent hepatocytes and suppress cancer development [36], a phenomenon known as "senescent surveillance" [37]. It seems that whether the effects are tumor-suppressive or tumorigenic depends on the stages of the tumor. For normal and precancerous tissue, SASP functions mainly as tumor-suppressive, leading to autocrine and paracrine senescent effects and immunosurveillance. For cancerous tissues, however, the same SASP factors increase cancer cells' tumorigenicity rather than inducing senescence in cancer cells. Furthermore, those premalignant senescent hepatocytes will develop into HCC. Likewise, Yoshimoto et al. discussed in the previous section, found that the DCA in mice promotes SASP phenotype in HSCs, leading to the release of pro-inflammatory and tumor factors and developing HCC after carcinogen exposure [53].

Regarding cell senescence's role in promoting NAFLD into HCC, Yilmaz et al. showed that hepatic senescence in fatty liver may be vital in transforming NAFLD into HCC [45]. Specifically, replicative senescence of hepatic steatotic cells leads to chronic hepatic injury. Additionally, they examined adipose tissue's role in NAFLD and its contribution to the transformation into HCC. Certain adipokines, hormones produced by adipose tissue, including adiponectin and leptin, have been shown to be involved in the development of insulin resistance, inflammation, and NAFLD, as well as the associated risk of HCC. Wang et al. indicated adipokines may be directly involved in HCC development, promoting tumor growth, invasiveness, and angiogenesis, suggesting that when inflammatory cells like macrophages are active in adipose tissue, they will work with adipokines to lead a cycle of macrophage recruitment, cytokine production, and inflammation [54]. Interestingly, Saxena et al. found an inverse relationship between adiponectin expression and human HCC tumor size [55]. Adiponectin increased phosphorylation of AMP-activated protein kinase (AMPK) and inhibited phosphorylation of mTOR; suggesting that they are activating a prime inhibitor of cell senescence and a key player with SIRT1 and inhibiting a main activator of cell senescence.

SIRT1

SIRT1 is a nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase that plays a key role as a post-translational regulator in modulating oxidative stress, mitochondrial function, inflammation, and lipid homeostasis [22-24]. It has been studied in many different diseases due to its significant role in oxidative regulation and several metabolic pathways. As a result, SIRT1 has been proposed as a potential therapeutic target for diseases affecting the lungs, kidneys, heart, brain, and liver [56]. Like how cell senescence becomes a hallmark of aging, SIRT1 levels also decrease with age [57]. Scheuermann et al. revealed that SIRT1 expression in old rat liver grafts was not upregulated in response to reperfusion and cold storage compared to the liver grafts from young rats [57]. They found that the diminished SIRT1 levels in the old liver grafts were associated with weakened mitochondrial function in the form of reduced tissue ATP, antioxidant defense, and metabolic function [57]. This may have implications when looking at cell senescence.

The downstream targets of SIRT1 are diverse, and key ones are involved in important determinants of cell senescence, including the DDR pathway and SASP (Figure 1). Research has shown that SIRT1 suppresses tumor suppressor p53 activity through deacetylation, which results in the downstream inhibition of the DDR pathway and cell senescence, including inhibition of CDKI p21 [22,44]. It also indirectly inhibits p53 by inactivating mTOR, which can regulate p53 activation and induce cell senescence outside the DDR pathway [58]. SIRT1 can both directly [19,23,59] and indirectly [23,39] inhibit NF-κB, the main controller of SASP. It can indirectly inhibit NF-κB by activating its downstream target AMPK, which is an important inhibitor of NF-κB [23], and by suppressing mTOR [39,58]. In addition to promoting p53 translation, mTOR also promotes IL-1α translation, and IL-1α in turn, promotes NF-κB transcriptional activity [39].

Not only does SIRT1 inhibit active players of cell senescence, but it is also involved in pathways that counter cell senescence as well. These include activating peroxisome proliferator-activated receptor alpha (PPARα) and its coactivator, PPAR gamma coactivator-1 alpha (PGC-1α), fork head box O (FOXO) proteins, and liver kinase B1 (LKB1). PPARα and PGC-1α regulate mitochondrial functions, like β-oxidation and fatty acid synthesis, and maintain mitochondrial homeostasis [19,22,60]. FOXO proteins work to regulate cell senescence by reducing ROS levels and DNA damage [22,61]. Lastly, LKB1 activates AMPK, which regulates lipid metabolism [62]. Additionally, AMPK is a critical

energy metabolism sensor for more than lipid metabolism. It has been shown to link metabolic disease and cancer by regulating the cell cycle, cell senescence, autophagy, and apoptosis in cancer advancement [63,64]. The active center of SIRT1 is in the binding region of NAD+ and NADH, the balance of these being the primary determinant of a cell's redox state. When the NAD+/NADH ratio is disrupted, SIRT1 can regulate proteins through deacetylation. Further investigation is needed to determine how diminished SIRT1 deacetylase activity leads to cell senescence and NAFLD and how overexpression may help prevent cell senescence and disease.

