The balance of protein farnesylation and geranylgeranylation during the progression of nonalcoholic fatty liver disease

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Protein prenylation is an essential posttranslational modification and includes protein farnesylation and geranylgeranylation using farnesyl diphosphate or geranylgeranyl diphosphate as substrates, respectively. Geranylgeranyl diphosphate synthase is a branch point enzyme in the mevalonate pathway that affects the ratio of farnesyl diphosphate to geranylgeranyl diphosphate. Abnormal geranylgeranyl diphosphate synthase expression and activity can therefore disrupt the balance of farnesylation and geranylgeranylation and alter the ratio between farnesylated and geranylgeranylated proteins. This change is associated with the progression of nonalcoholic fatty liver disease (NAFLD), a condition characterized by hepatic fat overload. Of note, differential accumulation of farnesylated and geranylgeranylated proteins has been associated with differential stages of NAFLD and NAFLD-associated liver fibrosis. In this review, we summarize key aspects of protein prenylation as well as advances that have uncovered the regulation of associated metabolic patterns and signaling pathways, such as Ras GTPase signaling, involved in NAFLD progression. Additionally, we discuss unique opportunities for targeting prenylation in NAFLD/hepatocellular carcinoma with agents such as statins and bisphosphonates to improve clinical outcomes.

Prenylation is a type of lipid modification wherein a farnesyl (15-carbon) or a geranylgeranyl (20-carbon) side chain is added to a C-terminal cysteine residue of a CaaX or CaaX-like motif, dependent on the characteristics of X (where C is cysteine, A is any aliphatic amino acid, and X is another amino acid); these modifications are called farnesylation and geranylgeranylation, respectively (1). Given the hydrophobicity of the lipids involved, prenylated proteins are anchored to cellular membranes in proximity to downstream signaling pathways involved in numerous cellular processes, including cell proliferation and differentiation, cell metabolism, and intracellular protein trafficking (2). Geranylgeranyl diphosphate synthase (GGPPS)² is the branch point enzyme in the mevalonate (MVA) pathway that is responsible for synthesizing GGPP from its substrate FPP, and abnormal expression of this enzyme affects the ratio of FPP to GGPP, disrupting the balance of protein farnesylation and geranylgeranylation (3–5).

The existence of imbalances in this system has a high correlation with the development of many diseases, including non-alcoholic fatty liver disease (NAFLD) and NAFLD-associated fibrosis. NAFLD refers to a clinical condition characterized by hepatic fat overload without alcoholism (6). It is strongly associated with obesity, diabetes, and insulin resistance and is considered a metabolic syndrome (7). NAFLD is classified into nonalcoholic fatty liver (NAFL, simple steatosis) and nonalcoholic steatohepatitis (NASH) (8). The simple steatosis in NAFL represents a state of imbalance where triglyceride deposition overpowers its consumption. Prolonged lipid accumulation and inflammation can progress to NASH, advanced liver fibrosis, cirrhosis, and, ultimately, hepatocellular carcinoma (HCC).

Although the pathogenesis of NAFLD has been investigated through extensive research and clinical studies, the molecular mechanism involved in the progression from NAFLD to HCC remains to be elucidated. Several central molecules/pathways related to the MVA pathway, including Ras-ERK1/2, PI3K-Akt, sterol regulatory element–binding protein 1 (SREBP), Rac, and AMPK, are activated during the progression of NAFLD to HCC. These changes give the cell features of proliferation, genomic instability, and immortalization, eventually promoting progression to HCC (Fig. 1).

