STATINS AND PROTEIN PRENYLATION IN CANCER CELL BIOLOGY AND THERAPY

Carmen García-Ruiz[1], Albert Morales[1], and Jose C. Fernández-Checa[1,2]

[1]Department of Cell Death and Proliferation, IIBB-CSIC, and Liver Unit-Hospital Clinic IDIBAPS, 08036-Barcelona, Spain; [2]Research Center for Alcoholic Liver and Pancreatic Diseases, Keck School of Medicine of the University of Southern California, Los Angeles, CA. USA.

Grant Support: The work was supported by CIBEREHD, Fundación Mutua Madrileña, Fundació la Marató de TV3, PI11/03025 (META) and grants PI09/0056 (Instituto de Salud Carlos III), SAF2009-11417, SAF2008-02199, SAF2011-23031 (Plan Nacional de I+D), Spain; and P50-AA-11999 (Research Center for Liver and Pancreatic Diseases, NIAAA/NIH).

Correspondence to: Carmen Garcia-Ruiz, cgrbam@iibb.csic.es, or José C. Fernandez-Checa, checa229@yahoo.com. Address: Centro Esther Koplowitz, C/ Rosselló 151, 08036-Barcelona, Spain.

ABSTRACT

The use of statins has scaled up to become one of the most prescribed medicines in the world and have been very useful in the manegement of cardiovascular diseases and related mortality. The disclosure of their chemical structure similar to that of hydroxy methyl glutaryl-CoA (HMG-CoA) revealed their ability to compete with and inhibit the rate-limiting enzyme HMG-CoA reductase that catalyzes the synthesis of mevalonate, which then serves as the precursor for isoprenoids and cholesterol in the mevalonate pathway. While most of the effects of statins are associated with the lowering of cellular cholesterol levels, it is clear that they also blunt the non-sterol branch of the mevalonate pathway, decreasing formation of isoprenoids and altering protein-prenylation, a critical event in the posttranslational modulation of proteins involved in the regulation of cell cylce progression, proliferation and signaling pathways. Randomized controlled trials for the prevention of cardiovascular diseases indicated that statins elicited provocative and unexpected benefits for reducing a number of different types of cancers, including colorectal carcinoma, melanoma, prostate and hepatocellular carcinoma, although in other cancer types the preclinical expectations of statins were dissapointing. In this review, we will describe the evidence and mechanisms underlying the potential beneficial use of statins and the role of protein prenylation in cancer prevention. Of relevance, the combination of statins with other anti cancer drugs may be a significant asset in malignancies resistant to current therapy.

INTRODUCTION

Statins are a class of small molecules that lower cholesterol levels by inhibiting the rate-limiting enzyme hydroxymethylglutaryl-CoA (HMG-CoA) reductase, which plays a central role in the production of cholesterol in the liver. The screening of fungal extracts for inhibitors of cholesterol biosynthesis led to the discovery in 1976 of compacting, also known as mevastatin, as the first small molecule that blocked the conversion of radiactive acetate in cholesterol in rat liver membranes[1]. The mechanism of action of this pioneering statin involved the competitive inhibition of HMG-Co reductase, which catalyzes the conversion of HMG-CoA into mevalonate [2]. Because statins are similar to HMG-CoA on a molecular level they take the place of HMG-CoA in the enzyme and reduce the rate by which it is able to produce mevalonate. Due to the critical role of cholesterol as a constituent of atherosclerotic plaques lining the walls of blood vessels and the association between increased cholesterol and cardiovascular disease, the use of statins to reduced plasma cholesterol levels has scaled up to become one of the most prescribed medicines in the world [3]. One of the benefits associated with the use of statins is obviously linked to decrease incidents of cardivascular events and heart attacks. In addition, increasing evidence in preclinical and epidemiological studies have indicated that another potential benefit of statins use is associated with lower incidence of cancer development, particularly colorectal carcinoma, melanoma, prostate and hepatocellular carcinoma [4]. Even though this outcome indicate a critical role for cholesterol lowering in the potential beneficial association of statins and cancer prevention, due to the nature of the mevalonate pathway the effect of statins are beyond just preventing cholesterol synthesis. Indeed, there are several potential mechanisms whereby statins could influence

the incidence and progression of cancer development and therapy response, including the regulation of non-sterol components that are synthesized in the mevalonate pathway including dolichol, ubiquinol and the isoprenoids farnesol and geranylgeraniol, which in turn regulate the function of key proteins involved in cell growth and proliferation [5]. In addition, statins inhibit the activation of the proteasome pathway, contributing to the maintenance of proteins that block cell cycle. Moreover, through cholesterol downregulation, statins may also regulate the function of the Hedgehog, a signaling pathway that besides its critical function as a morphogen can promote carcinogenesis as well. Hence in this review, we will briefly cover the evidence for the role of statins in cancer prevention, particularly colorectal carcinoma, pancreas and hepatocellular carcinoma, focusing on the mevalonate pathway and protein prenylation to fully understand the actions of statins beyond cholesterol regulation.

STATINS AND THE MEVALONATE PATHWAY

Through the pionering work of Akira Endo [1], the molecular mechanism of statins is the inhibition of HMG-CoA reductase, which sits at the apex of the molecular pathway of cholesterol synthesis in the so-called mevalonate pathway [2]. However, although this process is essential in the synthesis of cholesterol, it also generates a number of non-sterol molecules that play critical roles in many different cell processes by regulating key proteins post-translationally. In addition to its crucial role in membrane structure and properties, cholesterol has been described in the regulation of a number of cellular processes and pathways, including cancer biology and therapy [6,7,8]. The contribution of cholesterol in promoting cancer is well known and has been described for more than a century when John H. Webb suggested that cancer was due to the crystalization of

cholesterol from living cells [9]. Later observations confirmed the accumulation of cholesterol in tumors, suggesting that cholesterol is associated with the regulation of cell proliferation [10]. While the role of cholesterol in cancer has been considered previously [4,11,12], in this review we will focus primarily on the contribution of the non-sterol branch of the mevalonate pathway in cancer biology. In the mevalonate pathway acetyl-CoA is converted to HMG CoA, which is then transformed into mevalonate catalyzed by HMG-CoA reductase [2]. The phosphorylation of mevalonate yields 5-pyrophosphomevalonate, which is converted to isopentenyl pyrophosphate (IPP) (**Figure 1**). IPP can be reversibly transformed to dimethylallyl pyrophosphate (DMAPP), and the combination of both IPP and DMAPP yields the 10-carbon isoprenoid geranyl pyrophosphate (GPP). The sequential addition of 1 or 2 more IPP units to GPP generates the 15-carbon and the 20-carbon isoprenoids farnesyl pyrophosphate (FPP) and the geranylgeranyl pyrophosphate (GGPP), respectively. FPP branches into the non-sterol pathways, which contribute to the generation of other derivatives such as ubiquinol, dolichol, and the sterol pathway via conversion into squalene by squalene synthase, which catalyzes the first committed step in cholesterol synthesis. As it can be inferred from this metabolic picture, the inhibition of HMG-CoA reductase by statins not only blunts cholesterol synthesis but also depress the generation of isoprenoids impairing protein prenylation. This dual function of statins underlies their cholesterol-independent effects in a number of pathologies, and provides the basis for the efficacy of statins in reducing cardiac events beyond their effect in reducing cholesterol levels [13,14]. As indicated above, mevastatin, the first characterized statin, is a natural compound and was identified from Penicillium Citrinum, a mold that infects the Japanese orange [1]. Many

statins are derived from fungi, such as lovastatin (**Figure 2**) or made synthetically such as atorvastatin (**Figure 2**). The efficacy to block HMGCoA reductase depends on the structure and physical properties of statins. All available statins are lipophylic except pravastatin, a natural statin derived from fungi, and have a side chain with either an open-ring (acid) or closed-ring (lactone) structure. The latter is an inactive prodrug that is converted to the active form, β-hydroxy-acid, by carboxyesterases in the liver. It has been shown that HMG-CoA reductase is bound about 1000 times more effectively by statins with open-ring structures than by its natural substrate, HMG-CoA and exhibit a high potency in blocking the mevalonate pathway [12]. Unlike other statins, the active hydroxy metabolites of atorvastatin, particularly the o-hydroxy derivative, exhibit the same enzymatic inhibition of HMGCoA reductase as the parental statin. In addition, the active o-hydroxy derivative of atorvastatin has been described to prevent cholesterol domain formation by an antioxidant mechanism [15].

In addition to the regulation of mevalonate, which impacts on the modulation of cell cholesterol levels and protein-prenylation, statins also affect other cellular processes including the inhibition of the proteasome pathway [16,17], cell adhesion, migration and invasion via inhibition of the interaction between the integrin lymphocyte function-associated antigen 1 (LFA1) and intercellular adhesion molecule 1 (ICAM1) [18]. Giving the role of protein prenylation as a key mechanism involved in the regulation of cell differentiation, cell cycle progression and apoptosis by targeting specific proteins [19,20,21,22], in the following sections we will briefly describe the players involved in this important pathway which is of relevance in cancer cell biology.

PROTEIN-PRENYLATION

As described above the mevalonate pathway is responsible not only for the synthesis of cholesterol but also for the generation of non-sterol isoprenoids, FPP and GGPP, which bind to and regulate target proteins in a process named protein prenylation (**Figure 1**). FPP and GGPP are covalently attached to cysteine residues in CaaX motifs at the C-terminus of proteins, where C is a cysteine moiety next to two aliphatic residues with X being any aminoacid, leading to the farnesylation or geranylgeranylation of the target protein [23] (**Figure 3**). While farnesylation is catalyzed by farnesyltransferase (FTase), geranylgeranylation is catalyzed by two geranylgeranyltransferases, GGTase I and II. Once prenylated, proteins undergo postprenylation modifications, including the proteolytic processing that removes the aaX tripeptide catalyzed by the prenyl protease named Ras-converting enzyme 1 (RCE1) and the addition of a methyl group to the C-terminal prenylcysteine catalyzed by the enzyme isoprenylcysteine carboxyl methyltransferase (ICMT) which uses S-adenosyl-L-methionine as methyl donor [24]. From a functional perspective, prenylation of proteins is viewed as a tool that promotes their membrane attachment, binding to other signaling proteins and afford protection against proteolytic degradation [25]. The functional role of these posttranslational modifications depends on the nature of the targeted protein. For relevance in this review, many prenylated proteins are involved in various aspects of carcinogenesis, including cellular proliferation, apoptosis, angiogenesis and metastasis [26,27]. For instance, prenylation of proteins such as Ras, lamin B or centromere proteins (e.g. CENP-E and CENP-F) play a critical role in the regulation of cellular proliferation, while the prenylation of Rac, Rho and protein tyrosine phosphatase, PTP4A3 modulate apoptosis, angiogenesis and metastasis, respectively. Thus, given the important function of protein

prenylation in the regulation of a number of steps involved in carcinogenesis, several enzymes involved in protein prenylation have been targeted pharmacologically to modulate cancer cell biology [28,29,30]. Besides the development of inhibitors for FTase, GGTase I and II, RCE1, ICMT, other pharmacological agents regulating protein prenylation at different levels have been developed for potential anticancer effects, including those targeting mevalonate pyrophosphate decarboxylase (sodium phenylacetate and sodium phenylbutyrate), IPP isomerase, FPP synthase (aminobisphosphonates) and FTase (FTIs) and GGTase (GGTIs) inhibitors. In addition, statins by inhibiting HMG-CoA reductase impact negatively on the generation of isoprenoids, including FPP and GGPP, which in turn, regulate protein prenylation. However, as described below, the outcome with single-agent inhibition, including statins, in cancer cell biology did not meet the predicted expectations based on preclinical experience [31], providing the rational for combination therapy targeting the mevalonate pathway.

