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Targeting the Mevalonate Pathway in Cancer

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Abstract

The mevalonate synthesis inhibitors, statins, are mainstay therapeutics for cholesterol management and cardiovascular health. Thirty years of research has uncovered supportive roles for the mevalonate pathway in numerous cellular processes that support oncogenesis, most recently macropinocytosis. Central to the diverse mechanisms of statin sensitivity is an acquired dependence on one mevalonate pathway output, protein geranylgeranylation. New chemical prenylation probes and the discovery of a novel geranylgeranyl transferase hold promise to deepen our understanding of statin mechanism of action. Further, insights into statin selection and the counterproductive role of dietary geranylgeraniol highlight how we should assess statins in the clinic. Lastly, rational combination strategies preview how statins will enter the oncology toolbox.

Keywords

Cholesterol; Mevalonate; Statins; Prenylation; Combinations

Beyond Warburg Metabolism: Mevalonate Dependence

Altered metabolism is a hallmark of cancer cells [1]. However, it has been challenging to identify druggable, cancer-specific metabolic dependencies that apply to a broad range of tumor types. Growing evidence indicates that increased mevalonate pathway flux is a general feature of cancer [2]. Mechanistic insights underlying increased mevalonate flux have reignited the field with investigators uncovering regulatory roles for well-studied oncogenic drivers and tumor suppressors. Dependency on the mevalonate pathway is linked to several tumor phenotypes including macropinocytosis, a nutrient uptake pathway that is an attractive target in certain cancers [3]. A central motivation for targeting the mevalonate pathway is that selective, well-tolerated inhibitors already exist. Statins, which block synthesis of mevalonate by HMG-CoA-reductase (HMGCR), are prescribed to millions of people to treat hypercholesterolemia. This review focuses on recent advances in

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understanding of mevalonate pathway dependence in cancer and provides an update on preclinical translation of statins in oncology.

Mevalonate production is an essential biosynthetic step that provides the precursors for *de novo* synthesis of cholesterol and several other cellular components (Fig. 1). Cholesterol is the ubiquitous precursor to all sterol hormones in mammals and is a key component in membrane rigidity as well as membrane signal transduction. Interest in cholesterol biology led to the discovery of competitive HMGCR inhibitors in a screen of *Penicillium* molds, thus forming the first and foremost class of mevalonate pathway inhibitors: statins [4].

Statins reduce the pool of mevalonate that supplies the downstream isoprenoid building blocks of sterols, protein lipid tails and lipid-based cofactors long recognized to be important for cell growth [5]. Reduction in *de novo* cholesterol synthesis in hepatocytes leads to feedback increases in transcriptional activity of sterol regulatory-element binding protein (SREBP) and expression of low-density lipoprotein (LDL) receptors [6]. These receptors bind plasma LDL, reducing the atherogenic potential of lipoprotein-bound, plasma cholesterol. The natural product, lovastatin, was approved by the FDA in 1987 for the control of hypercholesterolemia, followed by the semi-synthetic statins like simvastatin and later by fully synthetic statins like pitavastatin.

A plethora of statins now exist with varying pharmacological properties [7–9]. Lipophilic prodrug statins such as lovastatin and simvastatin become activated when metabolized in the liver, the primary site of *de novo* cholesterol synthesis [10]. Hydrophilic statins such as rosuvastatin and pravastatin are active hydroxy acids that target to the liver by active uptake and thus reduce passive diffusion into other tissues. Some lipophilic statins—fluvastatin and pitavastatin—are non-cytochrome P450 3A4 substrates, potentially reducing drug-drug interactions and associated toxicities [7]. Long-acting statins such as atorvastatin, pitavastatin and rosuvastatin also have the benefit of reducing dosing frequency.

The cellular response to statins in cancer models are comparable amongst lipophilic statins *in vitro* [11,12]. Hydrophilic statins such as rosuvastatin and pravastatin are generally less potent due to reduced cell entry [13]. Lipophilic statins demonstrate cytostatic and/or cytotoxic effects at low micromolar ranges in various cancer types [14–18] and as such have been identified as drugs with high potential to be repurposed as oncology drugs [19,20]. However, oncologists often question the clinical relevance of statin effects on cancer cell lines *in vitro*, as the effective concentrations are 10-100 times greater than plasma levels (low to mid-nanomolar) that are typically achieved in patients taking statins at the safe dose for hypercholesterolemia [21,22]. This has piqued interest in improving statin efficacy through combination strategies [23].

The anti-cancer effects of statins are perceived to be on-target because they are generally reversed by supplementing the media with mevalonate or with downstream mevalonate pathway intermediates. Notably, rescue with geranylgeranyl pyrophosphate (GGPP) or its alcohol form geranylgeraniol (Fig. 1) is commonly observed whereas cholesterol or squalene supplementation usually has no effect. In cancers dependent on GGPP, the post-translational addition of the geranylgeranyl moiety onto GTPases is further implicated by studies that

reproduce the anti-cancer effects of statins using geranylgeranyltransferase inhibitors (GGTIs) or by genetic silencing of geranylgeranyltransferase. However, protein geranylgeranylation may not explain all of the anti-cancer effects of statins. Indeed, context-specific dependencies such as farnesylated HRAS in head and neck cancers and CXCR4-CXCL12 signaling in AML are driving farnesylation inhibitors into the clinic [24,25]. In other settings, loss of mevalonate pathways outputs, such as ubiquinone [26], heme A [27], and isopentenylated tRNAs necessary for selenoprotein maturation [28] support mechanisms for mitochondrially-generated reactive oxygen species (ROS). Collectively, translation of statins to clinical oncology will require careful consideration of molecular determinants of mevalonate dependency, statin pharmacokinetics and pharmacodynamics, and promising combination approaches.

Oncogenic Lesions Ramp up Mevalonate Demand

Mevalonate pathway flux is an absolute requirement for all cells. In contrast, increased mevalonate pathway demand is a hallmark of oncogenesis in which the availability of mevalonate pathway intermediates results in adaptive changes that promote tumor cell fitness (Fig. 2). Within this framework, we identify three lines of evidence for the cancer-supportive role of the mevalonate pathway: flux driven by oncogenes, flux tempered by tumor suppressors, and control achieved by inhibition of the mevalonate pathway.

Several cancer types demonstrate increased mevalonate metabolism and sensitivity to statin mediated inhibition [2]; however, the molecular mechanisms driving these changes are diverse. The most prominent regulators of mevalonate pathway gene expression are SREBPs. SREBP transcriptional activity is subject to multiple levels of post-translational control (Fig. 1) [29,30]. As reviewed previously, AKT and mTORC1 are signaling mediators that promote SREBP maturation [31]. This review also highlighted cooperative mutant p53:SREBP interactions [32] and oncogenic MYC:SREBP interactions [33] that promote transcription of mevalonate pathway genes, subsequently confirmed in tumors with MYC overexpression [34]. We highlight studies that have detailed additional roles for these and other oncogenes and tumor suppressors in controlling mevalonate pathway flux.

Oncogenes Drive Mevalonate Flux

Generation of HMG-CoA depends on acetyl-CoA and NADPH availability in the cytosol [35]. Thus, mevalonate synthesis is partially dependent on mediators of metabolic reprogramming in cancer such as those driven by PI3K-AKT and MYC.