Interestingly, the accumulation of differential amounts of farnesylated and geranylgeranylated proteins regulated by GGPPS has been associated with differential stages of NAFLD and NAFLD-associated fibrosis (4, 9). Statins, a class of com-

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The abbreviations used are: GGPPS, geranylgeranyl diphosphate synthase; NAFLD, nonalcoholic fatty liver disease; NASH, non alcoholic steatohepatitis; HCC, hepatocellular carcinoma; FPPS, farnesyl diphosphate synthase; GGPP, geranylgeranyl diphosphate; FPP, farnesyl diphosphate; MVA, mevalonate; FTase, farnesyltransferase; GGTase, geranylgeranyltransferase; FTI, farnesyltransferase inhibitor; DNL, de novo lipogenesis; GGTI, geranylgeranyltransferase inhibitor; HMGR, HMG-CoA reductase; DGBP, digeranyl bisphosphate; IRS, insulin receptor substrate; SREBP, sterol-regulatory element–binding protein; NBP, nitrogenous bisphosphate; QC, quality control; NAFL, nonalcoholic fatty liver; ERK, extracellular signal–regulated kinase; PI3K, phosphatidylinositol 3-kinase; AMPK, AMP-activated protein kinase; LKB1, liver kinase B1; MAPK, mitogen-activated protein kinase; HFD, high-fat diet; YAP, Yes-associated protein; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase.

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2 The abbreviations used are: GGPPS, geranylgeranyl diphosphate synthase; NAFLD, nonalcoholic fatty liver disease; NASH, non alcoholic steatohepatitis; HCC, hepatocellular carcinoma; FPPS, farnesyl diphosphate synthase; GGPP, geranylgeranyl diphosphate; FPP, farnesyl diphosphate; MVA, mevalonate; FTase, farnesyltransferase; GGTase, geranylgeranyltransferase; FTI, farnesyltransferase inhibitor; DNL, de novo lipogenesis; GGTI, geranylgeranyltransferase inhibitor; HMGR, HMG-CoA reductase; DGBP, digeranyl bisphosphate; IRS, insulin receptor substrate; SREBP, sterol-regulatory element–binding protein; NBP, nitrogenous bisphosphate; QC, quality control; NAFL, nonalcoholic fatty liver; ERK, extracellular signal–regulated kinase; PI3K, phosphatidylinositol 3-kinase; AMPK, AMP-activated protein kinase; LKB1, liver kinase B1; MAPK, mitogen-activated protein kinase; HFD, high-fat diet; YAP, Yes-associated protein; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase.

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pounds widely used to lower cholesterol, are inhibitors of HMG-CoA reductase (HMGCR, the upstream enzyme in the MVA pathway) and consequently alter the ratio of FPP/GGPP followed by the balance of protein prenylation (2). Considering the effects of several inhibitors targeting MVA pathway enzymes on immune control (66), metabolic disease (10), and cancer progression (11), protein prenylation can also affect the progression of NAFLD through processes such as metabolic reprogramming and signaling pathway activation. More importantly, identifying a drug targeting the prenylation balance can provide insights for prospective therapeutic strategies for NAFLD and HCC.

Protein prenylation

Anchorage to cellular membranes is a prerequisite for the biological function of many regulatory proteins, which can be located on the membrane surface or embedded in the lipid bilayer. Many peripheral proteins are targeted to membranes as a result of posttranslational modification with lipid moieties. Two types of isoprenoid lipids, FPP and GGPP, which are intermediates in the MVA pathway for cholesterol, terpene and terpenoid synthesis, are utilized for such modifications (Fig. 2, left). Proteins with cysteine residues typically found in the CaaX motif can be farnesylated with FPP or geranylgeranylated with GGPP. Either of these biochemical reactions depends upon the nature of the X residue. If X is serine, methionine, alanine, or glutamine, the protein is farnesylated; if X refers to leucine or isoleucine, the protein is geranylgeranylated (12).