MONOTHERAPY VS COMBINATION THERAPY TARGETING THE MEVALONATE PATHWAY AS AN ANTICANCER STRATEGY

Monotherapy

Although the use of statins is prevalent because elevated total cholesterol and low density lipoproteins (LDL) levels are major risk factors for coronary heart disease [32], and in most cases safe, there can be side effects associated with statin use, such as myopathy and hepatotoxicity [33], which are commonly speculated to be due to the depletion of nonsterol components of the mevalonate pathway [34]. Furthermore, statin use does not always reduce plasma LDL to desired levels [35], which is particularly important to

mediate the therapeutic potential of statins in cardiovascular events [36,37,38]. LDL is the major cholesterol-carrying lipoprotein in the blood and the liver is the major organ for LDL clearance via mechanisms essentially mediated by LDL receptor expressed in hepatocytes. LDL-LDL receptor interaction is responsible for most of LDL removed from the circulation and LDL receptor deficiency is the major cause for familial hipercolesterolemia [39]. However, proteins that regulate LDL receptor posttranscriptionally determine the response to statin-mediated LDL receptor overexpression. For instance, PCSK9 is a protein that plays a critical role in post-translational degradation of LDL receptor. PCSK9 is highly expressed in hepatocytes and small intestine and is a sterol-responsive gene, up-regulated by statins-mediated SREBP-2 activation [40]. This may explain why many patients undergoing statin treatment to increase LDL receptor expression cannot always attain their therapeutic goals.

Given the function of HMG-CoA reductase in the mevalonate pathway its inhibition by statins leads to a reduction of cellular FPP and GGPP levels, resulting in the impairment of protein prenylation. However, although such as dual function may account for the positive role of statins in regulating carcinogenesis, the chronic inhibition of HMG-CoA may lead to undesired effects in the mevalonate pathway, accounting for the dissociation between the effects observed in experimental models and clinical trials. For instance, HMG-CoA reductase levels are negatively regulated by complex transcriptional, translational and posttranslational feedback mechanisms controlled by both sterol and non-sterol products of the mevalonate pathway [2]. Reduction of isoprenoid and cholesterol levels by statins leads to upregulation of HMG-CoA reductase levels and, eventually, development of resistance [2,41]. I*n vitro* mechanistic studies of statins used

significantly higher concentrations than those that were therapeutically achievable in phase I trials or with standard anticholesterol dosing. Dose-limiting toxicities, including gastrointestinal side effects, myelotoxicity, myalgias, elevation of creatine phosphokinase and hepatotoxicity, precluded further dose increase in clinical trials [31]. Moreover, studies in experimental models indicated that statin effects are mostly conferred through a reduction in geranylgeranylation (specifically, geranylgeranylation of Rho and Rac) rather than a reduction in farnesylation [20,42]. Similar to the use of statins as monotherapy, sodium phenylbutyrate, which inhibits mevalonic pyrophosphate decarboxylase, exhibited limited activity in clinical trials at doses that were effective in experimental studies [43,44]. However, undesired neurotoxic effects prevented the possibility of using higher doses in clinical trials. Moreover, by inhibiting Ras farnesylation, FTIs inhibit multiple downstream pathways, such as Raf–MAPK kinases-ERK or Akt, Tiam1–Rac, which are involved in cellular survival and proliferation [45,46]. However, despite promising preclinical evidence, the antitumor activity of FTIs as single agents in most solid tumors has been disappointing, perhaps with the exception of hematological malignancies, in which FTIs exhibited a promising response [47]. A plausible explanation for the lack of efficiency of FTIs in cancer therapy is the occurrence of cross-prenylation of specific target proteins. For instance, it has been shown that both K-Ras and N-Ras can also be geranylgeranylated by GGTase I as an alternative method of prenylation [48], which underlies the refractoriness to the inhibition of K- and N-Ras farnesylation by FTIs. Unlike K-Ras and N-Ras, H-Ras is only farnesylated and cannot be geranylgeranylated. Moreover, K- or N-Ras-transformed cells exhibit decreased response to FTIs compared to H-Ras-transformed cells during

carcinogenesis, and it has been shown in a mouse model dependent on K-Ras that protein farnesylation was not required for lung carcinogenesis [49]. Thus, these findings underscore that although FTIs focused mainly on H-Ras activation, it is increasingly recognized that K-Ras, N-Ras and other proteins are more important than H-Ras in carcinogenesis.

The conversion of FPP into squalene by squalene synthase (SS) serves as the first committted step towards the synthesis of cholesterol, which requires extensive oxygen consumption. Inhibition of SS has attracted much interest as a pharmacological target as it implies the inhibition of cholesterol synthesis without depressing isoprenoid levels, and consequently, various compounds have been identified as inhibitors [50,51]. For instance, lapaquistat (TAK-475, Takeda), a SS inhibitor, progressed to phase III clinical trials, but studies were discontinued after the US Food and Drug Administration recommended suspension of studies with high-dose (100 mg/kg) monotherapy due to hepatotoxicity manifested as elevated levels of liver transaminases [51]. However, it is uncertain whether this outcome was due to an enzyme inhibitory class effect or whether it was specific to the drug. Unlike statins, inhibition of SS can result in the accumulation of both FPP and FPP metabolites, such as farnesol-derived dicarboxylic acids [52], which could contribute to the hepatotoxic effects observed with the high-dose monotherapy of lapaquistat. For instante, farnesol itself can be proapoptotic at high concentrations [53]. Interestingly, the combination therapy of SS inhibitors (e.g. lapaquistat) with statins would be expected to avoid potential accumulation of FPP metabolites. For instance, combination therapy of lapaquistat and statins showed additional LDL reduction compared with statins alone [51]. Of relevance, T-91485, the active metabolite of

lapaquistat, is capable of preventing statin-induced myotoxicity in a human skeletal muscle cell model [54]. Moreover, lapaquistat is able to prevent statin-induced myotoxicity in a guinea pig model [55]. In addition to the expected cholesterol depletion, other SS inhibitors have shown the potential for added benefits due to decreased triglyceride biosynthesis [56], most likely due to a farnesol-mediated mechanism [57]. Nitrogenous bisphosphonates (NBP; e.g., zoledronate and alendronate) are a second class of clinical drugs targeting the mevalonate pathway, and they are used for treatment of bone-related disorders such as osteoporosis. NBPs function by inhibition of FPP synthase, resultingin depletion of cellular levels of FPP and other downstream isoprenoids [58]. Bisphosphonates may be regarded as analogs of diphosphates, in which the central bridging oxygen atom (P-O-P) has been replaced with a carbon (P-C-P). This results in increased metabolic stability and allows chemical functionalization of the bisphosphonate core. Furthermore, the P-C-P linkage combined with an α-hydroxy group facilitates bone targeting [59], although other reports indicated the ability of nitrogenous bisphosphonates to decrease cholesterol levels in patients with osteoporosis and hyperlipidemias [60]. In addition, recent findings indicated the ability of statins and aminobisphosphonates to extend longevity in progeria [61]. Several human progerias, including Hutchinson-Gilford progeria syndrome (HGPS), are caused by the accumulation at the nuclear envelope of farnesylated forms of truncated prelamin A, which can be prevented by FTIs. However, alternative prenylation of laminA by geranylgeranyltransferase in the setting of FTase inhibition, could explain the low efficiency of FTIs in ameliorating the phenotypes of progeroid mouse models. Interestingly, recent studies show that a combination of statins and

aminobisphosphonates efficiently inhibits both farnesylation and geranylgeranylation of progerin and prelamin A and markedly improves the aging-like phenotypes of a mouse model of human premature aging [61].

Thus, since metabolites of the mevalonate pathway are a key tool for the posttranslational modification of proteins, their modulation may have far reaching consequences other than decreasing cholesterol levels, which ultimately may be of relevance in many pathophysiological processes including cancer biology and therapy.

Combination therapy

Given these pitfalls in the use of montherapy targeting the mevalonate pathway as a strategy for fighting cancer, other alternatives include combination therapy aiming at simultaneous inhibition of both geranylgeranylation and farnesylation, or concomitant reduction of isoprenoid availability and inhibition of prenylation and postprenylation enzymes.

Combinations of FTIs and GGTIs have been shown to induce synergistic cytotoxicity, apoptosis and disruption of Ras–MAPK signaling. In different myeloma cells, which often exhibit K- and N-Ras mutations, FTIs or GGTIs separately failed to block prenylation of K- and N-Ras, while GGTI–FTI combinations inhibited prenylation of all Ras isoforms and induced a more potent blockade of the Ras–MAPK signaling cascade [62]. GGTI–FTI combinations synergistically inhibit proliferation of multiple myeloma cell lines and primary cells, and induce apoptosis. Interestingly, dual prenylation inhibitors (DPIs) that block both FTase and GGTase enzymatic activities have been shown to induce apoptosis in PSN-1 pancreatic tumor cells by blocking K-Ras prenylation compared to either FTI or GGTI agents alone [63]. H- N- and K-Ras

prenylation exhibit differential susceptibility to FTI vs GGTIs inhibition. For instance, H and N-Ras prenynation is effectively inhibited by FTIs and only partially by GGTIs, whereas K-Ras prenylation requires both FTIs and GGTIs inhibition [64]. In addition, Rho proteins can also be alternatively prenylated, and FTIs alone are unable to inhibit Rho prenylation, whereas GGTI–FTI combinations are able to inhibit it effectively [62]. Thus, combined inhibition of geranylgeranylation and farnesylation can overcome the resistance conferred by cross-prenylation, thus potentiating the activity of either FTIs or GGTIs alone. Although FTI and GGTI combinations have demonstrated enhanced antiproliferative and proapoptotic activities *in vitro* (relative to FTI monotherapy), the concern with this particular combination therapy relates to its cytotoxicity, which is mainly due to the GGTIs. Indeed, DPIs which simultaneously inhibit GGTase I and FTase can overcome the toxicity of GGTIs and GGTI–FTI combinations. Interestingly, agents that mimic the CaaX moiety have been shown to exhibit significant inhibitory activity against GGTase I (besides FTase), and are well tolerated in Phase I and II clinical trials [65,66], suggesting they may be promising agents for cancer cell therapy.

Moreover, by inhibiting the mevalonate pathway and reducing the biosynthesis of cholesterol and isoprenoids, statins interfere with membrane synthesis and decrease the *N*-glycosylation of growth factor receptors such as the insulin-like growth factor receptor [41]. Although inhibition of protein farnesylation by FTIs might lead to increased levels of FPP, which can be used for the synthesis of cholesterol and dolichyl phosphate, the addition of statins would be expected to prevent this alternative pathway. Therefore, combinations of statins and FTIs act synergistically to inhibit malignant cell proliferation and induce apoptosis, causing a more effective inhibition of prenylation modifications by

decreasing isoprenoid levels and by inhibiting geranylgeranylation and farnesylation. For instance, in multiple myeloma cells, FTIs potentiate the ability of lovastatin to inhibit Rho, K- and N-Ras prenylation and MEK-MAPK activation, resulting in impaired cell migration and enhanced apoptosis [62]. *In vitro* doses of lovastatin combined with FTIs have been demonstrated to be at least as effective as double doses of lovastatin administered alone, indicating that statin–FTI combinations might be of relevance to achieve therapeutic effects without the potential toxicity of using higher doses of statins alone.

Since one of the mechanisms of resistance to statins is upregulation of HMG-CoA reductase levels, tumor cells exhibit a relative resistance of HMG-CoA reductase to sterol feedback mechanisms but are particularly sensitive to isoprenoid-mediated suppression [41]. Therefore, the combination of isoprenoids with statins can counteract the statin-induced upregulation of HMG-CoA reductase levels, leading to a more potent inhibition of the mevalonate pathway. For instance, it has been shown that the combination of lovastatin and the isoprenoid γ-tocotrienol synergistically inhibits growth of human DU145 and LNCaP prostate carcinoma and murine B16 melanoma cells [42].