Diminished SIRT1 Activity, Cellular Senescence, and NAFLD

There are few studies investigating whether the decreased SIRT1 activity induces cell senescence while looking at the liver, especially regarding the development of NAFLD and its progression. In other disease models, diminished SIRT1 has been shown to drive cell senescence. For instance, Chen et al. demonstrated that in vascular smooth muscle, cell-specific knockout of SIRT1 increased vascular cell senescence, upregulated p21 expression, and increased vascular inflammation [65]. However, with current research, our understanding of cell senescence, and knowledge about SIRT1, it is unclear how diminished SIRT1 levels may lead to hepatic and adipocytic senescence and lead to the development and progression of NAFLD, which needs further investigation.

In general, SIRT1 is downregulated in NAFLD patients [66]. One study induced hepatic steatosis using tamoxifen (TAM), a drug that inhibits SIRT1. Their results revealed that SIRT1-FOXO1 suppression permitted lipid synthetase activation and led to hepatic steatosis development [67]. Another study looking at the absence of SIRT1 analyzed liver-specific SIRT1 knockout mice that were fed a standard diet. These mice presented with decreased glucose production, insulin sensitivity, and increased hepatic cholesterol and free fatty acid [68]. Likewise, Purushotham et al. looked at the hepatocytes of mice with a specific SIRT1 gene deletion, which resulted in a damaged PPARα/PGC-1α pathway causing decreased fatty acid β-oxidation and resulting in the development of hepatic steatosis, endoplasmic reticulum stress, and inflammation [60]. Although metabolic dysregulation is strongly associated with cell senescence, the impact of adipocyte senescence on NAFLD is not well-defined. Cheng et al. used a systemic SIRT1 activity ablation mouse model ($SIRT1^{y/y}$ mice) to reveal that the loss of SIRT1 activity promotes NAFLD through mobilizing free fatty acids to the liver from the adipose tissue; however, whether this is related to adipocyte senescence is unclear [69]. Likewise, SIRT1 had a role in adipocytic cell senescence by directly reducing the expression of p53 and p21 [61]. Furthermore, mice studies lacking SIRT1 found macrophages in the liver and adipose tissues to be highly activated, promoting insulin resistance and metabolic syndrome, both highly relevant to NAFLD [70]. Decreased SIRT1 expression in the liver, due to factors such as aging [22] and a high-fatty diet [71], could lead to decreased oxidative metabolism, allowing for increased ROS, then oxidative stress and inhibition of SIRT1. It could also result in the damage of SIRT1 DNA and affect SIRT1's protective activity. Without SIRT1 activity, ROS will continue to accumulate, mitochondria function will diminish, and the DDR and NF-κB pathways will continue to be activated [70].

Examining how decreased SIRT1 activity leads to NAFLD through induction of cell senescence, Hua et al. showed that SIRT1's ameliorating effects on NASH in middle-aged $ApoE^{-/-}$ mice were associated with inflammatory cytokines IL-6 and TNF-α, lipid metabolism-related factors, including PPARα, and oxidative stress through LKB1, NF-κB and PGC1α pathways [56]. Looking at other players in cell senescence, CHK2, which is one of two checkpoint kinases in the DDR pathway that leads to the phosphorylation and activation of p53, can become activated via acetylation through the dissociation of it from SIRT1 when SIRT1 levels are deficient (Figure 1) [72]. Also, mTOR can continue to promote p53 activation and induce senescence in a manner independent of the DDR pathway [22]; it can promote IL-1α translation and result in the initiation of NF-κB transcriptional activity and promotion of SASP [39].

On the other hand, low SIRT1 will impact negative regulators of cell senescence, like FOXO proteins, LKB1, PPARα/PGC-1α, and AMPK, decreasing their levels and further impacting their own signal transduction pathways. Furthermore, positive promoters of cell senescence that are inhibited by SIRT1, like (p65/ NF-κB), p53, and (TSC2/mTOR), are activated. For instance, Chen et al. examined the hepatocyte of chronic-binge ethanol-fed mice, which induced alcoholic fatty liver, with hepatocyte-specific deletion of SIRT1, resulting in mTOR activation being promoted and exacerbating the development of fatty liver and liver injury [73]. Additionally, with low SIRT1 levels, antioxidant pathways are hindered, allowing for ROS and oxidative stress to take place. Therefore, it can be inferred that low SIRT1 activity and expression generate the ideal conditions for cell senescence to take place (Figure 1).