The connection of the mevalonate pathway to metabolic reprogramming is well illustrated by an acetyl-CoA tracing study in pancreatic cancer. In premalignant pancreatic acinar cells from a K-RAS-driven mouse model, aberrant activation of the AKT substrate ACLY increased abundance of acetyl-CoA, driving HMG-CoA synthesis as well as elevated expression of mevalonate pathway genes [36] (Fig. 2). Mevalonate pathway genes were also elevated in human pancreatic ductal adenocarcinoma (PDAC) samples. Different downstream metabolites played key roles at distinct stages of tumor progression. Induction of acinar-to-ductal metaplasia *in vitro* was suppressed by atorvastatin and rescued by supplementation of either mevalonate or cholesterol. In contrast, atorvastatin-mediated

inhibition of proliferation in human PDAC cell lines and murine cell lines was rescued by mevalonate and GGPP, but not by cholesterol. Thus, whereas cholesterol-rich membrane domains may promote oncogenic signaling in early dysplasia, mevalonate-derived lipid modifications on GTPases may coordinate dysregulated cellular processes that drive cancer survival in later stages (Fig. 3).

Hyperactivation of PI3K-AKT through loss of lipid phosphatase PTEN can promote dependency on mevalonate pathway intermediates that support macropinocytosis to meet increased metabolic demands (Fig. 2). A screen of *pten* null *Dictyostelium* identified mevalonate pathway inhibition to be synthetically lethal with loss of PTEN in *Dictyostelium* and verified in a human cell line [3]. This sensitivity extended beyond PTEN loss to K-RAS^{G12V}, which also hyperactivates PI3K-AKT signaling. Studies of normal and viral-oncogene-transformed breast organoids demonstrated selectivity of pitavastatin to tumor models. Pitavastatin inhibited macropinocytosis resulting in cell death due to amino acid starvation traced to loss of GGPP (Fig. 3). Loss of macropinocytosis could be rescued by GGPP and phenocopied by inhibiting GGPP production or the *Dictyostelium* prenyltransferase enzyme, GGTase1, implicating protein geranylgeranylation. However, the statin effect could only be partially rescued by overexpression of candidate GTPases, even when several members of the Ras, Rho, Rac and Rap families were co-expressed in their *Dictyostelium* model.

The oncogene MYC is a well-supported driver of metabolic reprogramming in cancer cells that promotes glutaminolysis and also intertwines with mevalonate synthesis in tumorigenesis [37]. Building on previous cancer stem cell studies [38–40], investigation of brain-tumor initiating cells (BTIC) has further connected MYC-reprogramming and mevalonate metabolism in maintaining stem-like populations [41]. MYC is overexpressed in 70% of gliomas and maintains BTIC to promote growth and self-renewal. In BTIC models, mevalonate pathway enzymes are highly expressed and are reduced upon differentiation or by MYC knockdown. shRNA-mediated knockdown of HMGCR in BTIC cells that were subsequently implanted in mice resulted in improved survival compared to mice implanted with a non-targeting shRNA control cell line. Statins preferentially inhibited viability and proliferation in BTIC, but not in matched differentiated glioma cells. Through induction of miR-33b, an important microRNA in cholesterol homeostasis [42], statins reduced MYC levels, exemplifying a feedforward relationship between MYC expression and mevalonate pathway flux [43] (Fig. 3).

Another common de-differentiation process in solid tumor cells is the epithelial-to-mesenchymal transition (EMT) that promotes invasiveness (Fig. 3). The EMT-inducing transcription factor ZEB1 promoted statin sensitivity in immortalized human mammary epithelial (MCF10A) cells, and expression of EMT markers correlates with statin sensitivity across a panel of cancer cell lines [44]. In an organoid model of EMT, statin treatment reduced dissemination upon expression of the transcription factor Twist1 [3]. Together these findings suggest the potential value of statins to suppress cancer cell invasion and metastasis. Consistent with a role for the mevalonate pathway in mesenchymal-transitioned cells, SREBP1 is an important factor in maintaining mesenchymal stem cells as well as supporting EMT in colorectal cancer [45]. This adaptation may be regulated by changes in the

extracellular matrix (ECM) during EMT, as culturing on soft ECM promoted SREBP1 processing through loss of a suppressive RhoA/AMPK mechanism (Fig. 2) [46]. In a phase II clinical trial of atorvastatin in breast cancer, RhoB, a tumor suppressing Rho family member, was increased and promoted the reversion of EMT, demonstrating that EMT can be targeted in the clinic [47,48].

Tumor Suppressors Quell Mevalonate Flux

A study of breast carcinogenesis showed that certain mutations of p53 confer gain-of-function control of mevalonate flux in cancer cells to impart a pro-invasive phenotype (Fig. 4) [32]. These mutations subvert a key function of wild-type (WT) p53 [49]. An essential tumor suppressor function of WT p53 is to reduce expression of mevalonate pathway genes. In models of colorectal and hepatocellular cancer, p53 loss promoted the maturation of SREBP-2 while p53 activation reduced levels of the mature form of SREBP-2 under sterol-depleted conditions. A p53 responsive mediator of retrograde sterol transport, *Abca1,* was identified as suppressor of SREBP-2 maturation, presumably by increasing sterol-levels at the endoplasmic reticulum [50]. Delivery of plasmids containing *Myc* and *Cas9* with *Tp53* sgRNAs to the liver showed that loss of p53 downregulated *Abca1* expression and subsequently increased SREBP-2 maturation compared to WT p53 tumors. p53 loss increased tumor growth in an atorvastatin-sensitive and mevalonate pathway-dependent manner; conversely, *Abca1* deletion in WT p53 tumors accelerated growth and reduced mouse survival. Thus, this study identified an important tumor-suppressive role of p53-induced *Abca1* on the tumor-supportive mevalonate pathway (Fig. 4).

The promyelocytic leukemia tumor-suppressor gene, *PML,* also inhibits mevalonate flux [51]. Deletion of the tumor suppressor phosphatase *PTEN* and *PML* were identified to frequently co-occur in human prostate cancers and were associated with an adverse prognosis and survival. Correspondingly, mice with prostate-specific deletion of *Pten* and *Pml* had higher penetrance of high-grade neoplasia and prostate cancer compared to loss of *Pten* alone, with a higher capacity to grow and self-renew. Tumors lacking *Pten* experienced feedback inhibition of MAPK pathway while the additional PML loss relieved feedback inhibition. *Pml* deletion increased SREBP processing corresponding to active MAPK signaling. Knockdown of SREBP1 or simvastatin treatment strongly inhibited the invasive phenotype. These results place SREBP activation and mevalonate pathway gene upregulation downstream of MAPK signaling (Fig. 4).

The primary cilium is a sensory organelle with tumor suppressive functions described in various epithelial cancers [52]. Loss of cilia is now recognized as an early oncogenic event that increases mevalonate pathway expression and promotes anchorage independence [53]. *In vitro* models of fibroblast transformation with various oncogenes led to cilia loss through reduction of Von Hippel Lindau (VHL), a tumor suppressor and component of primary cilium formation. Knockdown of ciliary components, *Tg737* or *Kif3a*, with the accompanying expression of K-RAS^{G12V} promoted anchorage-independent growth as well as tumor formation in nude mice. In the knockdown cells, ten percent of differentially regulated genes converged on the mevalonate pathway. Lovastatin treatment blocked enhanced colony formation as well as tumor-formation imparted by cilia loss, effects that

could be rescued with supplementation of FPP or GGPP. Cilia act as a rheostat for WNT signaling [54] and consequently loss of cilia promotes strong ß-catenin/TCF-mediated transcription. Interestingly, this study demonstrated that ß-catenin/TCF can complex with nuclear SREBP2 to strongly enforce mevalonate pathway expression (Fig. 4). Moreover, *VHL* loss was found to be synthetically lethal with mevalonate pathway inhibition in renal clear cell carcinoma, a tumor that demonstrates reduced primary cilia frequency [55,56]. Together, these results suggest that oncogenic lesions drive cilia loss to promote mevalonate pathway dependency which may be targeted by statins.