In addition to the beneficial combination of statins and aminobisphosphonates in premature aging and progeria [61], there is experimental data to support their synergistic effects in anticancer therapy, reflected by more efficient apoptosis induction and reduced tumor cell invasiveness in vitro as well as decreased in vivo metastasis [67,68]. The underlying mechanisms for this synergistic action of statins and aminobisphosphonates derive from their individual effect on the mevalonate pathway, namely, inhibition of HMG-CoA reductase (by statins) and FPP synthase and IPP isomerase (by

aminobisphosphonates), leading to decreased FPP synthesis and a reduction in cholesterol and dolichyl phosphate synthesis, which impact on the rate of protein prenylation and postprenylation and alteration in membrane dynamics, which in turn regulate different growth factors receptors [41]. In addition, the beneficial effect of the combination of statins and aminobisphosphonates as anticancer therapy may be exerted through mechanisms independent of the mevalonate pathway. While statins regulate cell invasion, migration and adhesion via modulation of LFA1 and ICAM1 [18], bisphosphonates contribute to the regulation of these processes by decreasing expression of integrins (e.g. avβ3 integrin) and inhibiting angiogenic factors such as VEGF, PDGF and FGF [69,70,71].

Consistent with the essential role of prenylation in protein biology and function, targeting this pathway may be also of relevance in cancer biology, particularly in combination with agents that inhibit postprenylation events. As alluded above, prenylated proteins undergo proteolytic processing such as the removal of the aaX tripeptide catalyzed by RCE1 and the addition of a methyl group catalyzed by ICMT. While inhibition of RCE1 has moderate antitumor effects, this process sensitizes tumor cells to FTI treatment. For instance, FTIs are more efficient in inhibiting cell growth of fibroblasts and skin carcinoma cells that are deficient in RCE1 activity [72]. Furthermore, although inhibition of ICMT alone does not completely abrogate downstream Ras signaling, such ERK1/2 and Akt activation [73,28], which is expected to have a minor role as anticancer strategy, the combination of ICMT inhibitors with FTIs or GGTIs or statins may be more effective in suppressing the growth of Ras-dependent tumors.

HEDGEHOG, CHOLESTEROL AND CANCER

Hedgehog (Hh) is a morphogen essential for embryonic development, but its overactivation is recognized as a key mechanism that fosters cancer development [74]. Of relevance here, there is evidence linking the Hh signaling with the mevalonate pathway, and that cholesterol is a key regulator of the Hh pathway. The Hh signaling pathway was first described in genetic studies of embryonic segmentation in Drosophila. However, it is highly conserved from insects to vertebrates. In mammals, three hedgehog homologs, Sonic (Shh), Indian (Ihh), and Desert hedgehog (Dsh) are known. Shh is the most commonly expressed and the best characterized homolog; it is crucial for the development and maintenance of the nervous system, axial skeleton, lungs, skin, hair, and stem cell populations. Shh is synthesized as a 45 kDa precursor protein that is auto-catalytically cleaved and covalently modified by palmitate and cholesterol [75]. Once Shh is secreted it binds to its receptor Patched 1 (Ptch1), a 12-transmembrane receptor for Shh, which functions as the key inhibitor of Shh signaling (**Figure 4**). Ptch1 inhibits Shh signaling by inhibiting activity of the 7-transmembrane receptor, Smoothened (Smo), a positive regulator of signaling [76]. The repression of Smo by Ptch1 inactivates downstream Shh effectors, namely the glioma-associated (Gli) family of transcription factors. The Gli transcription factors (Gli1, Gli2, Gli3) control the expression of Shh target genes including *Ptch1* and *Gli1* themselves, which provide negative and positive feedback regulation of Hh signaling, respectively. Binding of the Shh ligand to Ptch1 causes both Shh and Ptch1 to be sequestered into endocytic vesicles, which relieves the inhibition of Smo. As transcription factors, Gli can regulate target gene expression by direct association with a consensus binding site (5′-tgggtggtc-3′) located in the promoter region of the target genes. Direct downstream targets of Hh signaling include Bcl2,

Cyclin D1, Cyclin D2, FoxM1, FoxE1, Hip, and PDGFR among others [77,78]. As many of these genes are directly involved in cell cycle regulation (cyclins) and cell survival (Bcl2), their activation accounts for the contribution of Hh to tumor cell proliferation and hence cancer development.

The cholesterol connection to the Hh pathway is exerted at different levels. For instance, the Hh proteins are the only known family of proteins that have covalently bound cholesterol. In addition, the 12-transmembrane domains of Ptch1 include a five-transmembrane sterol-sensing domain that is also observed in several other proteins that participate in cholesterol metabolism, including SCAP [2]. Recent evidence suggested that Ptch1 could function as a mechanism for the efflux of cholesterol from cells, and that this novel action could contribute to Smo repression [79]. In the presence of cholesterol the inhibition of Ptch1 on Smo is relieved allowing the endocytic trafficking of Smo and Ptch1 to stimulate the transcriptional activation of target genes by the transcription factor Gli (**Figure 4**). This effect of cholesterol may be specific to the presence of the sterol in the plasma membrane, which may induce a conformational change in Ptch1 that alters its intracellular trafficking to allow Smo signaling. However, when cholesterol levels are low, as expected following the administration of statins or caused by genetic defects in the mevalonate pathway, the binding of Hh to Ptch1 fails to activate Smo, presumably due to insufficient membrane cholesterol to induce conformational change in Ptch1. Moreover, various enzymatic defects in the final steps of the cholesterol biosynthesis pathway give rise to multiple developmental anomalies due to impaired Hh signalling. For instance, vitamin D_3 is formed by the action of ultraviolet light on 7-dehydrocholesterol, an immediate precursor of cholesterol and the sterol that accumulates

in a relatively common genetic disorder, Smith–Lemli–Opitz syndrome (SLOS). Although predisposition for tumor development is not considered a feature associated with SLOS, a recent report described the first observed association of SLOS with malignant intracranial germ-cell tumor [80].

It is known that constitutive activation of the Hh pathway leads to tumorigenesis such as that seen in basal cell carcinomas and medulloblastoma. A variety of other human cancers, including brain, gastrointestinal, lung, breast and prostate cancers, also demonstrate inappropriate activation of this pathway [74]. Paracrine Hh signaling from the tumor to the surrounding stroma was recently shown to promote tumorigenesis. Moreover, this pathway has also been shown to regulate proliferation of cancer stem cells and to increase tumor invasiveness, indicating that targeted inhibition of Hh signaling may be effective in the treatment and prevention of many types of human cancers [81]. Based on these findings it could be hypothesized that statins may regulate Hh signaling pathway via their cholesterol lowering effects, which could have a putative impact on cancer development. However, this process needs adequate testing and validation in experimental models.

STATINS, COLORECTAL AND PANCREATIC CANCER

Although the effects of statins in cancer cell therapy have provided mixed results in some malignancies (e.g. breast cancer, melanoma), in the following section we will focus on specific types of cancer that have a major impact on health and in which statins use have shown more definitive outcomes. Colorectal cancer (CRC) is one of the most common cancers and the third cause of cancer-related deaths. While the causes leading to CRC are not completely understood, genetic factors and chronic intestinal inflammation (e.g.

inflammatory bowel disease) are major risk factors for CRC. Aberrant DNA methylation of CpG islands in the promoter regions of many genes occurs in human CRC and is associated with tumour suppressor gene silencing [82]. In addition, types of CRC that show extensive DNA methylation in the promoter regions of specific genes are associated with chemotherapy resistance [83]. Thus, given the health impact and death burden of CRC a better therapeutic approach is needed. In this regard, although the role of statins in CRC is controversial, there has been recent developments and evidence providing some hints for the beneficial effects of statins in CRC [84]. As described above, statins inhibit the key enzyme in the cholesterol-synthesis pathway, thereby reducing serum cholesterol levels; but the mevalonate pathway also generates intermediates that act as growth factors and are involved in cell survival pathways (**Figure 1**). Those dual and conflicting roles may help explain the mixed results of epidemiological studies on statins' effect on CRC [84]. Nevertheless, current data link statins with bone morphogenic protein (BMP) pathway, which regulates intestinal epithelial homoeostasis, differentiation, stem cell activation and CRC [85,86,87,88,89]. For instance, recent evidence indicated that statins inhibit cellular proliferation and induce apoptosis in CRC cells and in animal models via BMP regulation [90,91]. Indeed, the screening of compounds for their ability to enhance BMP expression for eventual use to enhance bone formation identified statins as the one of the most active agents [92]. Moreover, a recent study demonstrates the ability of statins to increase the chemosensitivity of CRC cells by inducing epigenetic reprogramming and reducing colorectal cancer cell stemness via the BMP [93]. Statins act as DNA methyltransferase inhibitors that upon demethylation of the BMP2 promoter activate BMP signaling, inducing differentiation of CRC cells and reducing their

stemness. Thus this recent evidence indicates that statins may be able to be used as differentiating agents in combined or adjuvant therapy in CRC exhibiting the CpG island methylator phenotype. Moreover, resistance of CRC to current chemotherapy relates to mutations in the v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS). Monoclonal antibodies (e.g. cetuximab and panitumumab) that target the epidermal growth factor receptor (EGFR) are effective in terms of response rate and progression-free survival in combination with standard cytotoxic chemotherapy in metastatic CRC [94,95]. However, mutations in KRAS, which occur in about 40% of CRC patients, is a major negative predictor for treatment response in patients receiving cetuximab [96,97]. Interestingly, a recent study reported that simvastatin overcomes cetuximab resistance in CRC cells with KRAS mutations by modulating BRAF activity and inducing apoptosis [98]. Despite these promising outcomes in preclinical studies, the recommendation of statin use for CRC in clinical practice is still far [99,100]. The contradictory results illustrate the central problem in evaluating claims for and against statins' potential as chemopreventive therapy, as most studies used data that are not specific for evaluating statins but rather are part of large epidemiological datasets, while other studies that found no association between statin usage and CRC need to take in consideration the time of statin usage and the age of patients.

Although somewhat lower compared to CRC, pancreatic cancer is still a major cause of cancer related deaths, particularly in developed countries [101]. The incidence and the failure of conventional chemotherapy to reduce the mortality associated with pancreatic cancer, dictate the need to identify and develop agents for pancreatic cancer chemoprevention and treatment. As with CRC, the association of statins with pancreatic

cancer risk is still unsettled and at variance with the findings reported in experimental approaches. For instance, antiproliferative effects of statins have been demonstrated in a number of *in vitro* as well as *in vivo* studies on pancreatic cancer cell lines regardless of Ras mutations [20,102,103,104]. Moreover, statins have been shown to sensitize pancreatic cancer cells to chemotherapeutic drugs by targeting the P2X7-Akt axis [105]. However, epidemiological studies evaluating the relationship between the use of statins and the risk of pancreatic cancer gave inconsistent findings. Some studies reported that the use of these drugs is inversely related to the risk of pancreatic cancer, whereas other studies found no or positive associations [106,107]. While several pitfalls may contribute to this unestablished scenario, including improper study designs, short follow-ups, or insufficient control for potential confounders, the type of statins used in these studies may be a critical factor. In addition to the lipophilicity of statins, other pharmacokinetic and pharmacodynamic properties might be responsible for differences in anticancer properties of individual statins. Indeed, substantial differences in antiproliferative effects on experimental pancreatic cancer cell lines with different statins used for clinical purposes have been described [108]. In this relevant study, it was found that the least efficient statins were pravastatin and atorvastatin, whereas rosuvastatin (despite its low lipophilicity) and cerivastatin were the most effective, and all statins (except pravastatin) inhibited intracellular Ras protein translocation [108]. Thus, these findings illustrate the need for proper design and controlled clinical studies to critically evaluate the role of statins in pancreatic cancer.