Promoting SIRT1, Inhibiting Cellular Senescence, and Preventing NAFLD

Promoting high levels of SIRT1 expression can ameliorate cell senescence and serve as a protective mechanism against disease [74]. Specifically, evidence shows that SIRT1 activation can help protect against obesity-induced hepatic steatosis and inflammation, seen in NAFLD and its progression [13]. As previously described, SIRT1 is a powerful regulator of essential homeostatic and metabolic pathways [19]. SIRT1 is integral for cell senescence inhibition, and the SIRT1/LKB1/AMPK pathway, in particular, has a great role in suppressing hepatic senescence [22]. Additionally, Cao et al. found that SIRT1 is able to significantly reduce ROS, a major trigger of cell senescence and mitochondrial dysfunction, and mitigate acute liver failure, which was deliberately induced in mice [75].

Kwon et al. discovered that SIRT1 inhibits CHK2, one of two upstream kinases of p53 in the DDR pathway via deacetylation

and protects cells by inhibiting the oxidative stress-dependent DDR pathway [72]. Similarly, SIRT1 deacetylation of p53 directly also renders it inhibited and leads to inhibition of DNA damage and cell senescence [22,23]. SIRT1 is also strongly involved in the antioxidant defense system, when it is active, it can regulate the PPARα and AMPK pathways, normalizing mitochondrial function and reducing oxidative stress preventing further cell injury and reducing liver damage [75]. Furthermore, by promoting PPARα activity and expression of its targets, there is a greater fatty acid β-oxidation and decreased lipogenesis [60].

SIRT1 also inhibits NF-κB, which has been demonstrated in multiple studies to drive inflammation and cell senescence. Additionally, SIRT1 can indirectly inhibit NF-κB through suppressing mTOR. Laberge et al. showed that reducing IL-1α in mice diminished NF-κB transcriptional activity, thereby suppressing SASP [39]. Furthermore, the impact of increasing SIRT1 upregulates genes relevant to metabolic functions, promotes insulin sensitivity, and reduces inflammatory gene expressions in animal adipose tissue [74]. As opposed to diminished expression, overexpression of SIRT1 can promote anti-senescent activity and prevent the development of NAFLD.

SIRT1 and HCC

There is conflicting research about whether SIRT1 is a tumor suppressor or tumor promoter. Specifically for HCC, some studies have shown that increased SIRT1 levels actually promote HCC, as opposed to ameliorating it as it does for NAFLD [13,76]. For instance, it has been found to be upregulated in HCC and promote its metastasis [76,77]. Additionally, its upregulation in HCC cells suppresses cellular differentiation, inhibits the senescence of cancerous cells through deacetylating p53, and promotes HCC tumorigenesis [78]. However, recent findings suggest that the localization of SIRT1 and the corresponding cell type determine whether SIRT1 acts as a tumor suppressor or promoter [19].

Several studies have examined SIRT1 expression in HCC cells and its role by examining SIRT1 knockdown cell lines. Similarly, Wang et al. showed that cancer stem cells from human HCC cell lines contained significantly higher expression of SIRT1 compared to normal human liver cells [79]. To study the effect of SIRT1 on liver cancer stem cells, they also looked at SIRT1 knockdown liver cancer stem cells. They found that SIRT1 knockdown increased the senescence features of the cancer stem cells. By inhibiting SIRT1, p53, p21, and p16 were activated in the cells with higher SA-β-gal expression and smaller HCC tumors compared to non-SIRT1 knockdown cells [79]. Portmann et al. took human HCC cell lines and non-tumor liver cells and found SIRT1 to be strongly expressed in the HCC cells; however other members of the SIRT family had either no significant difference in protein levels or lower protein levels compared with the normal cells [80]. Additionally, they looked at SIRT1 knockdown HCC cell lines and observed cell senescence, marked by the presence of SA-β-gal activity, in these cells compared to the non-SIRT1 knockdown cells.

SIRT1 has been shown to be an inhibitor of cell senescence in NAFLD development and progression. Perhaps, SIRT1 is being transformed and used to the cancer's advantage to prevent cancer cells from becoming senescent and continue to proliferate. Interestingly, Portmann et al. found that the overexpression of SIRT1 did not induce any tumors, suggesting that overexpression of SIRT1 does not initiate tumorigenesis [80]. At some point, SIRT1 expression is mutated to the cancer's advantage, like how tumor suppressor protein p53 is commonly mutated in cancers, and eventually becomes required for tumor formation and proliferation. It is unclear at what point HCC mutates the SIRT1 gene so that it is no longer protective and rather serves to prevent cancer cell senescence and promote tumorigenesis.