Protein prenylation depends on the activity of prenyltransferases. There are three prenyltransferases, all of which are heterodimeric enzymes containing α and β subunits. Farnesyltransferase (FTase) and geranylgeranyltransferase 1 (GGTase1) share the same α subunit but contain different β subunits. Both transferases recognize substrates with a CaaX sequence, the site where lipid modification occurs. Another prenyltransferase, GGTase2, is formed by RabGGTA (the α subunit) and RabGGTB (the β subunit). GGTase2 prenylates sites in additional C-terminal motifs, including CCXXX, CCXX, XCCX, XXCC, and XXCX. Unlike FTase and GGTase1, the prenylation by GGTase2 requires the participation of the Rab escort protein, an accessory protein involved in the recognition of Rab by GGTase2 (12). Distinct substrates have been identified for FTase (H-Ras, K-Ras, N-Ras, Ras2, Rap2, pre-Lamin A, Lamin B, RhoB, RhoE, and Rheb), GGTase1 (Rhoa, Rhob, RhoC, Rab8, Rab11, Rab13, Rac1, Rac2, Rab1, Rab13, Rac1, Rac2, RalA, Rap1B, and Cdc42), and GGTase2 (Rab GTPases) (13–17). In addition, recently, a new prenyltransferase, GGTase3, which consists of the α subunit PTAR1 and the β subunit of GGTase2 (RabGGTA), was identified. This enzyme geranylgeranylates FBXL2, a ubiquitin ligase, allowing it to associate with cell membranes (18) (Fig. 3).

After recognition, the aaX residues at the C terminus can be further removed by Ras-converting CaaX endopeptidase 1 (RCE1), and isoprenylcysteine carboxylyltransferase adds a methyl group to the isoprenoid-modified cysteine residue (12).

A large number of prenylated peptides in living cells without metabolic perturbation have been reported in a newly developed proteome-scale analysis (19). Hundreds of prenylated candidates have been identified by the development of isoprenoid analogues YnF and YnGG in combination with quantitative chemical proteomics, such as Ganab, K-Ras, N-Ras, Nos2, Nos3, Rab, Rac, and Rheb. Among these candidates, Ganab, Nos2, and Nos3 are involved in metabolic pathways, and K-Ras and N-Ras participate in thermogenesis, the insulin signaling pathway, and choline metabolism in cancer. In addition, Ras, RhoB, Rac, and liver kinase B1 (LKB1) are involved in the MAPK, PI3K/Akt, AMPK, and other signaling pathways, which are also engaged in metabolic regulation. These metabolism-related candidates give a hint that protein prenylation may influence metabolic state.
The balance of protein farnesylation and geranylgeranylation and its effect on altered metabolic states

The balance of protein farnesylation and geranylgeranylation is highly related to the activation state of GGPPS, the branch point enzyme in the MVA pathway. When GGPPS is activated, the GGPP/FPP ratio increases; consequently, protein geranylgeranylation is enhanced or vice versa, because the expression level and activity of FTase and GGTase normally do not change significantly. However, some proteins can alternatively undergo either type of prenylation under extreme conditions; for example, when either GGPP or FPP is unavailable, or $X$ is phenylalanine, the proteins can be either farnesylated or geranylgeranylated (15). In addition, both geranylgeranylation and farnesylation of H-Ras can occur when GGPPS is knocked out (3), and K-Ras can be geranylgeranylated in cells treated with farnesyltransferase inhibitors (FTIs) (17). Furthermore, several studies about these alternative prenylation patterns have been published (13–17), although the molecular mechanisms remain unclear.

Another possible effect by alteration of FPP/GPP ratio is the direct change of metabolic pathways in the cell. FPP is a key...
intermediate in cholesterol metabolism (2), whereas GGPP is crucial in the metabolism of dolichols (20), which are essential for protein glycosylation (21, 22). Considering the different $K_{\text{m}}$ of the enzymes for FPP or GGPP destination, the FPP/GGPP ratio change might also influence the synthesis of cholesterol, heme A, dolichol, etc. by FPP and/or ubiquinone, etc. by GGPP. Therefore, FPP and GGPP, as metabolites, may regulate cell functions with a different mechanism to maintain the metabolic homeostasis apart from protein prenylation.