STATINS IN METABOLIC LIVER DISEASES AND HEPATOCELLULAR CARCINOMA

Fatty liver disease is a common cause of chronic liver injury in industrialized countries, and encompasses a spectrum of disorders ranging from fatty liver (steatosis) to steatohepatitis that can progress to cirrhosis and eventually hepatocellular carcinoma (HCC). Due to the rising prevalence of obesity and type II diabetes worldwide metabolic liver diseases, in particular, nonalcoholic steatohepatitis (NASH) constitutes a global health concern. Although the mechanisms underlying NASH are not fully undertood it is known that the onset of hepatic steatosis sensitizes to secondary factors (e.g. hypoxia, inflammatory cytokines) leading to the characteristic features of NASH such as oxidative stress, inflammation, hepatocellular death and fibrosis. Moreover, disease can progress towards HCC and obesity and high body mass index (BMI) have been shown to increase the risk for cancer, particularly HCC [109]. For instance, men with a BMI of 35-40 exhibited a staggering 4-5-fold increase in relative HCC risk [109]. HCC is one of the most frequent tumors worldwide and is generally a fatal disease, as few patients are amenable to surgery because of late HCC diagnosis. Moreover, current treatment options offer no or very low survival in HCC patients not suitable for resection [110,111]. Hence, investigations of molecular mechanisms leading to HCC development and progression is required to identify new targets for its early diagnosis, chemoprevention and treatment. Aberrant lipogenesis is key for metabolic liver disease and HCC, with reports indicating upregulation of a number of lipogenic enzymes at the mRNA and protein level in human HCC [112,113,114]. Consistent with these findings, a recent report indicated increased lipogenic enzymes and activities in human HCC and cell lines, including the stimulation of cholesterol levels in HCC tissues [114]. Moreover, an association between liver fat accumulation and HCC development has been long known [115,116]. Concerning the

evolution of simple steatosis to more advanced stages of NASH, a novel role for cholesterol has emerged, particularly with its trafficking to mitochondrial membranes [117]. Indeed, mitochondrial cholesterol accumulation has been shown to sensitize to inflammatory cytokines, contributing to the transition from steatosis to steatohepatitis by regulating mitochondrial antioxidant defense mechanisms and mitochondrial membrane permeabilization [118,119]. Interestingly, in genetic models of obesity, treatment with atorvastatin prevented the increase of cholesterol in mitochondria and the sensitization of ob/ob mice to LPS-mediated liver injury [118]. In HCC cell lines and heterotopic xenografts HCC it has been shown that the increased trafficking of cholesterol to mitochonria modulates chemotherapy sensitivity, and that *in vivo* treatment with atorvastatin sensitized nude mice harboring HCC tumors to doxorubicin, suggesting that cholesterol play a role in HCC and that the use of statins may be of relevance in HCC [8,120]. Moreover, recent findings further showed the potential therapeutic effects of statins in hepatic carcinogenesis involving mechanisms related to decreased matrix metalloproteinase activity or inhibition of autophagy [121,122,123]. However, the experience of statins in human HCC has been conflicting. This is probably because in many trials, which examined the association between statins and cancer, including HCC, the impact of statins in cancer was a secondary end point. For instance, a population-based cohort study using the Danish Cancer Registry for the period of 1989-2002 found not significant increased or decreased rate ratios for any of the studied site-specific cancers including liver [124]. However, a systematic review of 17 randomised controlled trials, 10 cohort studies, and 15 case-control studies indicated that while statins had no effect on the overall incidence of lung or breast cancer, they seemed to protect from liver

cancer [125]. Moreover, a population-based case–control study in Taiwan over a thounsand cases of liver cancer and matched controls, indicated that the use of statins may reduce the risk of liver cancer [126]. However, trials addressing the potential therapeutic effect of statins (pravastatin) indicated a potential beneficial role in modulating HCC progression. For example, a clinical trial of 91 patients with advanced HCC reported that the median survival of 18 months of patients on pravastatin was twice that of controls [127]. Although this effect was not confirmed in a shorter cohort (51 patients) [128], another trial with 131 patients showed that the combination of chemoembolization with pravastatin improved survival of patients with advanced HCC in comparison to patients receiving chemoembolization alone [129]. Furthermore, a recent study using a large cohort of diabetics, whose risk of HCC is higher than average, concluded that the use of statins is associated with a significant reduction in the risk of HCC among patients with diabetes [130]. Collectively, these clinical studies strongly suggest the need to further explore the potential therapeutic relevance of statins alone or in combination with other treatments in HCC.

CONCLUDING REMARKS

Cancer-related morbidity is one of the leading causes of deaths in the world. Due to the social and health impact research on cellular and molecular pathways driving oncogenesis and carcinogenesis is one of the most active areas of biomedical investigation with the aim to characterize novel treatment options. Although the role of cholesterol as a cancer-promoting factor has been known for more than a century, the interest and potential relevance of cholesterol metabolism in cancer have increased with the use of statins. Emerging data in experimental models suggest that cholesterol upregulation promotes carcinogenesis. Cholesterol is a critical component of membrane bilayers that determine the structural and functional properties of cellular membranes. Its enrichment in particular organelles, such as mitochondria, may have an important impact in the regulation of mitochondrial function, cell death susceptibility and response to chemotherapy. However, the biological effects of statins may be broader than simply reducing cholesterol levels. By inhibiting HMG-CoA reductase statins impact negatively on the generation of isoprenoids, which in turn, regulate protein prenylation affecting a number of critical pathways in the regulation of cell cycle, proliferation, adhesion, which would be expected to regulate carcinogenesis. Thus, while statins have the potential of regulating widespread factors many of them essential for oncogenesis, a further understanding on the molecular processes promoting specific types of cancers may give valuable insights to design combination therapy to improve cancer therapy. For instance, in the case of HCC further research is needed to evaluate whether targeting the mevalonate pathway, using inhibitors that block different steps in the pathway, in combination with sorafenib may be more effective than either treatment alone.

ACKNOWLEDGMENTS

The work was supported by grants SAF2008-02199, SAF2009-11417 and SAF2011-23031 (Plan Nacional de I+D), by PI11/03025 (META) and CIBEREHD from the Instituto Carlos III, the Fundación Mutua Madrileña, the center grant P50AA11999 (Southern California Research Center for ALPD and Cirrhosis) from the National Institute on Alcohol Abuse and Alcoholism of NIH, USA, and Fundació La Marató de TV3. This work was carried out (in part) at the Esther Koplowitz Centre.

ABBREVIATIONS

BMI: body mass index

BMP: bone morphogenetic protein

BRAF: v-raf murine sarcoma viral oncogene homolog B1

CRC: Colorectal cancer

DMAPP: dimethylallyl pyrophosphate

DPI: dual prenylation inhibitor

Dsh: Desert hedgehog

EGFR: epidermal growth factor receptor

ERK: extracellular-signal-regulated kinase

FGF: fibroblast growth factor

FoxE1: forkhead box E1

FoxM1: forkhead box M1

FPP: farnesyl pyrophosphate

FT: farnesyltransferase

GGPP: geranylgeranyl pyrophosphate

GGT: geranylgeranyl transferase

Gli: glioma-associated

GPP: geranyl pyrophosphate

HCC: hepatocellular carcinoma

HGPS: Hutchinson-Gilford progeria syndrome

Hh: Hedgehog

Hip: hedgehog-interacting protein

HMG-CoA : hydroxymethylglutaryl-CoA

ICAM1: intercellular adhesion molecule 1

ICMT: isoprenylcysteine carboxyl methyltransferase

Ihh: Indian hedgehog KRAS

IPP: isopentenyl pyrophosphate

LFA1: lymphocyte function-associated antigen 1

MAPK: mitogen activated protein kinase

NASH: non-alcoholic steatohepatitis

NBP: Nitrogenous bisphosphonate

P2X7: purinergic receptor

PCSK9: Proprotein convertase subtilisin/kexin type 9

PDGF: platelet-derived growth factor

Ptch1: Patched 1

RCE1: Ras-converting enzyme 1

Shh: Sonic hedgehog

SLOS: Smith–Lemli–Opitz syndrome

Smo: Smoothened

SS: squalene synthase

VEGF: Vascular endothelial growth factor

Figure 1

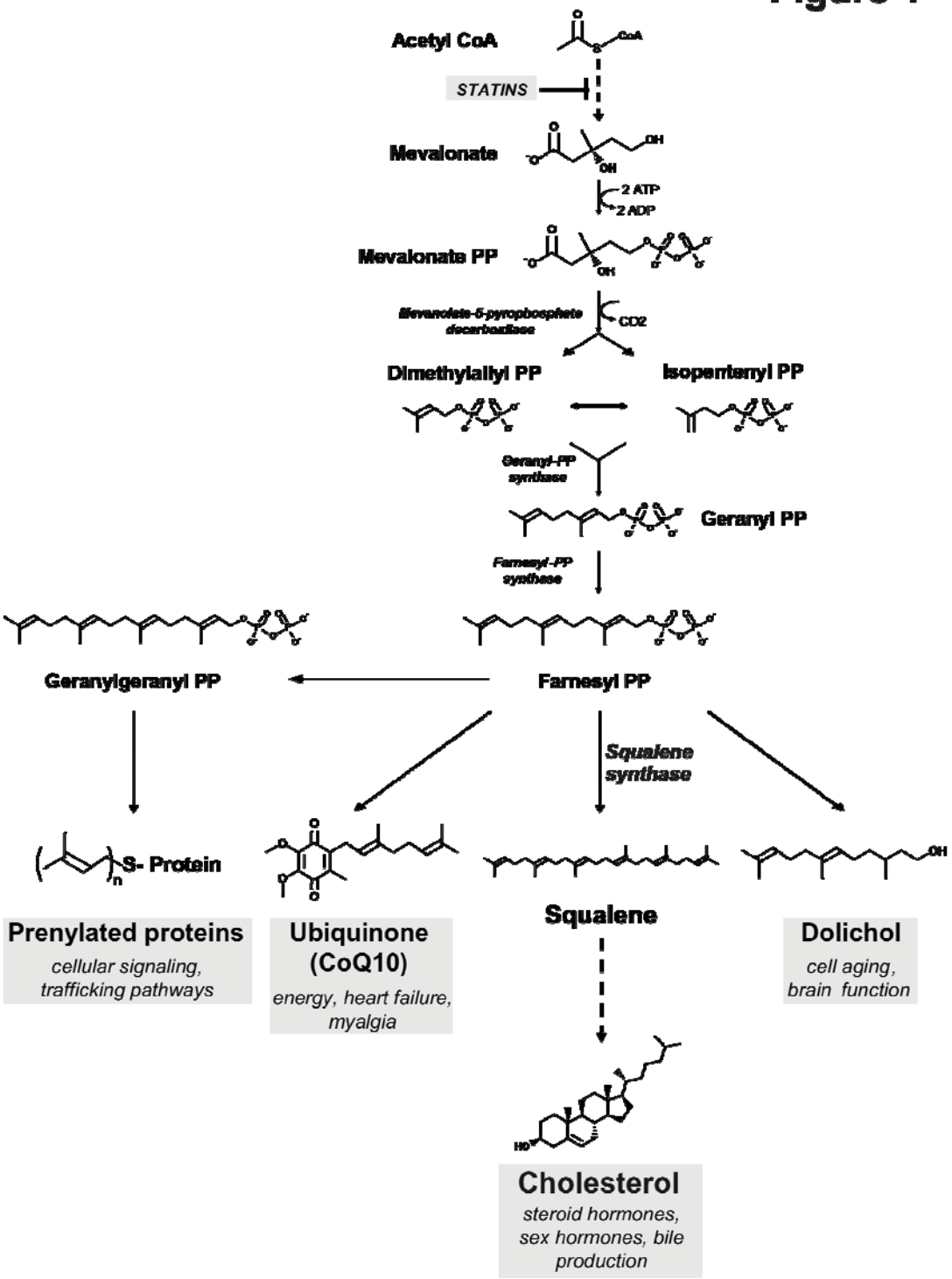
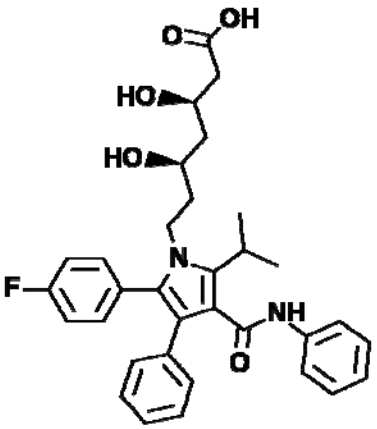
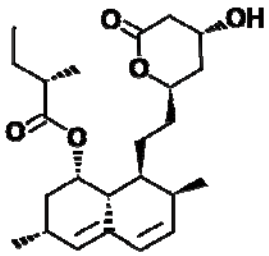