CAROTENOIDS

Carotenoids are a class of naturally occurring pigments found in plants, mainly colorful fruits and vegetables, algae, and photosynthetic bacteria [13,81,82]. Chemically speaking, they are classified as tetraterpenes, C40, and may be acyclic or modified into rings [83]. Additionally, they are divided into two main structural groups based on the presence of oxygen. Xanthophylls are the oxygenated carotenoid derivatives and carotenes are hydrocarbon-only carotenoids [17,82,84]. Carotenoids can also be distinguished by their ability to be metabolized into vitamin A. Those that contain an unsubstituted -ionone ring, like the well-known β-carotene, have pro-vitamin A activity [81]. Of the 600 carotenoids currently identified [82,83], only 50 are found in the human diet [82]. However, only 20 of the 50 are detectable in human serum [82]. Of these carotenoids, the most common are lycopene, β-carotene, α-carotene, lutein, zeaxanthin, and β-cryptoxanthin [17,83-85], accounting for >90% of carotenoids in the human body [17,81]. Carotenoids have been studied in both animals and humans across multiple disease types including, but not limited to, colon cancer [86], type 2 diabetes [87], cardiovascular disease [88], and NAFLD [25-28]. Carotenoids' anti-inflammatory properties prevent the positive feedback loop of pro-inflammatory mediators and activate the counteracting negative feedback loop [29]. Further investigation is needed to demonstrate how carotenoids can mechanistically increase SIRT1 directly and indirectly.

Carotenoid Impact on SIRT1, Cellular Senescence, and NAFLD

Emerging evidence shows that carotenoids can promote SIRT1 expression and activity, thereby inhibiting cell senescence and preventing NAFLD development in three main ways: 1) targeting ROS, 2) targeting enzyme NAMPT, increasing NAD+ production, and indirectly promoting SIRT1 activity, and 3) targeting SIRT1 gene expression and activity directly (Figure 3). Carotenoids, in general, serve as antioxidants. In liver studies, lycopene, a non-vitamin A carotenoid, has been shown to inhibit liver tumor initiations by scavenging ROS or upregulating SIRT1 and the antioxidant defense system [16]. Studies have investigated lycopene on NAFLD development [25-28], as it is one of the most potent antioxidants to neutralize ROS effectively and is a primary carotenoid in human tissue plasma [26,29]. For