GGPPS is highly abundant in the liver, adipose tissue, and muscle of mice with obesity and insulin resistance but is expressed at a relatively low level under normal conditions (23). Previous studies have shown that insulin can induce Rab geranylgeranylation by activating GGTase enzymes in 3T3-L1 preadipocytes and that abrogation of GGTase activity inhibits the phosphorylation of MAPK pathway components in 3T3-L1 preadipocytes (24). GGPP stimulates PPARY expression and adipogenesis (25). Inhibition of GGPPS by digeranyl bisphosphonate (DGBP) leads to reduced GGPP levels but accumulation of FPP, impairing protein geranylgeranylation in MC3T3-E1 preosteoblast cells (26). Our studies have also revealed that the MAPK/Egr-1/GGPPS/Ras axis plays an essential role in certain metabolic states, such as type 2 diabetes. Long-term insulin stimulation activates GGPPS and further activates K-Ras by enhancing its geranylgeranylation. Ras/MAPK/Erk1/2 signaling results in insulin receptor substrate-1 (IRS-1) phosphorylation, contributing to insulin resistance (27). Furthermore, we observed increased GGPPS expression in the skeletal muscles of mice with insulin resistance, and specific knockout of GGPPS in skeletal muscle improved systemic insulin sensitivity and glucose homeostasis by enhancing glucose uptake in skeletal muscle. These metabolic alterations mediated by ggpps knockout were achieved through decreased geranylgeranylation of RhoA, which further induced the phosphorylation of IRS-1 (28). Thus, the GGPPS/RhoA/Rho kinase/IRS-1 pathway mediates lipid-induced systemic insulin resistance in obese mice (29). In summary, GGPPS is crucial for the balance of protein farnesylation and geranylgeranylation in the regulation of metabolic states, which may influence other metabolic diseases, such as NAFLD/HCC.

**Metabolic states mediated by the prenylation balance in NAFLD/HCC progression**

Proliferative cancer cells often exploit nutrients, such as glucose, to support their energy demand and biomass synthesis. They tend to convert most glucose to lactate regardless of whether oxygen is present (aerobic glycolysis), referred to as the Warburg effect (30, 31). Although compared with fatty acid $\beta$-oxidation, glycolysis is an ineffective way to generate energy from glucose, hepatocytes rely more on glycolysis than on $\beta$-oxidation for energy during NAFLD/HCC progression (31). This alteration in metabolic control may eventually alter existing cell metabolism in a way that supports cell growth (32).

Our previous work revealed that GGPPS was highly expressed in the livers of NAFLD patients but down-regulated in HCC patients (33). Additionally, GGPPS was first up-regulated in the livers of mice with high-fat diet (HFD)-induced NAFLD and was then down-regulated after long-term HFD overload in NAFLD, which was associated with fibrosis, suggesting that the GGPPS-dependent protein prenylation balance mediates metabolic alterations during the development of NAFLD-associated fibrosis (Fig. 4). The balance of prenylation regulated by the two-phase change in GGPPS expression is associated with differential stages of NAFLD progression to HCC by influencing the activity of metabolic enzymes and signal transduction through the related signaling pathways.

During NAFLD progression, an HFD enhances hepatic lipid oxidation and glycolysis. Additionally, an HFD accelerates lipid accumulation by up-regulating the expression of GGPPS (4, 9), which alters the relative ratio of FPP to GGPP, thereby influencing SREBP activation. However, GGPPS is down-regulated during the progression of NAFLD to HCC, which drives NAFLD-associated fibrosis by promoting glycolysis and suppressing oxidative phosphorylation via the LKB1/AMPK axis (4). Hence, these factors promote the Warburg effect to support glycolysis and result in metabolic reprogramming during NAFLD progression, thus driving HCC progression.