Acetyl CoA
CoA
STATINS
Mevalonate
OH
2 ATP
2 ADP
Mevalonate PP
Mevalonate-5-pyrophosphate decarboxilase
CO2
Dimethylallyl PP
Isopentenyl PP
Geranyl-PP synthase
Geranyl PP
Farnesyl-PP synthase
Geranylgeranyl PP
Farnesyl PP
Squalene synthase
S- Protein
Squalene
OH
HO
Prenylated proteins
cellular signaling, trafficking pathways
Ubiquinone (CoQ10)
energy, heart failure, myalgia
Dolichol
cell aging, brain function
Cholesterol
steroid hormones, sex hormones, bile production

Figure 2

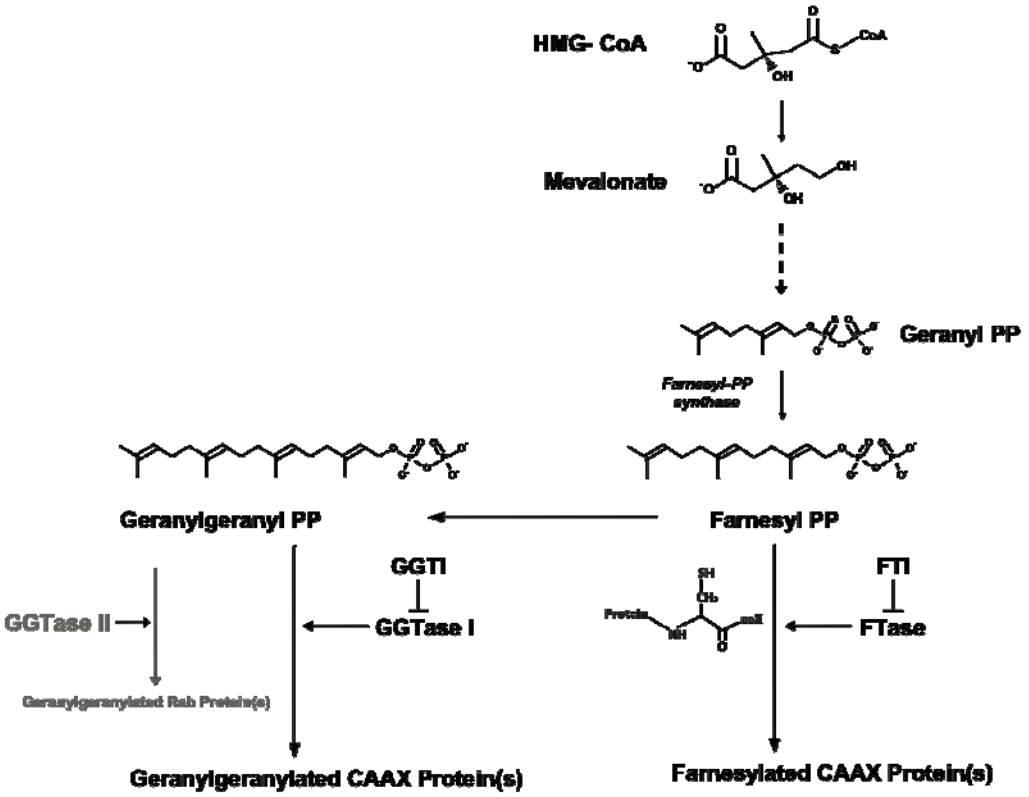


atorvastatin



lovastatin

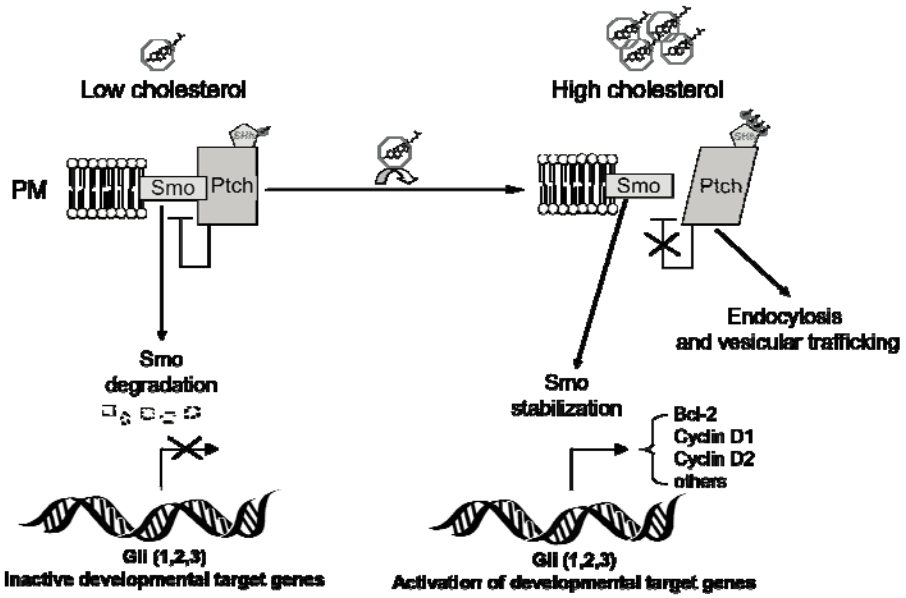
Figure 3



γ-subunits of the heterotrimeric G proteins
Rap 1
RhoA,B,C and G
Cdc42
Rac 1 and 2
R-Ras1 and R-Ras2/TC21
Ral Aand B
cGMP phosphodiesterase β
2′-3′ ologo (A) synthase 3′-phosphodiesterase
Inositol-1,4,5-triphosphate 5-phosphate type I

H-Ras, K4B-Ras, and N-Ras
Rap2
Nuclear Lamins A and B
Rho E
Pxf
Phosphorylase kinase α and β
PRL-1/PTP CAAX 1 and 2
Transducin γ
cGMP phosphodiesterase α
Rhodopsin kinase
YDJ1 homolog
Inositol-1,4,5-triphosphate

Figure 4


Low cholesterol
High cholesterol
PM
Smo
Ptch
Smo
Ptch
Endocylosis
and vesicular trafficking
Smo
degradation
Smo
stabilization
Bcl-2
Cyclin D1
Cyclin D2
others
Gli (1,2,3)
Inactive developmental target genes
Gli (1,2,3)
Activation of developmental target genes

REFERENCES:

[1] Endo, A.; Kuroda, M.; Tanzawa, K., Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B fungal metabolites, having hypocholesterolemic activity. *FEBS Lett* **1976,** *72* (2), 323-326.

[2] Goldstein, J. L.; Brown, M. S., Regulation of the mevalonate pathway. *Nature* **1990,** *343* (6257), 425-430.

[3] Fox, K. A.; Steg, P. G.; Eagle, K. A.; Goodman, S. G.; Anderson, F. A., Jr.; Granger, C. B.; Flather, M. D.; Budaj, A.; Quill, A.; Gore, J. M., Decline in rates of death and heart failure in acute coronary syndromes, 1999-2006. *JAMA* **2007,** *297* (17), 1892-1900.

[4] Demierre, M. F.; Higgins, P. D.; Gruber, S. B.; Hawk, E.; Lippman, S. M., Statins and cancer prevention. *Nat Rev Cancer* **2005,** *5* (12), 930-942.

[5] Edwards, P. A.; Ericsson, J., Sterols and isoprenoids: signaling molecules derived from the cholesterol biosynthetic pathway. *Annu Rev Biochem* **1999,** *68*, 157-185.

[6] Maxfield, F. R.; Tabas, I., Role of cholesterol and lipid organization in disease. *Nature* **2005,** *438* (7068), 612-621.

[7] van Meer, G.; Voelker, D. R.; Feigenson, G. W., Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol* **2008,** *9* (2), 112-124.

[8] Morales, A.; Mari, M.; Garcia-Ruiz, C.; Colell, A.; Fernandez-Checa, J. C., Hepatocarcinogenesis And Ceramide/Cholesterol Metabolism. *Anticancer Agents Med Chem* **2011**.

[9] Webb, J. H.; Cancer, its nature and treatment. *Lancet* **1901,** *ii*, 976 (letter).

[10] White, R. M.; On the ocurrence of crystals in tumours. *J Pathol Bacteriol* **1909,** *13*, 3-10.

[11] Newman, T. B.; Hulley, S. B., Carcinogenicity of lipid-lowering drugs. *JAMA* **1996,** *275* (1), 55-60.

[12] Chan, K. K.; Oza, A. M.; Siu, L. L., The statins as anticancer agents. *Clin Cancer Res* **2003,** *9* (1), 10-19.

[13] Ridker, P. M.; Rifai, N.; Pfeffer, M. A.; Sacks, F. M.; Moye, L. A.; Goldman, S.; Flaker, G. C.; Braunwald, E., Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. *Circulation* **1998,** *98* (9), 839-844.

[14] Gotto, A. M., Jr.; Whitney, E.; Stein, E. A.; Shapiro, D. R.; Clearfield, M.; Weis, S.; Jou, J. Y.; Langendorfer, A.; Beere, P. A.; Watson, D. J.; Downs, J. R.; de Cani, J. S., Relation between baseline and on-treatment lipid parameters and first acute major coronary events in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). *Circulation* **2000,** *101* (5), 477-484.

[15] Mason, R. P.; Walter, M. F.; Day, C. A.; Jacob, R. F., Active metabolite of atorvastatin inhibits membrane cholesterol domain formation by an antioxidant mechanism. *J Biol Chem* **2006,** *281* (14), 9337-9345.

[16] Shibata, M. A.; Kavanaugh, C.; Shibata, E.; Abe, H.; Nguyen, P.; Otsuki, Y.; Trepel, J. B.; Green, J. E., Comparative effects of lovastatin on mammary and prostate oncogenesis in transgenic mouse models. *Carcinogenesis* **2003,** *24* (3), 453-459.

[17] Rao, S.; Porter, D. C.; Chen, X.; Herliczek, T.; Lowe, M.; Keyomarsi, K., Lovastatin-mediated G1 arrest is through inhibition of the proteasome, independent of

hydroxymethyl glutaryl-CoA reductase. *Proc Natl Acad Sci U S A* **1999,** *96* (14), 7797-7802.

[18] Weitz-Schmidt, G.; Welzenbach, K.; Brinkmann, V.; Kamata, T.; Kallen, J.; Bruns, C.; Cottens, S.; Takada, Y.; Hommel, U., Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. *Nat Med* **2001,** *7* (6), 687-692.

[19] Simpson, K. J.; Dugan, A. S.; Mercurio, A. M., Functional analysis of the contribution of RhoA and RhoC GTPases to invasive breast carcinoma. *Cancer Res* **2004,** *64* (23), 8694-8701.