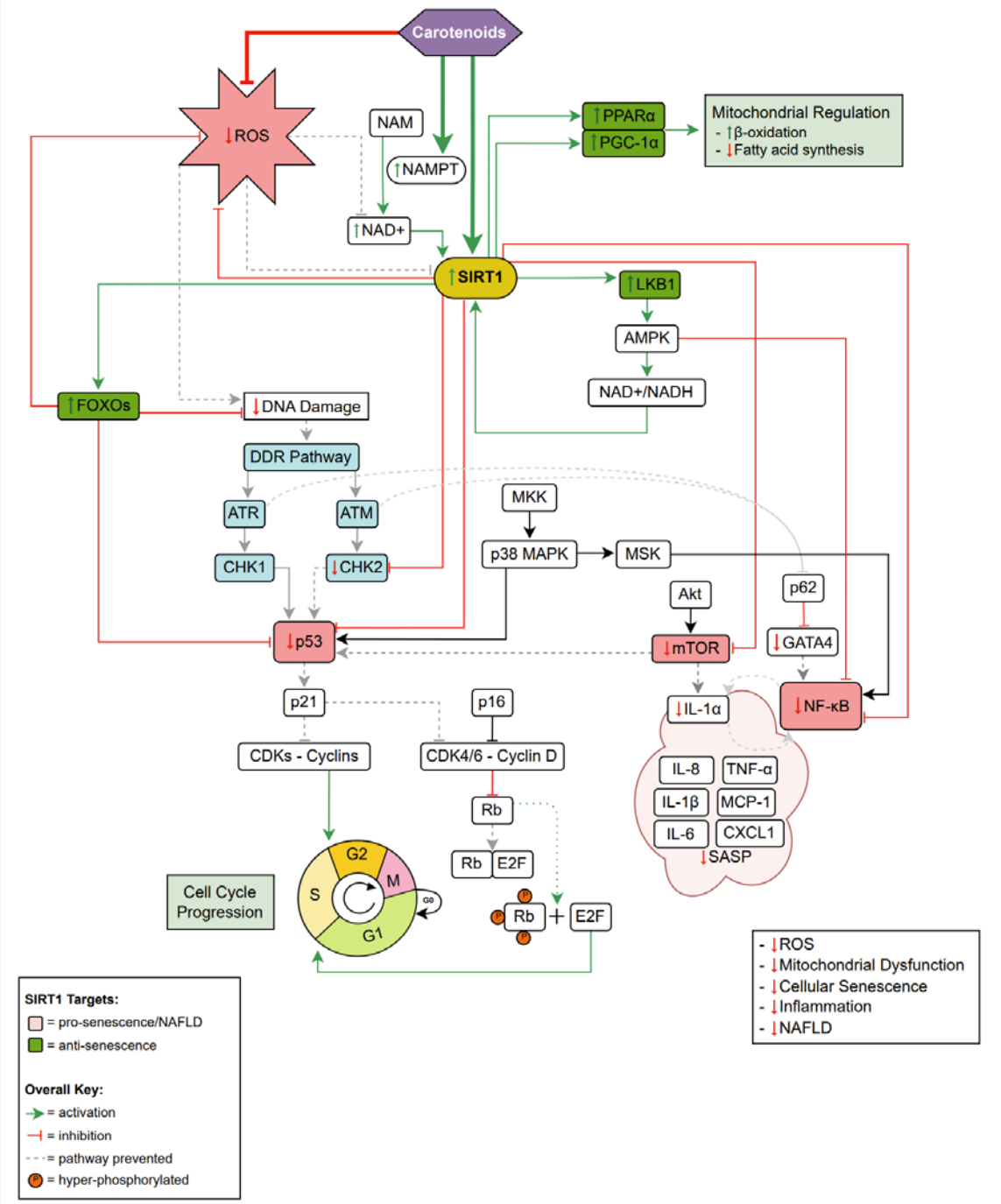

Figure 3 Proposed pathway of carotenoids directly and indirectly increasing sirtuin 1 (SIRT1) expression and activity leading to inhibition of cellular senescence. Green lines with arrowheads represent activation/enhancement. Red lines with perpendicular heads represent inhibition/suppression. Grey dotted lines represent pathways prevented. Green up/down arrows next to labels rep resent factors related to anti-senescence that are increasing/decreasing. Red up/down arrows next to labels represent factors related to pro-senescence that are increasing/decreasing.

Abbreviations: Akt: Protein kinase B; AMPK: AMP-activated protein kinase; ATM: Ataxia telangiectasia mutated; ATR: Ataxia telangiectasia and Rad3-related protein; CDK: Cyclin-dependent kinase; CHK1: Checkpoint kinase 1; CHK2: Checkpoint kinase 2; CXCL1: Chemokine (C-X-C motif) ligand 1; DDR: DNA damage response; E2F: E2 transcription factor; FOXO: Forkhead box O; G0: Resting phase (of the Cell Cycle); G1: Gap 1 (of the Cell Cycle); G2: Gap 2 (of the Cell Cycle); GATA4: GATA binding protein 4; IL: interleukin; LKB1: Liver kinase B1; M: Mitosis (of the Cell Cycle); MAPK: mitogen-activated protein kinase; MCP-1: Monocyte chemoattractant protein-1; mTOR: Mammalian target of rapamycin; NAD: Nicotinamide adenine dinucleotide; NAFLD: Nonalcoholic fatty liver disease; NAM: Nicotinamide; NAMPT: Nicotinamide phosphoribosyltransferase; NF-κB: Nuclear factor kappa B; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator-1 alpha; PPARα: Peroxisome proliferator-activated receptor alpha; Rb: Retinoblastoma protein; ROS: Reactive oxygen species; S: Synthesis (of the Cell Cycle); SA-β-gal: Senescence-associated beta-galactosidase; SASP: Senescence-associated secretory phenotype; SIRT1: Sirtuin 1; TNF-α: Tumor necrosis factor-alpha.

instance, lycopene has been shown to inhibit lipid peroxidation and ROS [26], thereby preventing hepatocyte ballooning and liver injury, and improve antioxidant capacity in adipocytes [26], which can help limit their senescence and therefore limit their contribution to NAFLD development.

NAMPT, the rate-limiting step for NAD+ synthesis in the salvage pathway [25,26], is pertinent to SIRT1 as its activation requires NAD+ hydrolysis. ROS can disrupt the NAD+/NADH balance, leading to less NAD+ available, thus negatively impacting SIRT1 activity and mitochondrial function. However, NAMPT overexpression has been shown to improve hyperlipidemia, glucose homeostasis, and adipose browning in obese mice [25]. Zhu et al. examined mice fed a high-fat diet and administered lycopene to determine its efficacy against NAFLD [26]. Compared to mice fed a high-fat diet without lycopene supplementation, they found that lycopene increased NAMPT expression and decreased acetylation of FOXO1, confirming the activation of SIRT1. Additionally, they found PPARα expression was significantly increased, aligning with inhibited lipogenesis and increased fatty acid β-oxidation. Furthermore, lycopene was shown to inhibit NF-κB and decrease pro-inflammatory factors, including IL-6, IL-1β, and TNF-α [26].