**FPP/GGPP and SREBP activation**

SREBP is a critical regulator for maintaining lipid homeostasis (34). SREBP precursors form a complex with SREBP cleavage–activating protein, which is retained by the insulin-induced gene-1/2 proteins in the endoplasmic reticulum in the presence of increased cellular sterol levels. Downstream effectors of SREBP are primarily encoded by genes involved in regulating lipid metabolism, particularly those associated with de novo lipogenesis (DNL), including FAS (35), ACC1 (35), and SCD1 (36). Moreover, these genes can mediate the inflammatory reaction in NAFLD, which is essential for HCC progression. Hence, SREBP down-regulation can reduce the inflammatory reaction and prevent the progression from NAFLD to NAS, cirrhosis, and even HCC. A recent study revealed that geranylgeranylated RhoA-dependent actomyosin contraction inhibits SREBP1 activation. FPP accumulation resulting from GGPPS deficiency inhibits DNL in hepatocytes, suppressing SREBP-1 expression and LXR activation by activating FXR/SHP signaling (37). Moreover, a recent report indicated that SREBP-1 couples mechanical cues and lipid metabolism via protein geranylgeranylation, indicating the role of isoprenoids in regulating SREBP activation in lipid metabolism (38). Moreover, our study showed that zoledronic acid, an inhibitor of FPPS, inhibits hepatic DNL and liver steatosis by suppressing RhoA prenylation-dependent SREBP-1c activation (39). Considering the alteration of the prenylation balance by GGPPS in NAFLD, this finding indicates that up-regulation of SREBP mediates inflammatory reactions and increases hepatic total cholesterol and triglyceride levels, consequently correlating with HCC progression (4).

**LKB farnesylation and AMPK activation**

As the LKB1-AMPK pathway is important for cells to maintain metabolic homeostasis by sensing the AMP/ATP levels (40), it is thought to control the GGPPS-regulated metabolic reprogramming process. Mechanistically, as we reported, Ggpps deficiency enhances the farnesylation of LKB1 and promotes metabolic reprogramming by regulating AMPK activity...
AMPK activation turns off ATP-consuming pathways and switches on ATP-producing pathways. Such metabolic alterations further induce hepatic inflammation through elevated macrophage infiltration and proinflammatory cytokine production. In addition, insulin resistance is frequently detected in patients with NAFLD; this state decreases AMPK activity and produces a hyperuricemic environment, resulting in hepatic ATP depletion and further favoring glycolysis (41). Thus, the GGPPS-regulated protein prenylation balance is a metabolic controller of fat overload–induced NAFLD and fibrosis development (4).

**Signaling pathways mediated by prenylation in NAFLD/HCC progression**

The progression from NAFLD to HCC is related to several signaling pathways involved in steatogenic, fibrogenic, proliferative, and proinflammatory signaling (42), such as the Ras/PI3K/AKT and Hippo–Yes–associated protein (YAP)/YAZ pathways. These signaling pathways have been reported to be regulated by the prenylation balance. Thus, an abnormal balance of protein prenylation may contribute to HCC progression from NAFLD via signal transduction through related signaling pathways.

**Ras signaling pathway**

The intracellular GTP-binding proteins involved in signal transduction comprise the largest family of prenylated proteins. The Ras protein is the most extensively studied small GTPase (11). As malignancy is associated with Ras mutation, Ras is a potential target for cancer therapy. For example, Ras mutations have been found in pancreatic cancer (90%) (43), thyroid cancer (50%) (44), acute myeloid leukemia (44%) (45), colon cancer (47%) (46), melanoma (36%) (47), and lung cancer (30%) (48). To date, three forms of mutated Ras have been identified: H-Ras, K-Ras, and N-Ras. K-Ras mutations occur more commonly in cancer than do N-Ras mutations, which are usually found in hematologic malignancies (49). These three different types of mutated Ras share over 90% sequence homology but vary in their association with the inner plasma membrane.
Differences in the balance of prenylation and membrane-anchored Ras isoforms can explain differences in the activation of K-Ras, N-Ras, and H-Ras. K-Ras and N-Ras can be geranylgeranylated when FTase is inhibited, whereas H-Ras can only be farnesylated by FTase (17). After prenylation, K-Ras bypasses the Golgi complex, yet N-Ras and H-Ras encounter palmitoyl acyltransferases on the cytoplasmic surface of the Golgi (52). The locations of the three isoforms at the cell membrane also differ; K-Ras and N-Ras are not associated with lipid rafts, although the GDP-bound form of H-Ras binds to lipid rafts (53).