Challenges to Identifying Determinants of Mevalonate Pathway Dependency

In various model systems statins suppress diverse cancer hallmarks including proliferation, survival, and invasion/metastasis [2]. Statins have context-specific effects on tumor cell biology; moreover, in a given system the key outputs of mevalonate flux often involve families of related proteins with overlapping and redundant functions [2,3,31]. The multitude of possible mechanisms make it challenging to define key downstream determinants of mevalonate pathway dependency (Fig. 1). Recent studies have proposed roles for ubiquinone (Coenzyme Q10) [26,57] or dolichol [58,59]. There are also studies of the addition of IPP to tRNA, a process known as isopentenylation, and its role in cancer [28]. While these studies may result in niche applications of statins, they do not explain the more widely reported dependency on GGPP, as ubiquinone and dolichol can be derived from FPP [60–63] (Fig. 1). GGPP is utilized for the conversion of Vitamin K1 to Vitamin K2 [64] by UBIAD1, an enzyme which also directs FIMGCR degradation [65,66]. GGPP-directed functions of UBIAD1 are not well studied in statin's anti-cancer effects. The newly discovered signaling molecule S-geranylgeranyl-L-glutathione is also now a recognized GGPP output; however, little is known about the enzyme responsible for synthesis of this compound [67]. While these new GGPP products will surely garner more interest, we will focus our attention on protein geranylgeranylation.

GTPases make up a substantial portion of geranylgeranylated proteins. A challenge in assessing GTPase activity as a determinant of mevalonate pathway dependency is that inhibition of prenylation does not necessarily cause a loss of protein function. In mouse macrophages lacking *Pggt1b* or treated with statins, Rac1 did not mislocalize to the cytosol and instead had increased interaction with Iqgap1, an adaptor protein that stabilizes GTP-bound Rho GTPases; the enhanced Rac1 activity resulted in inflammatory arthritis [68,69]. Knockdown of Rad or Iqgap1 rescued the inflammatory effects caused by *Pggt1b* knockout or statin treatment in macrophages. Further evidence that statins do not universally suppress GTPase function is the observation that statins activate the abovementioned RAS-ERK-miR33b axis in glioma stem cells [41]. Whether loss of prenylation resulted in increased association with RAS effectors, thus increasing ERK phosphorylation, requires further investigation.

It is also becoming apparent that *in vivo*, loss of protein geranylgeranylation may lead to cancer cell-extrinsic phenotypes, such as altered anti-cancer immunity. Early studies concluded that statins may be immunosuppressive, stifling initial excitement for using statins as cancer therapies [70]. However, in cynomolgus monkeys and various mouse

influenza and cancer vaccination models, lipophilic statins were able to act as an adjuvant [71]. The loss of Rab5 geranylgeranylation resulted in prolongation and enhancement of antigen presentation in dendritic cells. Recent findings related to the mevalonate pathway and anti-cancer immunity have rekindled interest for potential interventions using statins [72].

When assessing geranylgeranylated GTPases for identifying molecular determinants of mevalonate pathway dependency several questions arise (Outstanding Questions). Novel tools and insights are available to better investigate the roles of prenylated proteins in death mediated by mevalonate pathway inhibition. These tools include prenylation inhibitors and chemical probes to distinguish target proteins.

Farnesyl can be post-translationally added to proteins by farnesyltransferase (FTase) utilizing FPP, while GGPP is utilized by at least three distinct geranylgeranyltransferase (GGTase) complexes (Fig. 5). Protein prenylation depends on c-terminal sequences, such as the cysteine-aliphatic-aliphatic-x_{aa} (CAAX) motif [73]. The CAAX box amino acid sequence determines whether a protein is either geranylgeranylated or farnesylated. The identification of RAS and RHO as prenylation substrates galvanized researchers to generate inhibitors of the FTase (FTI) and GGTase 1 (GGTI) as a promising strategy to target these “undruggable” oncogenes [74–76].

Most prenyltransferase inhibitors are peptidomimetics of CAAX motifs that act by competitive inhibition [76,77]. These compounds have demonstrated considerable preclinical potential in cancer models and provided useful mechanistic insight in numerous studies to primarily implicate GGTase 1 substrates in statin anti-cancer effects. Flowever, the discovery of a new prenylation enzyme complex (GGTase 3; Fig. 5) [78] that includes the essential gene PTAR1 [79] suggests that the selectivity of GGTIs should be revisited. Novel methods such as *in vitro* prenylation assays which utilize recombinant prenyltransferase enzymes to incorporate a labeled probe into protein from lysed cells (after treatment with statins or prenylation inhibitors) may be useful to assess the general prenylation activity of specific prenyltransferases and the selectivity of inhibitors [80] (Fig. 5). Global approaches to examine the prenylome by mass spectrometry using click-able isoprenoid probes are also now available to assess prenylation dynamics [81,82]. These approaches will be useful in re-establishing the complete set of prenylated proteins and their associated prenyltransferases.

Harnessing the Potential of Statins in the Clinic

Statins have drawn interest from oncologists based on epidemiological data [83–99], as well as numerous studies showing anti-cancer effects—a term we use here to encompass cytotoxic, cytostatic, and anti-metastastic or differentiation-inducing effects--in cell line and animal models with minimal toxicity to normal cells and tissues [47,100–109]. However, the rigor of the epidemiological evidence has been challenged [110,111] and, as noted above, the concentrations of statins needed to suppress cancer cell survival *in vitro* are generally much higher than clinically achievable levels at prescribed doses in plasma and in tumors [22]. These caveats accentuate the need for well-designed prospective trials to properly assess efficacy.

Epidemiological studies of statins in oncology often assume that all statins are equivalent [83–99]. Statins have distinct pharmacological properties [9] and the choice of statin compound has tremendous impact on the potential to impact tumor initiation and cancer treatment. Some commonly prescribed statins (simvastatin, atorvastatin) accumulate in the liver but achieve only low nanomolar concentrations in other tissues and in plasma. Hydrophilic statins such as pravastatin require active transport to enter cells and such transporters are generally not expressed in tumor cells. The half-life of different statins in plasma also varies widely. Thus, retrospective studies that group all statins together might underestimate the potential of statins to prevent or treat cancer.

Recent work highlighted that statin choice has been a major limitation of previous statin clinical trials in oncology [112], arguing that the compound pitavastatin has unique properties with potential to greatly improve anti-cancer efficacy. Pitavastatin achieves peak plasma concentrations in the 200 – 500 nM range and has a longer half-life than other statins [113,114]. Moreover, on a molar basis the anti-cancer effect of pitavastatin in cell lines is greater than other lipophilic statins [11,12,115]. Another advantage is that pitavastatin is not metabolized by the cytochrome P450 enzyme CYP3A4; this property should reduce drug-drug interactions with other medications and cancer therapies, improving both safety and efficacy [114]. Prospective trials are needed to determine whether pitavastatin can achieve meaningful tumor control as a single agent or combined with other cancer therapies.