Li et al. looked at the effects of tomato powder, which contains a high concentration of lycopene, on NAFLD development and gut microbiome in beta-carotene-15,15'-oxygenase$^{-/-}$ (BCO1) and beta-carotene-9',10'-oxygenase$^{-/-}$ (BCO2) double knockout mice fed a high-fat diet alone or a high-fat diet in conjunction with tomato powder [28]. Metabolically, the enzymes BCO1 and BCO2 both catalyze lycopene to generate its biologically active metabolites, apo-lycopenoids, with BCO2 having preferential catalysis [28]. They found that steatosis severity and hepatic triglyceride levels for the tomato powder-fed group were significantly reduced compared to the high-fat diet alone group. These results were associated with increased AMPK phosphorylation, SIRT1 activity, and NAMPT expression, followed by decreased lipogenesis, decreased liver uptake of fatty acids, and increased fatty acid β-oxidation. Interestingly, they also found that the mRNA expression of pro-inflammatory genes TNF-α, IL-1β, and IL-6 were significantly decreased in both liver and mesenteric adipose tissue. These findings suggest that the concentrated lycopene tomato powder does not require carotenoid cleavage enzymes to inhibit NAFLD; rather it does so by increasing SIRT1 activity, and increasing adiponectin production [28]. Additionally, Ip et al. found that supplementation of lycopene and its active metabolite apo-10'-lycopenoic acid on high saturated fat diets in BCO2$^{-/-}$ knockout mice inhibited steatosis in male mice [27]. Apo-10'-lycopenoic acid ameliorated steatosis by activating SIRT1 signaling, followed by decreased lipogenesis, indicated by markers such as increased AMPK phosphorylation and increased downstream target FOXO1. Lycopene was shown to mitigate steatosis as well; however, the present study found it did so through inducing PPARα genes in mesenteric adipose tissue, increasing fatty acid β-oxidation and decreasing lipogenesis, and did not find evidence of modulations to SIRT1 activation [27]. However, previous studies have demonstrated a strong link between PPARα and SIRT1 and that PGC-1α [60], which is a main activator of PPARα, is directly modulated by SIRT1 [89]. Additionally, like the previous study, Chung et al. found increased SIRT1 activity and higher SIRT1 mRNA and protein levels due to apo-10'-lycopenoic acid in high-fat-fed mice compared to controls not given the lycopenoid supplementation [90]. These findings were associated with significantly decreased hepatic steatosis in the apo-10'-lycopenoic acid supplementation group. Altogether, the studies mentioned above reveal that carotenoid intervention can prevent the development of NAFLD through the direct or indirect upregulation of SIRT1 expression and activity.

Certain metabolic conditions, like type 2 diabetes and aging, are strong risk factors for NAFLD development. Hwang et al. investigated the effect of lutein, a xanthophyll carotenoid, on hyperglycemia-induced senescent retinal pigment epithelium cells, mimicking diabetic conditions [91]. The cells were a spontaneously immortalized cell line derived from human retinal pigment epithelium cells. Similar to Chung et al., Hwang et al. found that lutein treatment increased both SIRT1 mRNA and protein levels. This was associated with lutein significantly inhibiting the induced premature senescence and ROS production. Moreover, Li et al. investigated lycopene's effects in aging rats who displayed insulin resistance, which could lead to type 2 diabetes, and vascular damage. They found that SIRT1 plays a key role in restoring insulin transduction, as shown in lycopene increasing activation of it, as well as demonstrating that in SIRT1 knockdown mice, administering lycopene did not have the same effect and could not restore insulin transduction without SIRT1 [66].

Cigarette smoking is an independent risk factor for NAFLD. Rakic et al. recently demonstrated that the exposure of ferrets to the combination of cigarette smoking and tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) exposure resulted not only in steatosis but also NASH, characterized by severe inflammatory cell infiltration with concurrent fat accumulation in liver, hepatocellular ballooning degeneration, increased NF-κB expression, and increased bilirubin and aspartate aminotransferase (AST) levels, which closely resembles NASH in humans [92]. Using ferret, an excellent animal model for dietary carotenoid intervention studies for hepatic disease due to similarities in carotenoid absorption and accumulation [93], Aizawa et al. demonstrated that dietary lycopene feeding, inhibited cigarette smoking-induced NASH, and decreased fibrosis biomarkers, which were accompanied by increased expression of antioxidant enzymes [94].