[20] Kusama, T.; Mukai, M.; Iwasaki, T.; Tatsuta, M.; Matsumoto, Y.; Akedo, H.; Nakamura, H., Inhibition of epidermal growth factor-induced RhoA translocation and invasion of human pancreatic cancer cells by 3-hydroxy-3-methylglutaryl-coenzyme a reductase inhibitors. *Cancer Res* **2001,** *61* (12), 4885-4891.

[21] Malliri, A.; Collard, J. G., Role of Rho-family proteins in cell adhesion and cancer. *Curr Opin Cell Biol* **2003,** *15* (5), 583-589.

[22] Mazieres, J.; Tillement, V.; Allal, C.; Clanet, C.; Bobin, L.; Chen, Z.; Sebti, S. M.; Favre, G.; Pradines, A., Geranylgeranylated, but not farnesylated, RhoB suppresses Ras transformation of NIH-3T3 cells. *Exp Cell Res* **2005,** *304* (2), 354-364.

[23] Zhang, F. L.; Casey, P. J., Protein prenylation: molecular mechanisms and functional consequences. *Annu Rev Biochem* **1996,** *65*, 241-269.

[24] Ashby, M. N., CaaX converting enzymes. *Curr Opin Lipidol* **1998,** *9* (2), 99-102.

[25] Konstantinopoulos, P. A.; Papavassiliou, A. G., Multilevel modulation of the mevalonate and protein-prenylation circuitries as a novel strategy for anticancer therapy. *Trends Pharmacol Sci* **2007,** *28* (1), 6-13.

[26] Sahai, E.; Marshall, C. J., RHO-GTPases and cancer. *Nat Rev Cancer* **2002,** *2* (2), 133-142.

[27] Hutchison, C. J., Lamins: building blocks or regulators of gene expression? *Nat Rev Mol Cell Biol* **2002,** *3* (11), 848-858.

[28] Winter-Vann, A. M.; Casey, P. J., Post-prenylation-processing enzymes as new targets in oncogenesis. *Nat Rev Cancer* **2005,** *5* (5), 405-412.

[29] Appels, N. M.; Beijnen, J. H.; Schellens, J. H., Development of farnesyl transferase inhibitors: a review. *Oncologist* **2005,** *10* (8), 565-578.

[30] Peterson, Y. K.; Kelly, P.; Weinbaum, C. A.; Casey, P. J., A novel protein geranylgeranyltransferase-I inhibitor with high potency, selectivity, and cellular activity. *J Biol Chem* **2006,** *281* (18), 12445-12450.

[31] Hindler, K.; Cleeland, C. S.; Rivera, E.; Collard, C. D., The role of statins in cancer therapy. *Oncologist* **2006,** *11* (3), 306-315.

[32] Wilson, P. W.; D'Agostino, R. B.; Levy, D.; Belanger, A. M.; Silbershatz, H.; Kannel, W. B., Prediction of coronary heart disease using risk factor categories. *Circulation* **1998,** *97* (18), 1837-1847.

[33] Armitage, J., The safety of statins in clinical practice. *Lancet* **2007,** *370* (9601), 1781-1790.

[34] Beltowski, J.; Wojcicka, G.; Jamroz-Wisniewska, A., Adverse effects of statins - mechanisms and consequences. *Curr Drug Saf* **2009,** *4* (3), 209-228.

[35] El Harchaoui, K.; Akdim, F.; Stroes, E. S.; Trip, M. D.; Kastelein, J. J., Current and future pharmacologic options for the management of patients unable to achieve low-density lipoprotein-cholesterol goals with statins. *Am J Cardiovasc Drugs* **2008,** *8* (4), 233-242.

[36] Shepherd, J.; Barter, P.; Carmena, R.; Deedwania, P.; Fruchart, J. C.; Haffner, S.; Hsia, J.; Breazna, A.; LaRosa, J.; Grundy, S.; Waters, D., Effect of lowering LDL cholesterol substantially below currently recommended levels in patients with coronary heart disease and diabetes: the Treating to New Targets (TNT) study. *Diabetes Care* **2006,** *29* (6), 1220-1226.

[37] Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* **2001,** *285* (19), 2486-2497.

[38] Javed, U.; Deedwania, P. C.; Bhatt, D. L.; Cannon, C. P.; Dai, D.; Hernandez, A. F.; Peterson, E. D.; Fonarow, G. C., Use of intensive lipid-lowering therapy in patients hospitalized with acute coronary syndrome: an analysis of 65,396 hospitalizations from 344 hospitals participating in Get With The Guidelines (GWTG). *Am Heart J* **2010,** *160* (6), 1130-1136, 1136 e1131-1133.

[39] Goldstein, J. L.; Brown, M. S., The LDL receptor. *Arterioscler Thromb Vasc Biol* **2009,** *29* (4), 431-438.

[40] Maxwell, K. N.; Breslow, J. L., Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype. *Proc Natl Acad Sci U S A* **2004,** *101* (18), 7100-7105.

[41] Elson, C. E.; Peffley, D. M.; Hentosh, P.; Mo, H., Isoprenoid-mediated inhibition of mevalonate synthesis: potential application to cancer. *Proc Soc Exp Biol Med* **1999,** *221* (4), 294-311.

[42] Mo, H.; Elson, C. E., Studies of the isoprenoid-mediated inhibition of mevalonate synthesis applied to cancer chemotherapy and chemoprevention. *Exp Biol Med (Maywood)* **2004,** *229* (7), 567-585.

[43] Buckner, J. C.; Malkin, M. G.; Reed, E.; Cascino, T. L.; Reid, J. M.; Ames, M. M.; Tong, W. P.; Lim, S.; Figg, W. D., Phase II study of antineoplastons A10 (NSC 648539) and AS2-1 (NSC 620261) in patients with recurrent glioma. *Mayo Clin Proc* **1999,** *74* (2), 137-145.

[44] Harrison, L. E.; Wojciechowicz, D. C.; Brennan, M. F.; Paty, P. B., Phenylacetate inhibits isoprenoid biosynthesis and suppresses growth of human pancreatic carcinoma. *Surgery* **1998,** *124* (3), 541-550.

[45] Sebti, S. M., Protein farnesylation: implications for normal physiology, malignant transformation, and cancer therapy. *Cancer Cell* **2005,** *7* (4), 297-300.

[46] Lackner, M. R.; Kindt, R. M.; Carroll, P. M.; Brown, K.; Cancilla, M. R.; Chen, C.; de Silva, H.; Franke, Y.; Guan, B.; Heuer, T.; Hung, T.; Keegan, K.; Lee, J. M.; Manne, V.; O'Brien, C.; Parry, D.; Perez-Villar, J. J.; Reddy, R. K.; Xiao, H.; Zhan, H.; Cockett, M.; Plowman, G.; Fitzgerald, K.; Costa, M.; Ross-Macdonald, P., Chemical genetics identifies Rab geranylgeranyl transferase as an apoptotic target of farnesyl transferase inhibitors. *Cancer Cell* **2005,** *7* (4), 325-336.

[47] Zimmerman, T. M.; Harlin, H.; Odenike, O. M.; Berk, S.; Sprague, E.; Karrison, T.; Stock, W.; Larson, R. A.; Ratain, M. J.; Gajewski, T. F., Dose-ranging

pharmacodynamic study of tipifarnib (R115777) in patients with relapsed and refractory hematologic malignancies. *J Clin Oncol* **2004,** *22* (23), 4816-4822.

[48] Whyte, D. B.; Kirschmeier, P.; Hockenberry, T. N.; Nunez-Oliva, I.; James, L.; Catino, J. J.; Bishop, W. R.; Pai, J. K., K- and N-Ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors. *J Biol Chem* **1997,** *272* (22), 14459-14464.

[49] Mijimolle, N.; Velasco, J.; Dubus, P.; Guerra, C.; Weinbaum, C. A.; Casey, P. J.; Campuzano, V.; Barbacid, M., Protein farnesyltransferase in embryogenesis, adult homeostasis, and tumor development. *Cancer Cell* **2005,** *7* (4), 313-324.

[50] Seiki, S.; Frishman, W. H., Pharmacologic inhibition of squalene synthase and other downstream enzymes of the cholesterol synthesis pathway: a new therapeutic approach to treatment of hypercholesterolemia. *Cardiol Rev* **2009,** *17* (2), 70-76.

[51] Elsayed, R. K.; Evans, J. D., Emerging lipid-lowering drugs: squalene synthase inhibitors. *Expert Opin Emerg Drugs* **2008,** *13* (2), 309-322.

[52] Bostedor, R. G.; Karkas, J. D.; Arison, B. H.; Bansal, V. S.; Vaidya, S.; Germershausen, J. I.; Kurtz, M. M.; Bergstrom, J. D., Farnesol-derived dicarboxylic acids in the urine of animals treated with zaragozic acid A or with farnesol. *J Biol Chem* **1997,** *272* (14), 9197-9203.

[53] Joo, J. H.; Jetten, A. M., Molecular mechanisms involved in farnesol-induced apoptosis. *Cancer Lett* **2010,** *287* (2), 123-135.

[54] Nishimoto, T.; Tozawa, R.; Amano, Y.; Wada, T.; Imura, Y.; Sugiyama, Y., Comparing myotoxic effects of squalene synthase inhibitor, T-91485, and 3-hydroxy-3-

methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors in human myocytes. *Biochem Pharmacol* **2003,** *66* (11), 2133-2139.

[55] Nishimoto, T.; Ishikawa, E.; Anayama, H.; Hamajyo, H.; Nagai, H.; Hirakata, M.; Tozawa, R., Protective effects of a squalene synthase inhibitor, lapaquistat acetate (TAK-475), on statin-induced myotoxicity in guinea pigs. *Toxicol Appl Pharmacol* **2007,** *223* (1), 39-45.

[56] Hiyoshi, H.; Yanagimachi, M.; Ito, M.; Saeki, T.; Yoshida, I.; Okada, T.; Ikuta, H.; Shinmyo, D.; Tanaka, K.; Kurusu, N.; Tanaka, H., Squalene synthase inhibitors reduce plasma triglyceride through a low-density lipoprotein receptor-independent mechanism. *Eur J Pharmacol* **2001,** *431* (3), 345-352.

[57] Hiyoshi, H.; Yanagimachi, M.; Ito, M.; Yasuda, N.; Okada, T.; Ikuta, H.; Shinmyo, D.; Tanaka, K.; Kurusu, N.; Yoshida, I.; Abe, S.; Saeki, T.; Tanaka, H., Squalene synthase inhibitors suppress triglyceride biosynthesis through the farnesol pathway in rat hepatocytes. *J Lipid Res* **2003,** *44* (1), 128-135.

[58] van Beek, E.; Pieterman, E.; Cohen, L.; Lowik, C.; Papapoulos, S., Farnesyl pyrophosphate synthase is the molecular target of nitrogen-containing bisphosphonates. *Biochem Biophys Res Commun* **1999,** *264* (1), 108-111.

[59] Nancollas, G. H.; Tang, R.; Phipps, R. J.; Henneman, Z.; Gulde, S.; Wu, W.; Mangood, A.; Russell, R. G.; Ebetino, F. H., Novel insights into actions of bisphosphonates on bone: differences in interactions with hydroxyapatite. *Bone* **2006,** *38* (5), 617-627.

[60] Guney, E.; Kisakol, G.; Ozgen, A. G.; Yilmaz, C.; Kabalak, T., Effects of bisphosphonates on lipid metabolism. *Neuro Endocrinol Lett* **2008,** *29* (2), 252-255.

[61] Varela, I.; Pereira, S.; Ugalde, A. P.; Navarro, C. L.; Suarez, M. F.; Cau, P.; Cadinanos, J.; Osorio, F. G.; Foray, N.; Cobo, J.; de Carlos, F.; Levy, N.; Freije, J. M.; Lopez-Otin, C., Combined treatment with statins and aminobisphosphonates extends longevity in a mouse model of human premature aging. *Nat Med* **2008,** *14* (7), 767-772.