Diet also has an under-appreciated role in the efficacy of statins in cancer. Unlike statins' impact on cholesterol control, their effect on cancer cell proliferation and survival depends on reduced pools of GGPP. Diets rich in geranylgeraniol provide an exogenous nutrient that can potentially restore these pools. This hypothesis is supported by controlled experiments where diet composition was varied in mouse tumor models [116]. Again, prospective trials in human patients will be needed to determine whether a diet low in geranylgeraniol (low flax, sunflower, and olive oils) can support anti-cancer efficacy of statins. As retrospective trials support statin efficacy in humans, it may not be necessary to test dietary intervention; instead, dietary journals or diet surveys from patients may be useful to assess whether diet confounds statin efficacy.

Improving Statin Efficacy: A Focus on Mitochondria

Tumor cells can activate diverse mechanisms to compensate for inhibition of single targets in signaling and metabolic pathways but can be more susceptible to combined inhibition of multiple survival mechanisms. Rational, mechanism-based combinations have potential to lower the dose needed to achieve a therapeutic effect (increased potency) and to obtain synergistic efficacy in blocking tumor cell proliferation and survival. Through detailed studies of statin responses in cancer cells, recent studies have revealed novel strategies to employ statins more effectively in combination regimens. A recent review [23] provided an in-depth discussion of statin combinations, including with chemotherapy and with molecular targeted therapies (for example: SREBP inhibition, anti-androgens, EGFR inhibition, HDAC inhibition) and immunotherapies. Here we highlight recent studies that evaluate mechanism-based combinations leveraging statin effects at the mitochondria [117].

Several mevalonate pathway outputs support mitochondrial function, including ubiquinone, heme A, and vitamin K2 (Fig. 1). Ubiquinone is the most well characterized for its function in the mitochondria. It is a lipophilic electron carrier in the electron transport chain, shuttling electrons from complex I and complex II to complex III (Fig. 6). A recent study has uncovered a unique role for ubiquinone in maintaining pyrimidine synthesis in p53-deficient colorectal cancer spheroids [26]. Growth of the spheroid tumors mimicked metabolic stress resulting in upregulation of the mevalonate pathway and ubiquinone. Statins reduced mitochondrial function while increasing ROS in p53-mutant spheroid cultures, resulting in apoptotic death. Generation of ROS resulted from dihydroorotate dehydrogenase, an essential enzyme for pyrimidine synthesis, which used ubiquinone as an electron transporter. Rescue was achieved by supplementation of mevalonate, ubiquinone and pyrimidines. In a parallel study, ubiquinone was confirmed to be depleted by statins in pancreatic cancer cell lines with p53 null mutations and *in vivo* in Kras-driven, p53 null mice resulting in ROS generation [57]. A compensatory increase in xCT, a redox balancing cystine/glutamate antiporter, was prevented with a clinical MEK inhibitor, selumetinib (Fig. 6). The combination of statins and selumetinib synergized to induce apoptosis in vitro and in vivo.

In a high-throughput gene expression screen of p53 wild type spheroid models, oxidative phosphorylation inhibitors were identified to target quiescent cells. In response, quiescent cells upregulated mevalonate pathway genes. The combined addition of oxidative phosphorylation inhibitors and statins induced synergistic cell death in quiescent multicellular spheroid models [118]. The statin combination functioned independently of cellular proliferation in this model and instead appeared to act on cellular energetics. Curiously, ubiquinone, GGPP, and squalene only partially rescued from this combination, suggesting multiple mevalonate pathway outputs mediate this combination. GGTI-298 fully recapitulated the effects of statins, indicating that protein geranylgeranylation may be central to the mechanism of this combination.

Statins predominantly induce cell death via apoptosis. In a CRISPR drop-out screen of metabolic pathways to identify apoptosis-sensitizing pathways, knockout of mevalonate pathway genes sensitized AML cells to venetoclax by hindering the response to oxidative stress as well as hampering oxidative phosphorylation due to limited isoprenoid precursors important for glutathione peroxidation and heme biosynthetic steps, respectively (Fig. 1) [119]. It should be noted that statins have also been characterized to sensitize to ferroptosis, a programmed cell death characterized by lipid peroxidation, through the reduction of a glutathione peroxidase, GPX4 (Fig. 6), and possibly ubiquinone [120,121]; however, no evidence exists that statins by themselves induce ferroptosis [122,123]. The resulting apoptotic sensitization to venetoclax may be due to mitochondrial dysfunction caused by statins as well as blockade of a mitochondrial protective response supported by the mevalonate pathway in *C. elegans.* [27].

Recent studies found that statins sensitize cancer cells to intrinsic apoptosis by the novel class of apoptosis-inducing drugs, BH3 mimetics [124,125]. Such agents induce rapid apoptosis when a cell is mitochondrially primed for death [126]. A BCL2-selective inhibitor, venetoclax, has changed the treatment landscape for chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML) [127–130]. However, venetoclax yields variable or no

response in other blood cancers [131] and resistance can develop overtime in CLL patients [132]. BH3 mimetics targeting other BCL2 family members (BCL-XL, MCL-1) are also in development [133]. Genetic and chemical dissection of intermediary metabolism demonstrated that mevalonate pathway inhibition can overcome resistance to BH3 mimetics in blood cancer cell lines [134]. Furthermore, synergy of statins with venetoclax occurred in a range of blood cancer cell lines and primary cells including CLL, AML and B-cell lymphoma [124]. The mechanism involved statin-induced upregulation of the pro-apoptotic protein PUMA, which can bind and inhibit all pro-survival members of the BCL2 family (Fig. 6). Notably, PUMA upregulation by statins in these systems was independent of p53, potentially adding value in blood cancers where p53 inactivation is common and associated with high risk of relapse. In addition, a retrospective analysis of venetoclax clinical trials in CLL showed that patients that were concurrently taking statins for cholesterol control were significantly more likely to achieve a complete response to venetoclax, and longer progression-free survival, with no difference in adverse events. When considering such retrospective analyses, one can speculate that these trials did not capture the full potential of statins as oncology drugs for at least two reasons: (i) the statin drugs most commonly prescribed for cholesterol control do not have optimal pharmacological properties to exert biological effects on blood cancer cells; (ii) the subjects did not have any dietary controls such as limiting intake of foods rich in geranylgeraniol.

Concluding Remarks

Understanding the roles of mevalonate pathway components in carcinogenesis will advance continuing efforts to yield a clinically targetable metabolic dependency. A major hurdle in the repurposing of statins as oncology drugs is the identification of the adaptive features that reflect increased cancer cell dependency on mevalonate flux. Identifying these features will help reveal molecular determinants of statin sensitivity (i.e. biomarkers) that can guide clinical practice. It is evident that mevalonate pathway dependence is a general feature across several types of cancer; however, it is unlikely that there is a single unifying reason for this dependence. Indeed, there may be several mechanisms across different types of cancer and even between different patients with the same cancer. The mevalonate pathway casts a broad net that entangles many different proteins and pathways. Novel tools are now available to help parse the effects of mevalonate pathway inhibition on a global scale. As we learn more about GTPase biology, these tools will be powerful for identifying key prenylated proteins that coordinate multi-protein processes in the cell.