Like lycopene, β-cryptoxanthin is a type of xanthophyll carotenoid that can also be cleaved by BCO1 and BCO2 and has been shown to upregulate SIRT1. Recently, Chiaverelli et al. examined the lung tissue of both BCO1$^{-/-}$/BCO2$^{-/-}$ double knockout mice and wild type mice supplemented with β-cryptoxanthin for 14 days, then exposed to cigarette smoke for a subsequent 14 days to induce inflammatory lung disease [95]. They found that treatment of both β-cryptoxanthin and its metabolite 3-OH-β-apo-10'-carotenal prevented lipopolysaccharide-induced inflammatory response in a dose-dependent manner, which

were associated with the upregulation of SIRT1. Additionally, β-cryptoxanthin was also associated with lower expression of IL-6 and TNF-α [95]. Since comparable disease of β-cryptoxanthin and its metabolite can attenuate lipopolysaccharide-induced inflammatory responses, the modulation of SIRT1 with β-cryptoxanthin against inflammation could be due to the biological action of β-cryptoxanthin as its own intact molecule.

The studies discussed reveal the protective effect of carotenoids against cellular senescence and NAFLD development through the potential mechanisms of scavenging ROS, increasing NAMPT expression, and upregulating SIRT1. Therefore, carotenoid intervention may help prevent the onset of other risk factors that are related to NAFLD.

Carotenoids and HCC

Studies have found that dietary consumption of carotenoids leads to increased serum levels of carotenoids, which is associated with decreased development of cancers and diseases [81,82,96,97]. For HCC development, carotenoid consumption has been shown to lower the risk for HCC significantly. The 2020 Singapore Chinese Health Study examined a prospective cohort of more than 60 thousand Chinese people aged 45-74 at enrollment at risk for HCC development. They meticulously followed their tomato intake for a mean of 17.6 years. They discovered that a higher tomato intake was associated with lower risk of HCC after adjustment for potential confounders [98]. Additionally, a recent meta-analysis of 15 animal studies found that lycopene significantly reduced the incidence and multiplicity of HCC, suggesting lycopene inhibits the initiation and progression of HCC in animal models. They further showed that lycopene's anti-inflammatory effect can inhibit HCC development and progression, including inhibition of the senescence-associated transcription factor NF-κB and its related cytokines, including TNF-α, IL-1β, and IL-6 [16].

Currently, there is little research regarding carotenoids' effect on the roles of SIRT1 and cell senescence in HCC development. Two studies have examined SIRT1 expression in induced HCC in mice and carotenoid supplementation. The first study, conducted by Ip et al., demonstrated that apo-10'-lycopenoic acid supplementation in diet inhibited high-fatty diet-promoted HCC development in $BCO1^{-/-}$ and $BCO2^{-/-}$ double knockout mice exposed to diethylnitrosamine (DEN), a known hepatic carcinogen [99]. The mice were injected with DEN at two weeks old to initiate HCC and started being fed a high-fat diet to further promote tumorigenesis with or without apo-10'-lycopenoic acid supplementation at six weeks of age for 24 weeks. Compared to the high-fatty diet-only group, the mice with apo-10'-lycopenoic acid supplementation significantly reduced hepatic tumorigenesis and multiplicity and decreased inflammatory factors, including TNF-α, IL-6, and NF-κB p65 protein expression, compared to the control group. They also found an upregulation of SIRT1. Interestingly, p21 was increased, and cyclin D, which can be inhibited by p21, was decreased, suggesting that cell cycle arrest and perhaps cell senescence took place.

Similarly, Xia et al. examined lycopene-rich tomato powder supplementation in mice following the same intervention timeline as the previous study regarding DEN injection and supplementation feeding [64]. They also looked at $BCO1^{-/-}$ and $BCO2^{-/-}$ double knockout mice, but were fed either a high-fatty diet alone or a high-fatty diet with tomato powder supplementation. Similar to the findings in Ip et al., they found decreased HCC development, incidence of HCC, tumor multiplicity, and tumor volume, along with decreased pro-inflammatory biomarkers, including IL-6, IL-1β, and MCP-1 for the tomato powder supplementation group [64]. There was also increased mRNA expression of both SIRT1 and NAMPT, decreased acetylation of FOXO1, and increased phosphorylated AMPK, indicating that SIRT1 had anti-tumorigenic properties. Additionally, they found that tomato feeding increased expressions of PPARα and its coactivator PGC-1α [64], which are associated with decreased lipogenesis and increased fatty acid β-oxidation.

Furthermore, β-cryptoxanthin is another carotenoid that has been used in diet supplementation to determine its impact on induced HCC in mice. Lim et al. examined normal wild-type mice and $BCO1^{-/-}$ and $BCO2^{-/-}$ double knockout mice injected with DEN at two weeks and then fed either a high carbohydrate diet with or without β-cryptoxanthin [100]. Like the previous studies, both wild-type and knockout mice fed with β-cryptoxanthin had significantly lower steatosis presentation, tumor size, total tumor volume, and HCC multiplicity. With β-cryptoxanthin administration, they found an association between increased p53 acetylation (the active form of p53) and decreased cyclin D1 (which can activate CDK4/6 to drive cell progression) [100], perhaps indicating that cell senescence was induced.