Once activated by prenylation, Ras acts as an upstream master regulator, directly activating downstream pathways involved in various cellular functions. Two pathways, the PI3K/AKT pathway and the Raf/MAPK/ERK pathway, mediate tumor cell proliferation, migration, and metastasis. Activation of PI3K/AKT signaling by growth factors increases the expression of SREBP1 and SREBP2 (54–58), which results in elevated lipid and cholesterol production and progression of NAFLD (59–61). Moreover, PI3K activity may be decreased with inhibition of the MVA pathway, possibly through decreased Ras prenylation (62).

Several studies have revealed that prenylation inhibition may be an efficient therapeutic strategy for cancer via inhibition of the Ras signaling pathway. Simultaneous knockout of prenyltransferase β subunits (both Fntb and Pggt1b) suppresses K-Ras–induced lung tumor progression more efficiently than deletion of either subunit alone (63). In addition, conditional Fntb or Pggt1b deficiency reduces K-Ras-G12D–induced lung cancer formation in mice (63), suggesting that FTase and GGTTase1 are targets for cancer therapy.

Other Ras GTPase superfamily signaling pathways

All proteins in the Ras GTPase superfamily are localized to the membrane by prenylation, and several studies have shown that statin treatment decreases the prenylated and membrane-associated forms of Ras, Rho, Rac, Rap, and Rab subfamily proteins (64). In pancreatic cancer with K-Ras mutation (90%) (49), pathways mediated by geranylgeranylated RapA and RapB correlate much more strongly with malignancy onset than do MEK or AKT pathways (65). In addition, geranylgeranylated RhoC performs an essential function in tumor metastasis (65, 66). A recent study reported that GGPPS inhibition therapy can be a novel strategy for the treatment of pancreatic ductal adenocarcinoma by disrupting Rab geranylgeranylation to induce the unfolded protein response pathway (67), which also occurs in HCC (68).

Furthermore, the Rho subfamily GTPase Rac1 is required for the induction of K-Ras–driven lung cancer in mice (69). Importantly, geranylgeranylated cell division cycle 42 (Cdc42) and Rac are downstream targets of Ras in mediating fibroblast transformation (70). Atorvastatin, which blocks FPP and GGPP production by inhibiting HMGCR, prevents HCC development by decreasing Rac1 prenylation to inhibit MYC phosphorylation (71). As MYC is a potent oncogene that causes transformation in various cancer types, inhibition of MYC phosphorylation can induce sustained regression of HCC (72). Thus, the MVA pathway is important in this MYC-driven HCC model.

Another Ras GTPase superfamily protein, RhoB, is both geranylgeranylated (70%) and farnesylated (30%) under physiological conditions. It has been shown that geranylgeranylated RhoB suppresses Ras-induced transformation and that inhibition of RhoB farnesylation contributes to FTI-induced apoptosis (73). However, both geranylgeranylated RhoB and farnesylated RhoB suppress tumor activity in several human epithelial cancer cells (74). A previous study also showed that the oncogene YAP and transcriptional coactivator with PDZ-binding motif (TAZ) require GGPP-mediated RhoA prenylation to be functional (75). Mechanistically, the balance of prenylation maintains GGPP levels, regulating the nuclear localization of YAP and TAZ through RhoA prenylation (76). Another study demonstrated that treatment with atorvastatin or geranylgeranyl transferase inhibitors (GGTIs) increased the levels of Lats1 and Lats2, two important tumor suppressors involved in the Hippo–YAP/TAZ pathway, which further controls cell proliferation and metabolism (77). Therefore, the balance of prenylation participates in signal transduction through pathways involved in NAFLD/HCC progression.