While the search for mechanisms and biomarkers of mevalonate dependence continues, statins are ready to be applied in well-designed prospective clinical trials, with pitavastatin likely leading the charge. The combination of pitavastatin with BH3 mimetics in the setting of blood cancers is one promising approach, since one BH3 mimetic (venetoclax) is already standard of care in certain hematologic malignancies (a phase I study of pitavastatin combined with venetoclax for patients with AML or CLL is recruiting: NCT04512105). Insights on diet providing geranylgeraniol that undermines statin efficacy highlights the critical need of dietary logging to provide context for individual patient differences. Identification of biomarkers of mevalonate pathway dependence and assessing rational

combination strategies may mitigate the need to adjust dosing schedules from standards used in hypercholesterolemia, allowing for statins to be translated to clinical oncology drugs.

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Outstanding Questions

1. Are the anti-cancer effects of mevalonate pathway inhibition mediated by the loss of a unique geranylgeranylated protein or a redundant family of geranylgeranylated proteins?
2. If a redundant family is involved, does loss of geranylgeranylation affect one pathway through overlapping functions or multiple pathways with complimentary functions to mediate the anti-cancer effects?
3. Are statin anti-cancer effects due to loss of function or gain of function properties of the non-geranylgeranylated product or products?
4. Are these targets actionable *in vivo*?
5. What markers can be used to identify a cancer that's sensitive to the identified mechanism?

Highlights

The mevalonate pathway provides precursors for synthesis of not only cholesterol, but also for isoprenoid lipids involved in protein prenylation.

Oncogenic lesions drive increased mevalonate pathway flux to meet the greater demand for pathway intermediates that support cancer cell hallmarks.

Statins suppress mevalonate production and are widely used medications for cardiovascular disease, with unrealized potential for cancer therapy. By suppressing protein prenylation, statins interfere with many cellular processes that support cancer hallmarks such as proliferation, survival, and metastasis.

Statin choice, dietary considerations, and rational combinations are crucial for successful application of statins in oncology.

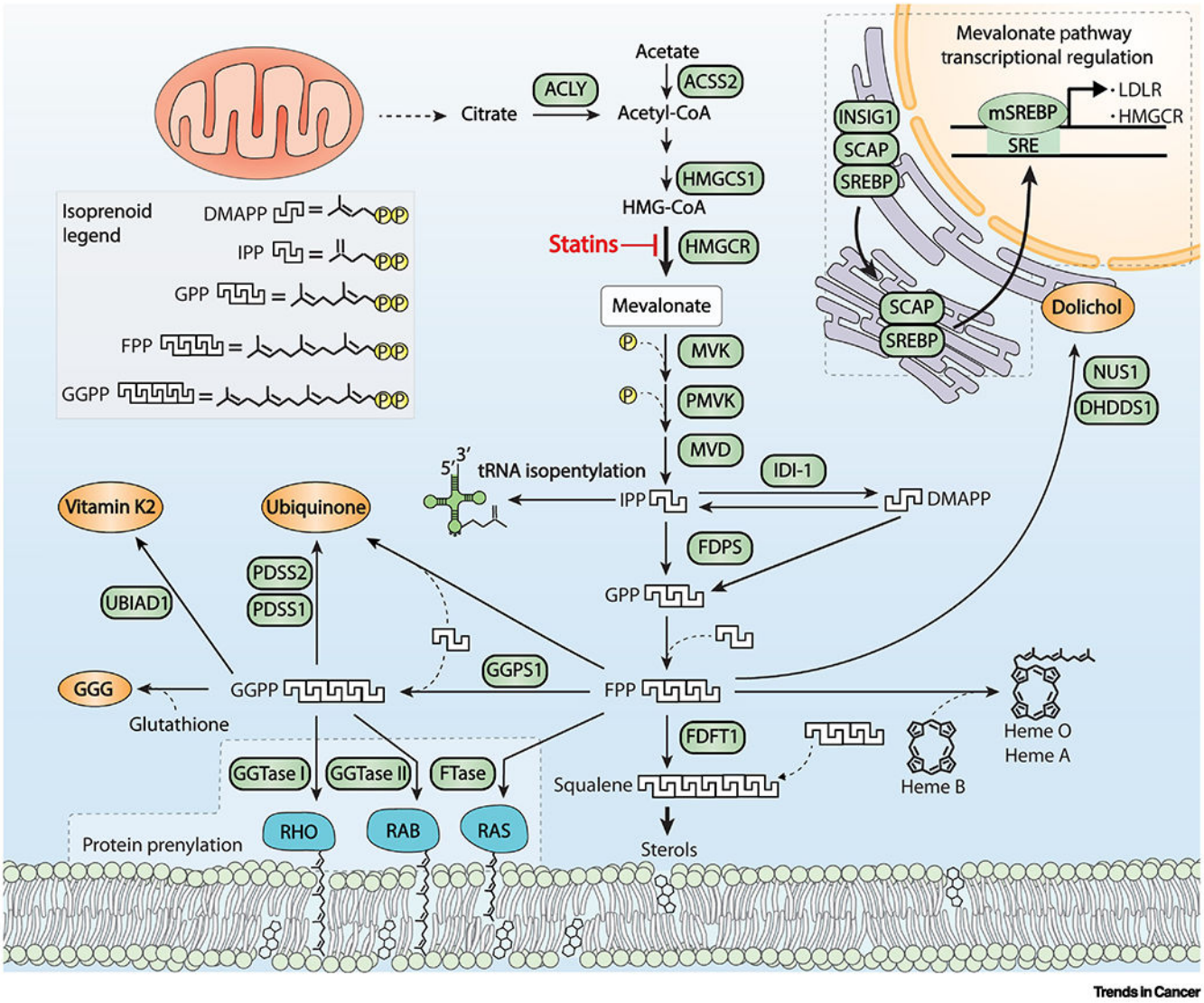

Figure 1. Inhibition of the mevalonate pathway by statins affects several outputs and lipid-regulating transcription factors.

The synthesis of mevalonate begins with a two-step condensation of three acetyl-CoA molecules to produce 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA). HMG-CoA is the substrate of the committed step enzyme, HMG-CoA reductase (HMGCR), that generates mevalonate. Mevalonate is further processed by two phosphorylation steps and decarboxylation to synthesize isopentenyl pyrophosphate (IPP), which can be isomerized to dimethylallyl pyrophosphate (DMAPP). These two molecules are building blocks for larger isoprenoid pyrophosphates. The synthesis of the 15-carbon isoprenoid farnesyl pyrophosphate (FPP) is a major branch point in the mevalonate pathway. FPP can be shuttled to the production of cholesterol through the synthesis and cyclization of the 30-carbon isoprenoid, squalene. FPP is also used in the synthesis of dolichol phosphate, an oligosaccharide carrier for asparagine glycosylation and mannose-donor for GPI-anchor production. The direct addition of FPP to biomolecules can occur during the formation of

ubiquinone (Coenzyme Q) and Heme A of cytochrome c oxidase. Finally, FPP is converted to geranylgeranyl pyrophosphate (GGPP) by geranylgeranyl diphosphate synthase 1 (GGPS1). Both FPP and GGPP can be post-translationally added to proteins, especially RHO, RAS and RAB family proteins, in a process known as prenylation by farnesyltransferase (FTase) and geranylgeranyltransferase (GGTase) respectively. GGPP also can be used for the synthesis of ubiquinone, the conversion of Vitamin K1 to Vitamin K2, and the generation of a newly discovered glutathione conjugate, s-geranylgeranyl-l-glutathione (GGG). SREBP maturation (mSREBP) is important for supporting mevalonate pathway gene expression.