Studies show carotenoid intervention to be significantly associated with decreased HCC tumorigenesis, proliferation, and volume, with evidence of increasing SIRT1 and decreasing inflammatory factors [64,99]. However, Ip et al. showed an increased expression of SIRT1 and p21 [99], an important player of the DDR pathway and cell senescence. Although Lim et al. did not explore SIRT1 levels, they also showed upregulation of p53 and decreased cyclin D1 [100], which could be attributed to p53 activation of p21, leading to the downward effects of cell cycle arrest. These results are intriguing as increased SIRT1 expression is associated with the downregulation of cell senescent player's p53 and p21. Further investigation is needed to see exactly how carotenoids are helping HCC, such as examining the activity and expression of SIRT1 and senescent cell markers at specific stages of HCC progression, comparing affected sections to non-affected sections, and examining human subjects compared to animal models.

CONCLUSION

Current evidence from animal and human studies point to cell senescence being the cause of NAFLD development and the driver of its progression into a more serious disease, NASH, and can be followed by cirrhosis and HCC. In particular, examining rodent and human liver cell lines, hepatocyte senescence has been shown to promote hepatic fat accumulation through

decreased fatty acid β-oxidation and increased lipogenesis, facilitating the development of NAFLD. Additionally, adipocyte senescence plays a role in NAFLD development as the over-secretion of pro-inflammatory cytokines and chemokines from senescent adipocytes will eventually go into circulation, affecting inflammatory processes in the liver. Furthermore, approximately 60% of hepatic triglycerides in human subjects are found to be from adipose origin. As NAFLD progresses, however, further investigation is needed to examine what types of senescence are protective versus harmful. For instance, hepatocyte senescence has been shown to promote NAFLD development and progression into NASH, as well as activate HSCs, which can lead to fibrosis. Evidence reveals a significant correlation between hepatocyte senescence and both NAFLD severity and fibrosis progression. However, HSC senescence may be protective against disease as it can lead to their own clearance by the immune system and produce fewer ECM components necessary for fibrosis. Consequently, senescence can be protective against or a trigger for HCC, which seems to have a significant dependence on the type and stage of the cell.

Targeting cell senescence has great potential for preventing NAFLD and its progression. SIRT1 could be a likely target for therapeutic intervention as it is integral in multiple activating and inhibitory pathways in cell senescence. Studies reveal the impact of diminished SIRT1 activity on activating senescent factors and inducing hepatic fat accumulation. On the other hand, overexpression of SIRT1 activity has been shown to induce anti-senescent activity and prevent NAFLD development. As the role of senescence in HCC development and progression is unclear, so is the role of SIRT1 and HCC. Looking at human HCC cell lines, SIRT1 is overexpressed and cell senescence is suppressed in these cells, suggesting that SIRT1 may promote HCC progression. However, evidence shows that the overexpression of SIRT1 does not initiate the tumors. Further investigation is needed to examine if and at what point HCC mutates the SIRT1 gene so that it is advantageous to cancer as opposed to the body to prevent senescence in cancer cells and promote their proliferation.

Regarding SIRT1 targeting, carotenoids have been shown to both directly and indirectly promote the activity and expression of SIRT1. As antioxidants, they scavenge ROS and inhibit lipid peroxidation, preventing the diminishing of SIRT1 activity and therefore limiting cell senescence and disease development. Carotenoids can also increase NAMPT expression, creating more available NAD+, and thus increasing SIRT1 activity. Lastly, carotenoids can directly increase SIRT1 gene expression and activity, particularly increasing SIRT1 mRNA and protein levels. Therefore, with SIRT1 as a target, carotenoids could prevent cell senescence and mitigate NAFLD in a multifaceted way. For HCC, further research needs to be performed, especially regarding SIRT1 and different cancer stages, to determine whether there is an optimal time for increasing SIRT1 expression versus inhibiting it and if SIRT1 can be induced or suppressed depending on specific cell type.

Until more clinical studies examine the specific point of transformation for SIRT1 in HCC, marking when overexpression is beneficial to the person versus the cancer, as well as better illustrate the impacts of senescence in NASH, carotenoid intervention seems like it would be most effective in NAFLD during NAFL stages, allowing reversal of the disease and prevention of progression. Furthermore, it is important to note that a majority of the studies described are animal studies. For carotenoid intervention, human clinical trials are needed to determine proper dosing.

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