Other factors involved in protein prenylation in NAFLD/HCC progression

Proinflammatory cytokine release regulated by protein prenylation

Inflammation plays a key role in extracellular matrix deposition and fibrosis, which further leads to HCC (78). The progression from NAFLD to fibrosis requires glucose, lipid, and amino acid metabolic reprogramming in response to a stressful microenvironment (79). Such metabolic changes further contribute to hepatic inflammation via enhanced proinflammatory cytokine production and macrophage infiltration. In a previous study, we illustrated a glycolysis-inflammation regulatory network in hepatocytes, referring to reprogrammed metabolism and inflammation in hepatocytes, with macrophages collectively accelerating progression from NAFLD to fibrosis (4). Further evidence indicates that proinflammatory cytokines, such as interleukin-6, C-reactive protein, C-peptide, and adiponectin, are essential for the development of fibrosis from NASH (31). Previous work has shown that statins can reduce C-reactive protein production stimulated by interleukin-6 (80), further inhibiting inflammatory reactions and hepatic tissue fibrosis. Overall, proinflammatory cytokine release regulated by altered protein prenylation is essential for the progression of NAFLD to fibrosis.

Rab geranylgeranylation and mitochondrial function

Mitochondrial quality control (QC) plays an important role in HCC progression, as mitochondrial dysfunction is commonly found in HCC (81); moreover, by eliminating damaged mitochondria, mitophagy is required for mitochondrial QC (82). Therefore, accumulation of damaged mitochondria is attributed to defective mitophagy, a metabolic defect in patients with NASH (83). Defective mitophagy and mitochondrial biosynthesis can promote fibrosis, which is one of the most important factors during HCC progression. Rab has a cru-
cular role in membrane trafficking, particularly vesicle formation, transport, fusion, cargo selection, and sorting, which are regulated by prenylation. Indeed, as a mitophagy effector downstream of the ubiquitin E3 ligase Parkin, Rab7 is fundamental in mitophagy (84). In our study, Ggpps deletion impaired Rab7 geranylgeranylation and mitophagy, consequently causing defects in mitophagy in hepatocytes, suggesting that dysregulation of Rab7 geranylgeranylation is a susceptibility factor for NAFLD/fibrosis progression. Thus, Ggpps-mediated prenylation in mitochondrial QC is important during HCC progression (4). Furthermore, we analyzed the CaaX motifs in mitochondria-related proteins of mice and humans and found several potential mitochondrial prenyltransferase substrates, including Rab24 (85), Rab32 (86), and Rab35 (87), which may also influence mitochondrial function.

**FPP/GGPP as ligands promoting the progression of NAFLD to HCC**

The metabolic changes that occur in tumors not only provide energy for cell division but also generate metabolites for downstream signaling. The isoprenoid and sterol metabolites produced by the MVA pathway also perform signaling functions. FPP and GGPP, as metabolites of the MVA pathway, can act as ligands and directly bind to proteins, thus acting as individual molecules rather than prenylation agents. FPP can directly interact with FXR to control the activity of downstream pathways (88), whereas GGPP binds with Skp2 (28) and PPARγ (25) (Fig. 2, right). Currently, we are working to identify some proteins to which GGPP/FPP bind, which may predict metabolic properties and NAFLD progression.

**Drugs targeting the MVA pathway in NAFLD/HCC progression**

As mentioned above, the balance of prenylation determines the progression from NAFLD to HCC. Thus, identifying a drug-targeting mediator of the prenylation balance involved in the MVA pathway can provide insight into prospective targeted therapies for NAFLD and HCC.

The MVA pathway is one of the most commonly clinically targeted biochemical pathways in human diseases, and inhibitors of the MVA pathway, such as statins and bisphosphonates, are extensively used in clinical trials (90). Statins, including lovastatin and atorvastatin, inhibit HMGCR, lowering the plasma cholesterol level in the treatment of hypercholesterolemia and atherosclerosis (91, 92). HCC is also responsive to statins (93), perhaps due to the hepatotropic pharmacology of these drugs (94). In addition, reports indicate that statins can act as adjuvants to stimulate the immune system and boost immunity as potent cancer therapeutics targeting immune activation (95, 96). Statins can also reduce cancer stemness, targeting cancer stem cells without impairing the function of innate immunity. Mechanistically, statins attenuate the prenylation of Ras family proteins, which is also highly clinically significant in HCC (97, 98). In addition, simvastatin decreases the synthesis of FPP and GGPP and exhibits anticancer effects against HCC by inducing G0/G1 arrest through up-regulation of p27 (99).