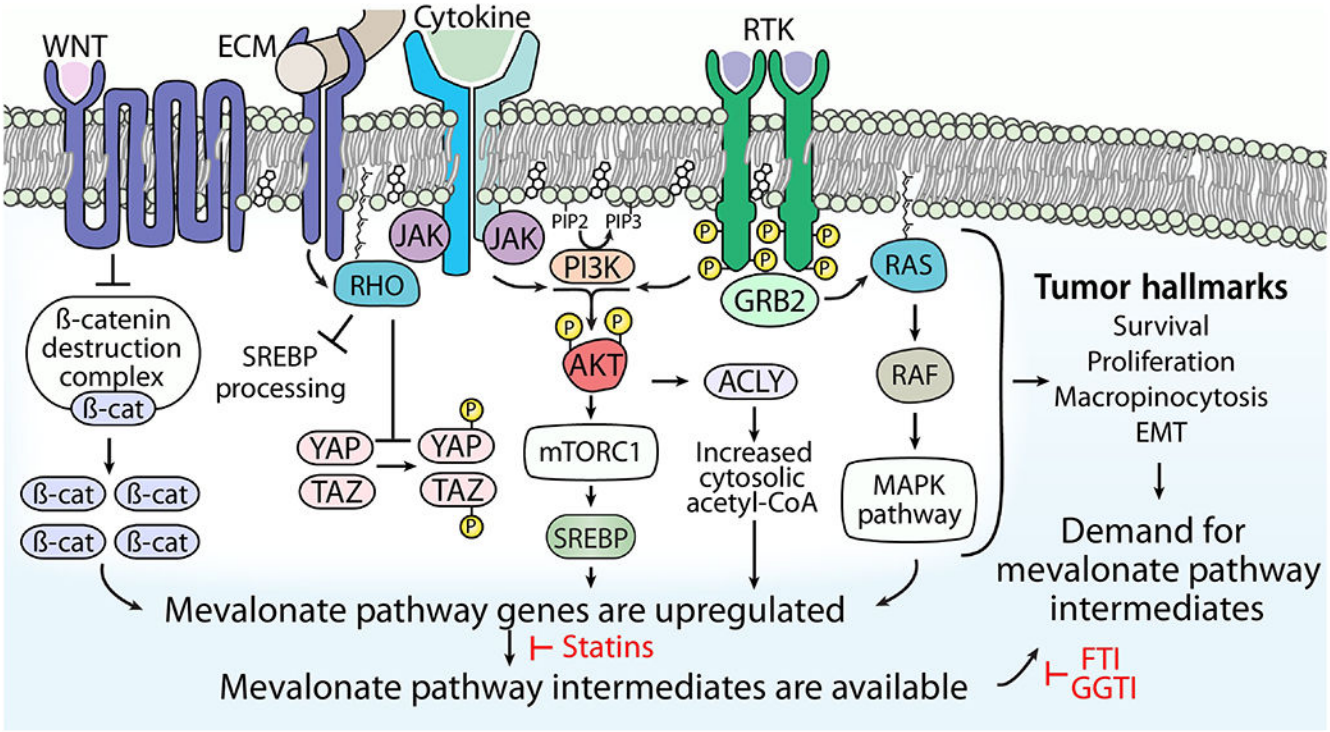

Figure 2. Oncogenic signaling pathways play a dual role in generating tumor hallmarks that increase demand for mevalonate pathway intermediates while concurrently upregulating mevalonate pathway meet the demand.

The mevalonate pathway supports signaling by cholesterol rich microdomains. Cilia act as a rheostat for WNT signaling. When cilia loss occurs, ß-catenin (ß-cat) interacts with SREBP2 to enforce expression of the mevalonate pathway to support tumorigenesis. Adhesion signaling from the surrounding extracellular matrix (ECM) finely controls YAP/TAZ localization to promote survival in epithelial cells while reduced adhesion signaling, as encountered by mesenchymal cells, strongly promotes SREBP processing and epithelial-to-mesenchymal transition (EMT). PI3K-AKT signaling, a major mediator of Warburg metabolism, can activate mTORC1-mediated SREBP processing as well as ACLY-mediated conversion of citrate to acetyl-CoA for mevalonate synthesis. The MAPK pathway may also contribute to SREBP maturation as well as promote several tumor hallmarks. Statins affect tumor hallmarks by blocking synthesis of necessary intermediates, while farnesyltransferase inhibitors (FTI) and geranylgeranyltransferase inhibitors (GGTI) block the use of specific intermediates (prenylation substrates) to meet the demand generated by tumor hallmarks, like macropinocytosis.