[62] Morgan, M. A.; Sebil, T.; Aydilek, E.; Peest, D.; Ganser, A.; Reuter, C. W., Combining prenylation inhibitors causes synergistic cytotoxicity, apoptosis and disruption of RAS-to-MAP kinase signalling in multiple myeloma cells. *Br J Haematol* **2005,** *130* (6), 912-925.

[63] Lobell, R. B.; Omer, C. A.; Abrams, M. T.; Bhimnathwala, H. G.; Brucker, M. J.; Buser, C. A.; Davide, J. P.; deSolms, S. J.; Dinsmore, C. J.; Ellis-Hutchings, M. S.; Kral, A. M.; Liu, D.; Lumma, W. C.; Machotka, S. V.; Rands, E.; Williams, T. M.; Graham, S. L.; Hartman, G. D.; Oliff, A. I.; Heimbrook, D. C.; Kohl, N. E., Evaluation of farnesyl:protein transferase and geranylgeranyl:protein transferase inhibitor combinations in preclinical models. *Cancer Res* **2001,** *61* (24), 8758-8768.

[64] Sun, J.; Qian, Y.; Hamilton, A. D.; Sebti, S. M., Both farnesyltransferase and geranylgeranyltransferase I inhibitors are required for inhibition of oncogenic K-Ras prenylation but each alone is sufficient to suppress human tumor growth in nude mouse xenografts. *Oncogene* **1998,** *16* (11), 1467-1473.

[65] Reid, T. S.; Long, S. B.; Beese, L. S., Crystallographic analysis reveals that anticancer clinical candidate L-778,123 inhibits protein farnesyltransferase and geranylgeranyltransferase-I by different binding modes. *Biochemistry* **2004,** *43* (28), 9000-9008.

[66] Lobell, R. B.; Liu, D.; Buser, C. A.; Davide, J. P.; DePuy, E.; Hamilton, K.; Koblan, K. S.; Lee, Y.; Mosser, S.; Motzel, S. L.; Abbruzzese, J. L.; Fuchs, C. S.; Rowinsky, E. K.; Rubin, E. H.; Sharma, S.; Deutsch, P. J.; Mazina, K. E.; Morrison, B. W.; Wildonger, L.; Yao, S. L.; Kohl, N. E., Preclinical and clinical pharmacodynamic assessment of L-778,123, a dual inhibitor of farnesyl:protein transferase and geranylgeranyl:protein transferase type-I. *Mol Cancer Ther* **2002,** *1* (9), 747-758.

[67] Budman, D. R.; Calabro, A., Zoledronic acid (Zometa) enhances the cytotoxic effect of gemcitabine and fluvastatin: in vitro isobologram studies with conventional and nonconventional cytotoxic agents. *Oncology* **2006,** *70* (2), 147-153.

[68] Andela, V. B.; Rosenblatt, J. D.; Schwarz, E. M.; Puzas, E. J.; O'Keefe, R. J.; Rosier, R. N., Synergism of aminobisphosphonates and farnesyl transferase inhibitors on tumor metastasis. *Clin Orthop Relat Res* **2002,** (397), 228-239.

[69] Vincenzi, B.; Santini, D.; Avvisati, G.; Baldi, A.; Cesa, A. L.; Tonini, G., Statins may potentiate bisphosphonates anticancer properties: a new pharmacological approach? *Med Hypotheses* **2003,** *61* (1), 98-101.

[70] Caraglia, M.; Santini, D.; Marra, M.; Vincenzi, B.; Tonini, G.; Budillon, A., Emerging anti-cancer molecular mechanisms of aminobisphosphonates. *Endocr Relat Cancer* **2006,** *13* (1), 7-26.

[71] McCarty, M. F., Suppression of dolichol synthesis with isoprenoids and statins may potentiate the cancer-retardant efficacy of IGF-I down-regulation. *Med Hypotheses* **2001,** *56* (1), 12-16.

[72] Bergo, M. O.; Ambroziak, P.; Gregory, C.; George, A.; Otto, J. C.; Kim, E.; Nagase, H.; Casey, P. J.; Balmain, A.; Young, S. G., Absence of the CAAX endoprotease Rce1: effects on cell growth and transformation. *Mol Cell Biol* **2002,** *22* (1), 171-181.

[73] Bergo, M. O.; Gavino, B. J.; Hong, C.; Beigneux, A. P.; McMahon, M.; Casey, P. J.; Young, S. G., Inactivation of Icmt inhibits transformation by oncogenic K-Ras and B-Raf. *J Clin Invest* **2004,** *113* (4), 539-550.

[74] Park, K. S.; Martelotto, L. G.; Peifer, M.; Sos, M. L.; Karnezis, A. N.; Mahjoub, M. R.; Bernard, K.; Conklin, J. F.; Szczepny, A.; Yuan, J.; Guo, R.; Ospina, B.; Falzon, J.; Bennett, S.; Brown, T. J.; Markovic, A.; Devereux, W. L.; Ocasio, C. A.; Chen, J. K.; Stearns, T.; Thomas, R. K.; Dorsch, M.; Buonamici, S.; Watkins, D. N.; Peacock, C. D.; Sage, J., A crucial requirement for Hedgehog signaling in small cell lung cancer. *Nat Med* **2011,** *17* (11), 1504-1508.

[75] Hooper, J. E.; Scott, M. P., Communicating with Hedgehogs. *Nat Rev Mol Cell Biol* **2005,** *6* (4), 306-317.

[76] Quinn, A. G.; Epstein, E., Jr., Patched, hedgehog, and skin cancer. *Methods Mol Biol* **2003,** *222*, 85-95.

[77] Athar, M.; Tang, X.; Lee, J. L.; Kopelovich, L.; Kim, A. L., Hedgehog signalling in skin development and cancer. *Exp Dermatol* **2006,** *15* (9), 667-677.

[78] Regl, G.; Kasper, M.; Schnidar, H.; Eichberger, T.; Neill, G. W.; Philpott, M. P.; Esterbauer, H.; Hauser-Kronberger, C.; Frischauf, A. M.; Aberger, F., Activation of the BCL2 promoter in response to Hedgehog/GLI signal transduction is predominantly mediated by GLI2. *Cancer Res* **2004,** *64* (21), 7724-7731.

[79] Bidet, M.; Joubert, O.; Lacombe, B.; Ciantar, M.; Nehme, R.; Mollat, P.; Bretillon, L.; Faure, H.; Bittman, R.; Ruat, M.; Mus-Veteau, I., The hedgehog receptor patched is involved in cholesterol transport. *PLoS One* **2011,** *6* (9), e23834.

[80] Oslejskova, H.; Horinova, V.; Sterba, J.; Pavelka, Z.; Babovic-Vuksanovic, D.; Dubska, L.; Valik, D., Malignant intracranial germinoma in Smith-Lemli-Opitz syndrome: cholesterol homeostasis possibly connecting morphogenesis and cancer development. *J Pediatr Hematol Oncol* **2008,** *30* (9), 689-691.

[81] Ng, J. M.; Curran, T., The Hedgehog's tale: developing strategies for targeting cancer. *Nat Rev Cancer* **2011,** *11* (7), 493-501.

[82] Toyota, M.; Ahuja, N.; Ohe-Toyota, M.; Herman, J. G.; Baylin, S. B.; Issa, J. P., CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci U S A* **1999,** *96* (15), 8681-8686.

[83] Jover, R.; Nguyen, T. P.; Perez-Carbonell, L.; Zapater, P.; Paya, A.; Alenda, C.; Rojas, E.; Cubiella, J.; Balaguer, F.; Morillas, J. D.; Clofent, J.; Bujanda, L.; Rene, J. M.; Bessa, X.; Xicola, R. M.; Nicolas-Perez, D.; Castells, A.; Andreu, M.; Llor, X.; Boland, C. R.; Goel, A., 5-Fluorouracil adjuvant chemotherapy does not increase survival in patients with CpG island methylator phenotype colorectal cancer. *Gastroenterology* **2011,** *140* (4), 1174-1181.

[84] Hede, K., Hints that statins reduce colon cancer risk finally being put to the test. *J Natl Cancer Inst* **2011,** *103* (5), 364-366.

[85] Hardwick, J. C.; Van Den Brink, G. R.; Bleuming, S. A.; Ballester, I.; Van Den Brande, J. M.; Keller, J. J.; Offerhaus, G. J.; Van Deventer, S. J.; Peppelenbosch, M. P.,

Bone morphogenetic protein 2 is expressed by, and acts upon, mature epithelial cells in the colon. *Gastroenterology* **2004,** *126* (1), 111-121.

[86] Kodach, L. L.; Wiercinska, E.; de Miranda, N. F.; Bleuming, S. A.; Musler, A. R.; Peppelenbosch, M. P.; Dekker, E.; van den Brink, G. R.; van Noesel, C. J.; Morreau, H.; Hommes, D. W.; Ten Dijke, P.; Offerhaus, G. J.; Hardwick, J. C., The bone morphogenetic protein pathway is inactivated in the majority of sporadic colorectal cancers. *Gastroenterology* **2008,** *134* (5), 1332-1341.

[87] Kodach, L. L.; Bleuming, S. A.; Musler, A. R.; Peppelenbosch, M. P.; Hommes, D. W.; van den Brink, G. R.; van Noesel, C. J.; Offerhaus, G. J.; Hardwick, J. C., The bone morphogenetic protein pathway is active in human colon adenomas and inactivated in colorectal cancer. *Cancer* **2008,** *112* (2), 300-306.

[88] Auclair, B. A.; Benoit, Y. D.; Rivard, N.; Mishina, Y.; Perreault, N., Bone morphogenetic protein signaling is essential for terminal differentiation of the intestinal secretory cell lineage. *Gastroenterology* **2007,** *133* (3), 887-896.

[89] He, X. C.; Zhang, J.; Li, L., Cellular and molecular regulation of hematopoietic and intestinal stem cell behavior. *Ann N Y Acad Sci* **2005,** *1049*, 28-38.

[90] Agarwal, B.; Rao, C. V.; Bhendwal, S.; Ramey, W. R.; Shirin, H.; Reddy, B. S.; Holt, P. R., Lovastatin augments sulindac-induced apoptosis in colon cancer cells and potentiates chemopreventive effects of sulindac. *Gastroenterology* **1999,** *117* (4), 838-847.

[91] Kodach, L. L.; Bleuming, S. A.; Peppelenbosch, M. P.; Hommes, D. W.; van den Brink, G. R.; Hardwick, J. C., The effect of statins in colorectal cancer is mediated

through the bone morphogenetic protein pathway. *Gastroenterology* **2007,** *133* (4), 1272-1281.

[92] Mundy, G.; Garrett, R.; Harris, S.; Chan, J.; Chen, D.; Rossini, G.; Boyce, B.; Zhao, M.; Gutierrez, G., Stimulation of bone formation in vitro and in rodents by statins. *Science* **1999,** *286* (5446), 1946-1949.

[93] Kodach, L. L.; Jacobs, R. J.; Voorneveld, P. W.; Wildenberg, M. E.; Verspaget, H. W.; van Wezel, T.; Morreau, H.; Hommes, D. W.; Peppelenbosch, M. P.; van den Brink, G. R.; Hardwick, J. C., Statins augment the chemosensitivity of colorectal cancer cells inducing epigenetic reprogramming and reducing colorectal cancer cell 'stemness' via the bone morphogenetic protein pathway. *Gut* **2011,** *60* (11), 1544-1553.

[94] Van Cutsem, E.; Kohne, C. H.; Hitre, E.; Zaluski, J.; Chang Chien, C. R.; Makhson, A.; D'Haens, G.; Pinter, T.; Lim, R.; Bodoky, G.; Roh, J. K.; Folprecht, G.; Ruff, P.; Stroh, C.; Tejpar, S.; Schlichting, M.; Nippgen, J.; Rougier, P., Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* **2009,** *360* (14), 1408-1417.