On the other hand, GGTase1 and FTase are required for tumor progression and maintenance (69, 100). GGTIs have been shown to be efficient in treating several cancers and inflammatory diseases (101), and one such agent, GGTI-2418, has been assessed in Phase I clinical trials (102). Two FTIs that have advanced to Phase III clinical trials are lonafarnib (102) and tipifarnib (103–105). However, these drugs could not improve the outcomes of advanced pancreatic cancer (103), advanced colon cancer (104), advanced non-small-cell lung cancer (102), or acute myeloid leukemia (105). More importantly, as H-, N-, and K-Ras require prenylation for their transforming activities (106), inhibition of prenylation exhibits significant therapeutic efficacy in ~30% of humans cancers driven by Ras mutations (102, 107). The results of these collective drug studies indicate that the MVA pathway can be considered a druggable target for HCC therapy.

**Prospective applications**

Although statins exhibit therapeutic efficacy in NAFLD/HCC, long-term use of statins is associated with adverse effects, such as myopathy (108) and liver injury (109). These effects might develop because statins suppress the MVA pathway from its initiation, thus leading to isoprenoid depletion, which was later demonstrated to be essential for cellular functions. Approximately 300 proteins related to cellular functions, such as membrane trafficking and signal transduction, in the human proteome are modified by prenyltransferases (110). Therefore, to prevent these side effects, other therapies combining different types and doses of MVA pathway inhibitors should be evaluated to achieve the best treatment effect without influencing protein prenylation. In addition, other inhibitors, such as nitrogenous bisphosphonates (NBPs) and DGBP, hold great promise for the treatment of NAFLD by targeting the balance of protein farnesylation and geranylgeranylation.

NBPs, including zoledronic acid, as potent inhibitors of FPPs, are widely used to treat osteoporosis and metastatic bone disease and may also be applied in cancer (111). Similar to statins, zoledronic acid reduces the intracellular levels of both FPP and GGPP, leading to inhibition of protein prenylation (112). Intriguingly, it depletes GGPP more efficiently than FPP (113). This effect might be explained by the observation that GGPPS contains three NBP-binding sites that result in its inhibition by NBP treatment (114); alternatively, it might arise due to the suppression of squalene synthase by NBPs (114, 115), which may ultimately restore the FPP level. Researchers have also demonstrated the cellular effects of NBPs in promoting apoptosis and autophagy, which are mainly mediated via downstream GGPP depletion and decreased protein geranylgeranylation (114, 116). In our previous study, zoledronic acid, an NBP, inhibited FPPS and reduced lipid deposition in the liver, as demonstrated in both mice with HFD-induced hepatic steatosis and ob/ob mice (39). Considering the changes observed in NAFLD progression, such inhibitors can be used in clinical trials on HCC prevention and treatment.

In addition to developing inhibitors of HMGCR and FPPS, some research groups have recently developed several dialkyl bisphosphonate inhibitors of GGPPS, including DGBP, a potent inhibitor of GGPP synthesis (IC50 = 200 nM) (117). Through depletion of intracellular GGPP, DGBP specifically impairs protein geranylgeranylation without changing farnesy-
lation (118). However, other studies have shown that DGBP administration causes intracellular FPP accumulation (26, 89). In addition, another study demonstrated that RAM2061, a potent GGPPPS inhibitor, is efficient against pancreatic ductal adenocarcinoma (67). In summary, inovation with GGPPS inhibitors provides a NAFLD-HCC therapy with improved selectivity. More studies investigating the balance of farne-sylation and geranylgeranylation in NAFLD progression are required to promote the development of NAFLD-HCC therapies.

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