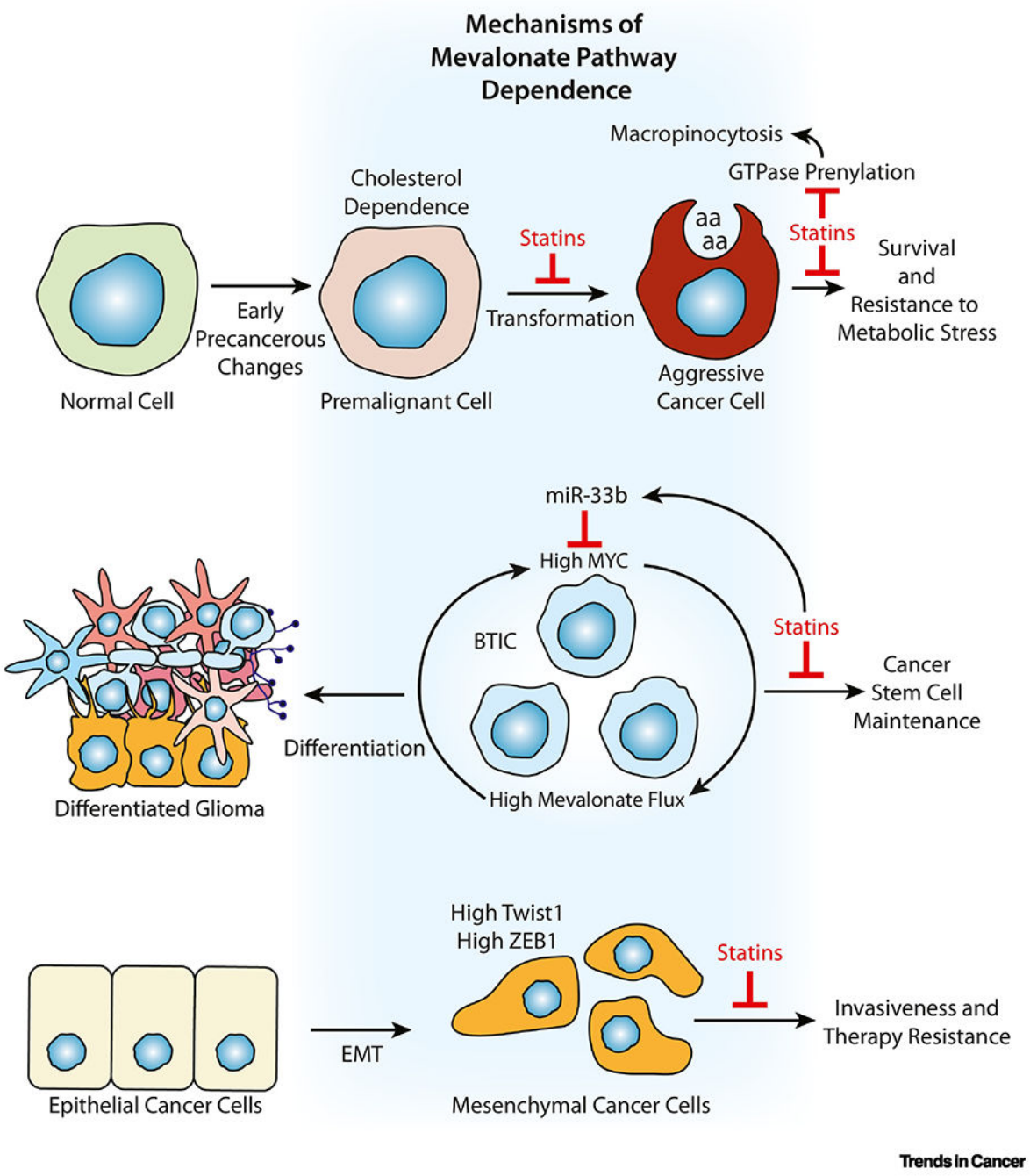

Figure 3. Novel insights uncover diverse mechanisms driving mevalonate pathway dependence.

Cholesterol may support early premalignant cell signaling, while strong evidence bolsters a role for protein prenylation in macropinocytosis. The cancer stem cell dependence on mevalonate pathway intermediates is mechanistically tied to a feedforward loop that promotes MYC expression and cancer stem cell maintenance. This feedforward loop can be disrupted by statin treatment. Lastly, the mesenchymal state has demonstrated a unique sensitivity to statins in various cancer models.

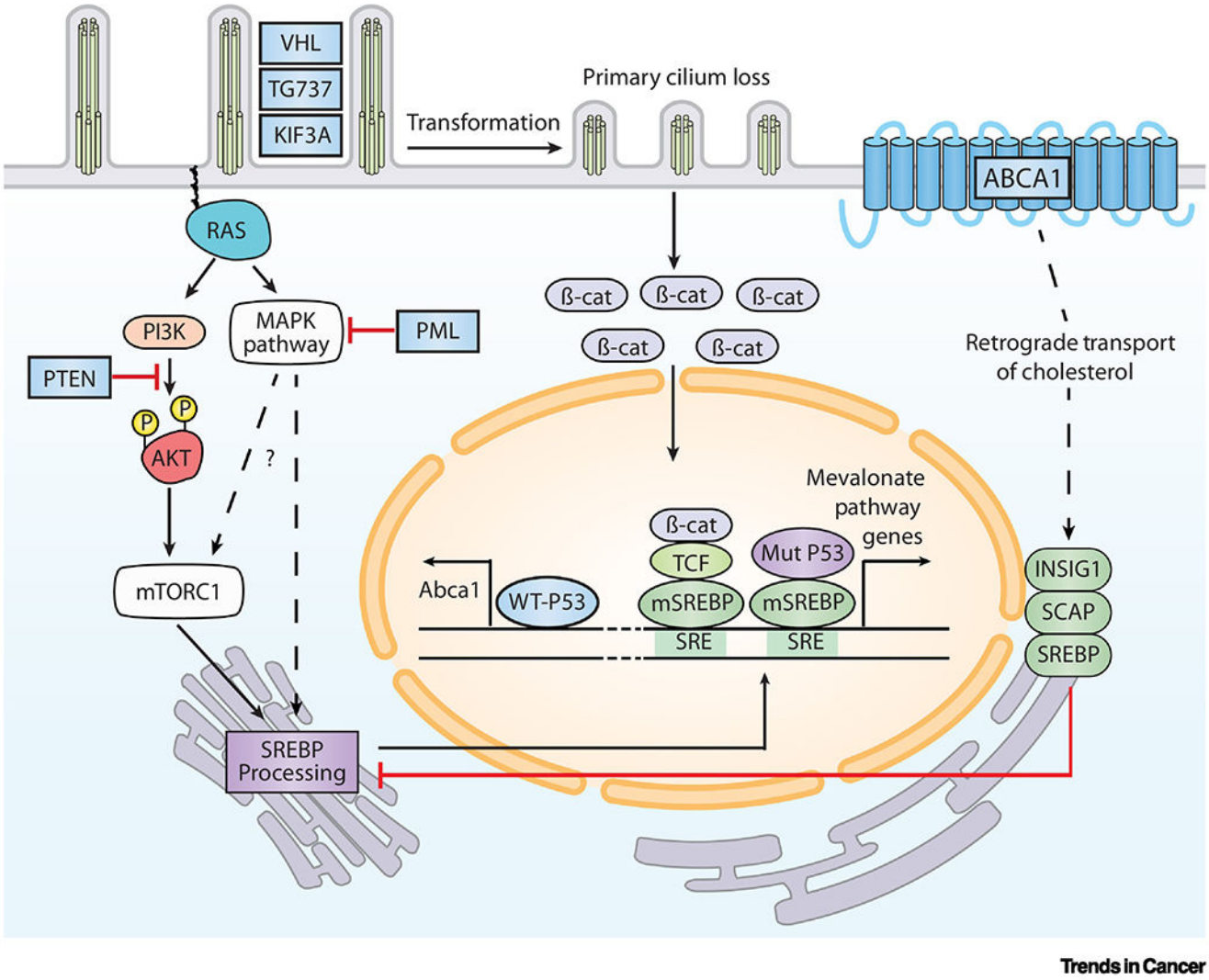

Figure 4. Tumor suppressors act as a checkpoint for mevalonate pathway flux by suppressing SREBP activity.

Tumor suppressors are in blue boxes with thick lines. Primary cilia, supported by VHL, Tg737, and Kif3a, are lost after transformation resulting in loss of their tumor suppressive function as a WNT rheostat and leading to enforced SREBP transcriptional activity. Wild type p53 suppresses the mevalonate by transcription of Abca1, a retrograde transporter of cholesterol that can suppress SREBP maturation. Certain p53 mutations may increase mSREBP activity through direct interaction. PTEN dampens PI3K-AKT-mTORC1 signaling, and thus mTORC1-mediated SREBP maturation; however, co-deletion of PTEN and the tumor suppressor PML is required in prostate cancer to promote the lipogenic SREBP program.