[95] Tabernero, J.; Van Cutsem, E.; Diaz-Rubio, E.; Cervantes, A.; Humblet, Y.; Andre, T.; Van Laethem, J. L.; Soulie, P.; Casado, E.; Verslype, C.; Valera, J. S.; Tortora, G.; Ciardiello, F.; Kisker, O.; de Gramont, A., Phase II trial of cetuximab in combination with fluorouracil, leucovorin, and oxaliplatin in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* **2007,** *25* (33), 5225-5232.

[96] Di Nicolantonio, F.; Martini, M.; Molinari, F.; Sartore-Bianchi, A.; Arena, S.; Saletti, P.; De Dosso, S.; Mazzucchelli, L.; Frattini, M.; Siena, S.; Bardelli, A., Wild-type

BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* **2008,** *26* (35), 5705-5712.

[97] Karapetis, C. S.; Khambata-Ford, S.; Jonker, D. J.; O'Callaghan, C. J.; Tu, D.; Tebbutt, N. C.; Simes, R. J.; Chalchal, H.; Shapiro, J. D.; Robitaille, S.; Price, T. J.; Shepherd, L.; Au, H. J.; Langer, C.; Moore, M. J.; Zalcberg, J. R., K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* **2008,** *359* (17), 1757-1765.

[98] Lee, J.; Lee, I.; Han, B.; Park, J. O.; Jang, J.; Park, C.; Kang, W. K., Effect of simvastatin on cetuximab resistance in human colorectal cancer with KRAS mutations. *J Natl Cancer Inst* **2011,** *103* (8), 674-688.

[99] Poynter, J. N.; Gruber, S. B.; Higgins, P. D.; Almog, R.; Bonner, J. D.; Rennert, H. S.; Low, M.; Greenson, J. K.; Rennert, G., Statins and the risk of colorectal cancer. *N Engl J Med* **2005,** *352* (21), 2184-2192.

[100] Vinogradova, Y.; Coupland, C.; Hippisley-Cox, J., Exposure to statins and risk of common cancers: a series of nested case-control studies. *BMC Cancer* **2011,** *11*, 409.

[101] Li, D.; Morris, J. S.; Liu, J.; Hassan, M. M.; Day, R. S.; Bondy, M. L.; Abbruzzese, J. L., Body mass index and risk, age of onset, and survival in patients with pancreatic cancer. *JAMA* **2009,** *301* (24), 2553-2562.

[102] Kusama, T.; Mukai, M.; Iwasaki, T.; Tatsuta, M.; Matsumoto, Y.; Akedo, H.; Inoue, M.; Nakamura, H., 3-hydroxy-3-methylglutaryl-coenzyme a reductase inhibitors reduce human pancreatic cancer cell invasion and metastasis. *Gastroenterology* **2002,** *122* (2), 308-317.

[103] Muller, C.; Bockhorn, A. G.; Klusmeier, S.; Kiehl, M.; Roeder, C.; Kalthoff, H.; Koch, O. M., Lovastatin inhibits proliferation of pancreatic cancer cell lines with mutant as well as with wild-type K-ras oncogene but has different effects on protein phosphorylation and induction of apoptosis. *Int J Oncol* **1998,** *12* (3), 717-723.

[104] Sumi, S.; Beauchamp, R. D.; Townsend, C. M., Jr.; Pour, P. M.; Ishizuka, J.; Thompson, J. C., Lovastatin inhibits pancreatic cancer growth regardless of RAS mutation. *Pancreas* **1994,** *9* (5), 657-661.

[105] Mistafa, O.; Stenius, U., Statins inhibit Akt/PKB signaling via P2X7 receptor in pancreatic cancer cells. *Biochem Pharmacol* **2009,** *78* (9), 1115-1126.

[106] Bonovas, S.; Filioussi, K.; Sitaras, N. M., Statins are not associated with a reduced risk of pancreatic cancer at the population level, when taken at low doses for managing hypercholesterolemia: evidence from a meta-analysis of 12 studies. *Am J Gastroenterol* **2008,** *103* (10), 2646-2651.

[107] Bradley, M. C.; Hughes, C. M.; Cantwell, M. M.; Murray, L. J., Statins and pancreatic cancer risk: a nested case-control study. *Cancer Causes Control* **2010,** *21* (12), 2093-2100.

[108] Gbelcova, H.; Lenicek, M.; Zelenka, J.; Knejzlik, Z.; Dvorakova, G.; Zadinova, M.; Pouckova, P.; Kudla, M.; Balaz, P.; Ruml, T.; Vitek, L., Differences in antitumor effects of various statins on human pancreatic cancer. *Int J Cancer* **2008,** *122* (6), 1214-1221.

[109] Calle, E. E.; Rodriguez, C.; Walker-Thurmond, K.; Thun, M. J., Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* **2003,** *348* (17), 1625-1638.

[110] Motola-Kuba, D.; Zamora-Valdes, D.; Uribe, M.; Mendez-Sanchez, N., Hepatocellular carcinoma. An overview. *Ann Hepatol* **2006,** *5* (1), 16-24.

[111] Thorgeirsson, S. S.; Grisham, J. W., Molecular pathogenesis of human hepatocellular carcinoma. *Nat Genet* **2002,** *31* (4), 339-346.

[112] Yahagi, N.; Shimano, H.; Hasegawa, K.; Ohashi, K.; Matsuzaka, T.; Najima, Y.; Sekiya, M.; Tomita, S.; Okazaki, H.; Tamura, Y.; Iizuka, Y.; Nagai, R.; Ishibashi, S.; Kadowaki, T.; Makuuchi, M.; Ohnishi, S.; Osuga, J.; Yamada, N., Co-ordinate activation of lipogenic enzymes in hepatocellular carcinoma. *Eur J Cancer* **2005,** *41* (9), 1316-1322.

[113] Yamashita, T.; Honda, M.; Takatori, H.; Nishino, R.; Minato, H.; Takamura, H.; Ohta, T.; Kaneko, S., Activation of lipogenic pathway correlates with cell proliferation and poor prognosis in hepatocellular carcinoma. *J Hepatol* **2009,** *50* (1), 100-110.

[114] Calvisi, D. F.; Wang, C.; Ho, C.; Ladu, S.; Lee, S. A.; Mattu, S.; Destefanis, G.; Delogu, S.; Zimmermann, A.; Ericsson, J.; Brozzetti, S.; Staniscia, T.; Chen, X.; Dombrowski, F.; Evert, M., Increased lipogenesis, induced by AKT-mTORC1-RPS6 signaling, promotes development of human hepatocellular carcinoma. *Gastroenterology* **2011,** *140* (3), 1071-1083.

[115] Caldwell, S. H.; Crespo, D. M.; Kang, H. S.; Al-Osaimi, A. M., Obesity and hepatocellular carcinoma. *Gastroenterology* **2004,** *127* (5 Suppl 1), S97-103.

[116] El-Serag, H. B.; Rudolph, K. L., Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* **2007,** *132* (7), 2557-2576.

[117] Garcia-Ruiz, C.; Mari, M.; Colell, A.; Morales, A.; Caballero, F.; Montero, J.; Terrones, O.; Basanez, G.; Fernandez-Checa, J. C., Mitochondrial cholesterol in health and disease. *Histol Histopathol* **2009,** *24* (1), 117-132.

[118] Mari, M.; Caballero, F.; Colell, A.; Morales, A.; Caballeria, J.; Fernandez, A.; Enrich, C.; Fernandez-Checa, J. C.; Garcia-Ruiz, C., Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. *Cell Metab* **2006,** *4* (3), 185-198.

[119] Mari, M.; Colell, A.; Morales, A.; Caballero, F.; Moles, A.; Fernandez, A.; Terrones, O.; Basanez, G.; Antonsson, B.; Garcia-Ruiz, C.; Fernandez-Checa, J. C., Mechanism of mitochondrial glutathione-dependent hepatocellular susceptibility to TNF despite NF-kappaB activation. *Gastroenterology* **2008,** *134* (5), 1507-1520.

[120] Montero, J.; Morales, A.; Llacuna, L.; Lluis, J. M.; Terrones, O.; Basanez, G.; Antonsson, B.; Prieto, J.; Garcia-Ruiz, C.; Colell, A.; Fernandez-Checa, J. C., Mitochondrial cholesterol contributes to chemotherapy resistance in hepatocellular carcinoma. *Cancer Res* **2008,** *68* (13), 5246-5256.

[121] Miyaki, T.; Nojiri, S.; Shinkai, N.; Kusakabe, A.; Matsuura, K.; Iio, E.; Takahashi, S.; Yan, G.; Ikeda, K.; Joh, T., Pitavastatin inhibits hepatic steatosis and fibrosis in non-alcoholic steatohepatitis model rats. *Hepatol Res* **2011,** *41* (4), 375-385.

[122] Yang, P. M.; Liu, Y. L.; Lin, Y. C.; Shun, C. T.; Wu, M. S.; Chen, C. C., Inhibition of autophagy enhances anticancer effects of atorvastatin in digestive malignancies. *Cancer Res* **2010,** *70* (19), 7699-7709.

[123] Taras, D.; Blanc, J. F.; Rullier, A.; Dugot-Senant, N.; Laurendeau, I.; Vidaud, M.; Rosenbaum, J., Pravastatin reduces lung metastasis of rat hepatocellular carcinoma via a coordinated decrease of MMP expression and activity. *J Hepatol* **2007,** *46* (1), 69-76.

[124] Friis, S.; Poulsen, A. H.; Johnsen, S. P.; McLaughlin, J. K.; Fryzek, J. P.; Dalton, S. O.; Sorensen, H. T.; Olsen, J. H., Cancer risk among statin users: a population-based cohort study. *Int J Cancer* **2005,** *114* (4), 643-647.

[125] Kuoppala, J.; Lamminpaa, A.; Pukkala, E., Statins and cancer: A systematic review and meta-analysis. *Eur J Cancer* **2008,** *44* (15), 2122-2132.

[126] Chiu, H. F.; Ho, S. C.; Chen, C. C.; Yang, C. Y., Statin use and the risk of liver cancer: a population-based case-control study. *Am J Gastroenterol* **2011,** *106* (5), 894-898.

[127] Kawata, S.; Yamasaki, E.; Nagase, T.; Inui, Y.; Ito, N.; Matsuda, Y.; Inada, M.; Tamura, S.; Noda, S.; Imai, Y.; Matsuzawa, Y., Effect of pravastatin on survival in patients with advanced hepatocellular carcinoma. A randomized controlled trial. *Br J Cancer* **2001,** *84* (7), 886-891.

[128] Lersch, C.; Schmelz, R.; Erdmann, J.; Hollweck, R.; Schulte-Frohlinde, E.; Eckel, F.; Nader, M.; Schusdziarra, V., Treatment of HCC with pravastatin, octreotide, or gemcitabine--a critical evaluation. *Hepatogastroenterology* **2004,** *51* (58), 1099-1103.

[129] Graf, H.; Jungst, C.; Straub, G.; Dogan, S.; Hoffmann, R. T.; Jakobs, T.; Reiser, M.; Waggershauser, T.; Helmberger, T.; Walter, A.; Walli, A.; Seidel, D.; Goke, B.; Jungst, D., Chemoembolization combined with pravastatin improves survival in patients with hepatocellular carcinoma. *Digestion* **2008,** *78* (1), 34-38.

[130] El-Serag, H. B.; Johnson, M. L.; Hachem, C.; Morgana, R. O., Statins are associated with a reduced risk of hepatocellular carcinoma in a large cohort of patients with diabetes. *Gastroenterology* **2009,** *136* (5), 1601-1608.