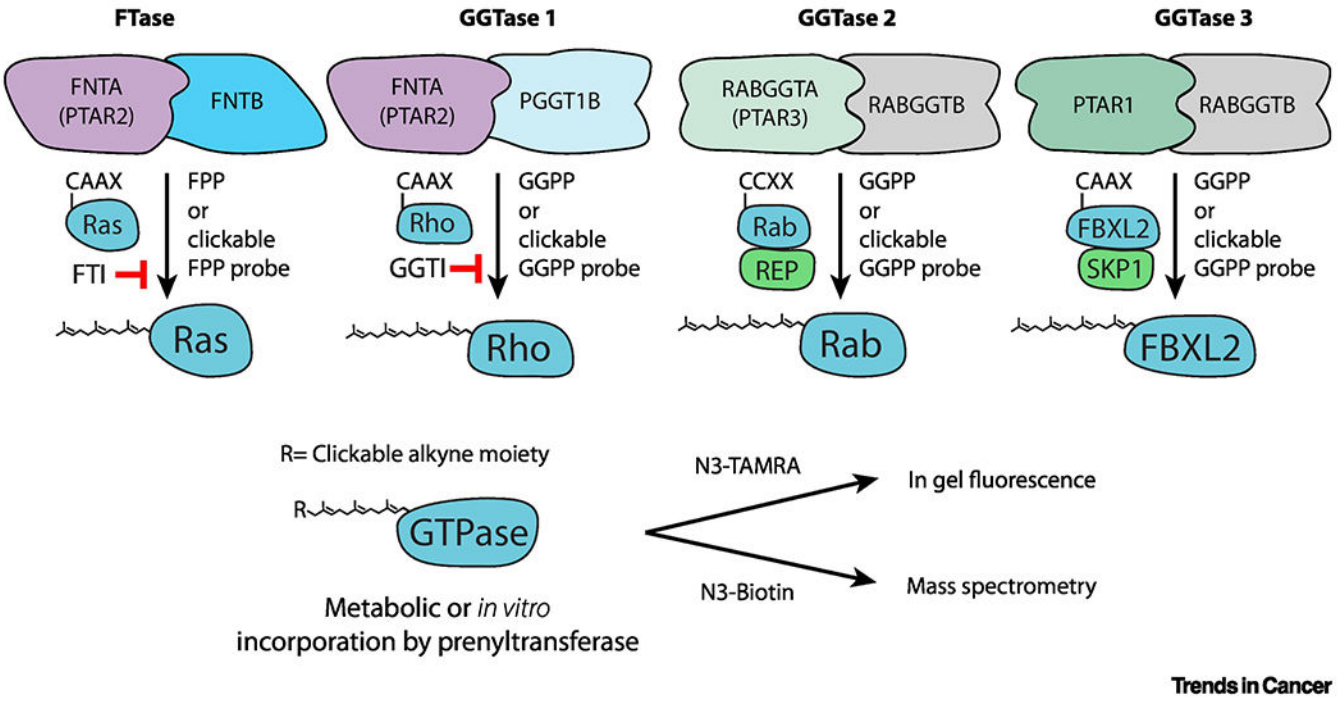

Figure 5. Prenyltransferase biology is key to identifying targets of statin anti-cancer effects.

Prenyltransferases mediate the prenylation of proteins through recognition of CAAX or dicysteine motifs. There is some overlap in the composition of individual complexes: FNTA pairs with different catalytic subunits (FNTB, PGGT1B) to form FTase and GGTase I; the catalytic subunit RABGGTB pairs with different subunits (RABGGTA/PTAR3, PTAR1) to form GGTase 2 (also known as RabGGTase) and GGTase 3. Chaperone proteins REP and SKP1 are respectively required for geranylgeranylation by GGTase 2 and newly identified GGTase 3. FTase inhibitors (FTI) have clinical potential in tumors driven by H-RAS, which is exclusively farnesylated. Currently available GGTase inhibitors (GGTI) competitively inhibit GGTase I by acting as a CAAX peptidomimetic though their activity towards GGTase 3 is not well characterized. Clickable prenylation probes provide tools to assess the substrates of each prenyltransferase using either targeted or global approaches using fluorescent tags like tetramethylrhodamine (TAMRA) azide (N3-TAMRA) or biotin-labeling with biotin azide (N3-Biotin).

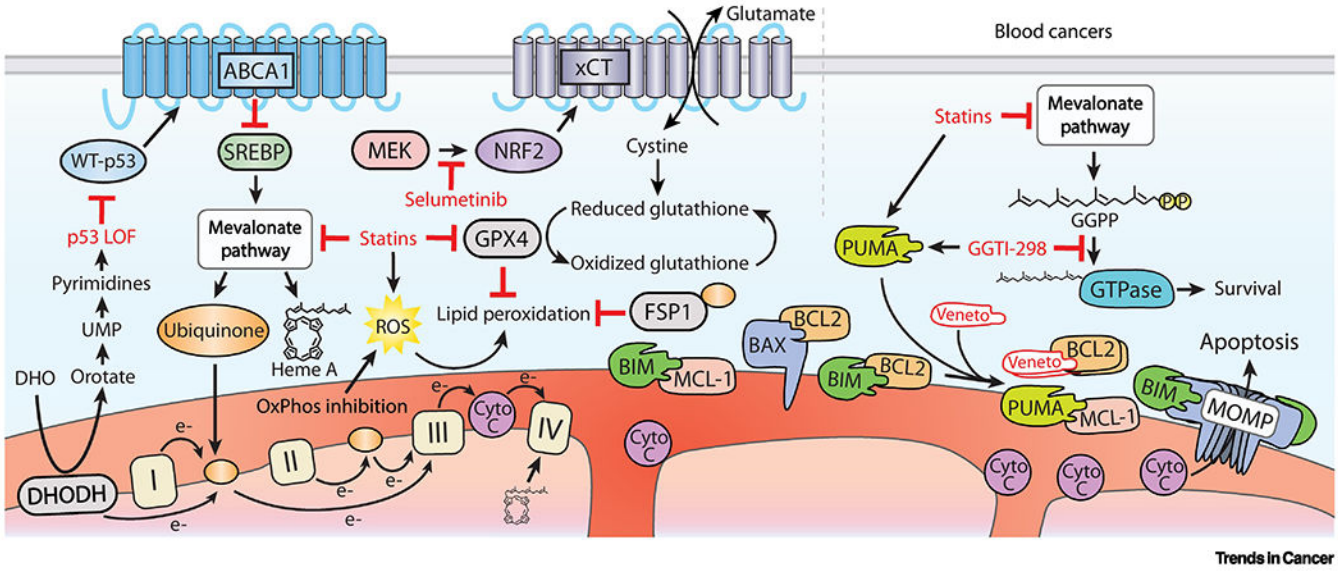

Figure 6. Rational combinations enhancing the effects of statins at the mitochondria.

The mevalonate pathway supports mitochondrial function by providing ubiquinone and heme A that support the electron transport chain and geranylgeranyl pyrophosphate (GGPP) for GTPases that promote survival. Cancers that have lost p53 function (p53 LOF) depend on the mevalonate pathway to support pyrimidine synthesis through the ubiquinone-reducing, dihydroorotate (DHO) dehydrogenase (DHODH) enzyme. Statins induce the production of reactive oxygen species. A MEK-NRF2-mediated compensatory increase of the cystine/glutamate antiporter, xCT, provides resistance to oxidation by supporting glutathione synthesis, which can be prevented by combining statins with the clinical MEK inhibitor, selumetinib. Furthering oxidative phosphorylation (Ox Phos) dysfunction with Ox Phos inhibitors synergize with statins to induce cell death. GPX4 and FSP1 depend on mevalonate pathway outputs to prevent lipid peroxidation and sensitization to ferroptosis. Inhibition of protein geranylgeranylation in blood cancers (via statins or GGTIs) results in the increase of p53-upregulated modulator of apoptosis (PUMA) in a p53 independent manner. Statin-induced PUMA sensitizes to the clinical BCL-2 inhibitor, venetoclax (Veneto), by disrupting anti-apoptotic interactions of BCL-2 as well as MCL-1 Release of the BH3 activator, BIM, induces mitochondrial outer membrane permeability (MOMP) resulting in cytochrome c (Cyto C) release and blood cancer apoptosis.