REVIEWS

The interplay between cell signalling and the mevalonate pathway in cancer

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Abstract | The mevalonate (MVA) pathway is an essential metabolic pathway that uses acetyl-CoA to produce sterols and isoprenoids that are integral to tumour growth and progression. In recent years, many oncogenic signalling pathways have been shown to increase the activity and/or the expression of MVA pathway enzymes. This Review summarizes recent advances and discusses unique opportunities for immediately targeting this metabolic vulnerability in cancer with agents that have been approved for other therapeutic uses, such as the statin family of drugs, to improve outcomes for cancer patients.

Acetyl-CoA
An essential metabolite that is used to drive many cellular processes, including the tricarboxylic acid (TCA) cycle, fatty acid and sterol biosynthesis, and acetylation of histones.

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Cancer cells reprogramme their metabolism to provide energy and the essential building blocks required to maintain their aberrant survival and growth[1–5]. This reprogramming may occur through either mutations in metabolic enzymes (for example, isocitrate dehydrogenases (IDHs)[6,7]) or alterations in cell signalling owing to oncogenic events and/or the remodelled tumour microenvironment. These activated signalling cascades in turn deregulate the expression[8,9] and/or the activity of enzymes in key metabolic pathways[10], including the mevalonate (MVA) pathway (FIGS 1,2).

The MVA pathway[11] uses acetyl-CoA, NADPH and ATP to produce sterols and isoprenoids that are essential for tumour growth[12] (FIGS 1,2). The production of acetyl-CoA occurs following glucose, glutamine or acetate consumption, which are often increased in cancer cells[4,5,13,14]. NADPH is produced from a variety of sources, including the pentose phosphate pathway, malic enzyme and IDHs[15,16]. Therefore, the MVA pathway is highly integrated into the overall metabolic state of cancer cells (FIG. 1). The transcription of genes encoding MVA pathway enzymes is primarily controlled by the sterol regulatory element-binding protein (SREBP) family of basic helix–loop–helix leucine zipper (bHLH-LZ) transcription factors. When intracellular sterol levels are high, the SREBPs are maintained in an inactive state at the endoplasmic reticulum (ER), where some MVA pathway enzymes are also localized. In response to sterol deprivation, a feedback response is initiated that leads to the SREBPs, along with their binding partner SREBP cleavage-activating protein (SCAP), dissociating from the insulin-induced genes (INSIGs) and translocating from the ER to the Golgi (FIG. 3). At the Golgi, the SREBPs are sequentially cleaved by site-1 protease and site-2 protease (S1P and S2P) and they translocate to the nucleus where they bind to sterol regulatory elements (SREs) in the promoters of their target genes and activate the transcription of MVA pathway genes to restore sterol and isoprenoid levels[12,17].

The importance of MVA pathway metabolites to the survival of cancer cells has been highlighted by recent studies that have identified a large number of MVA pathway enzymes as essential for the survival of several cancer cell lines[18–20]. Additionally, numerous studies have shown that the statin family of drugs, which inhibit the initial flux-controlling enzyme of the MVA pathway, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), decrease growth and increase apoptosis in many cancer types *in vitro* and *in vivo*[21–25]. These observations point to the MVA pathway as being a key dependency in tumours, and one that is readily targetable.

The MVA pathway has been suggested by some studies to be oncogenic. Early work in chronic lymphocytic leukaemia (CLL) showed that MVA can stimulate replication in primary leukaemic cells[26]. In another study, overexpression of the catalytic domain of HMGCR in primary mouse embryonic fibroblasts cooperated with $HRAS^{G12V}$ to promote foci formation, suggesting that HMGCR is a metabolic oncogene[27]. In addition, the direct infusion of MVA into mice harbouring breast cancer cell xenografts caused an increase in tumour growth[28]. Data from primary patient samples also suggest a role for the MVA pathway in promoting tumorigenesis, with a higher expression of MVA pathway genes correlating with poor prognosis in breast cancer[27]. Collectively, this evidence indicates that the MVA pathway has a key role in cancer.

In this Review, we discuss recent evidence demonstrating that the MVA pathway is deregulated in cancer through aberrant cell signalling, which in turn establishes a tumour vulnerability that can be therapeutically targeted to improve outcomes for cancer patients.

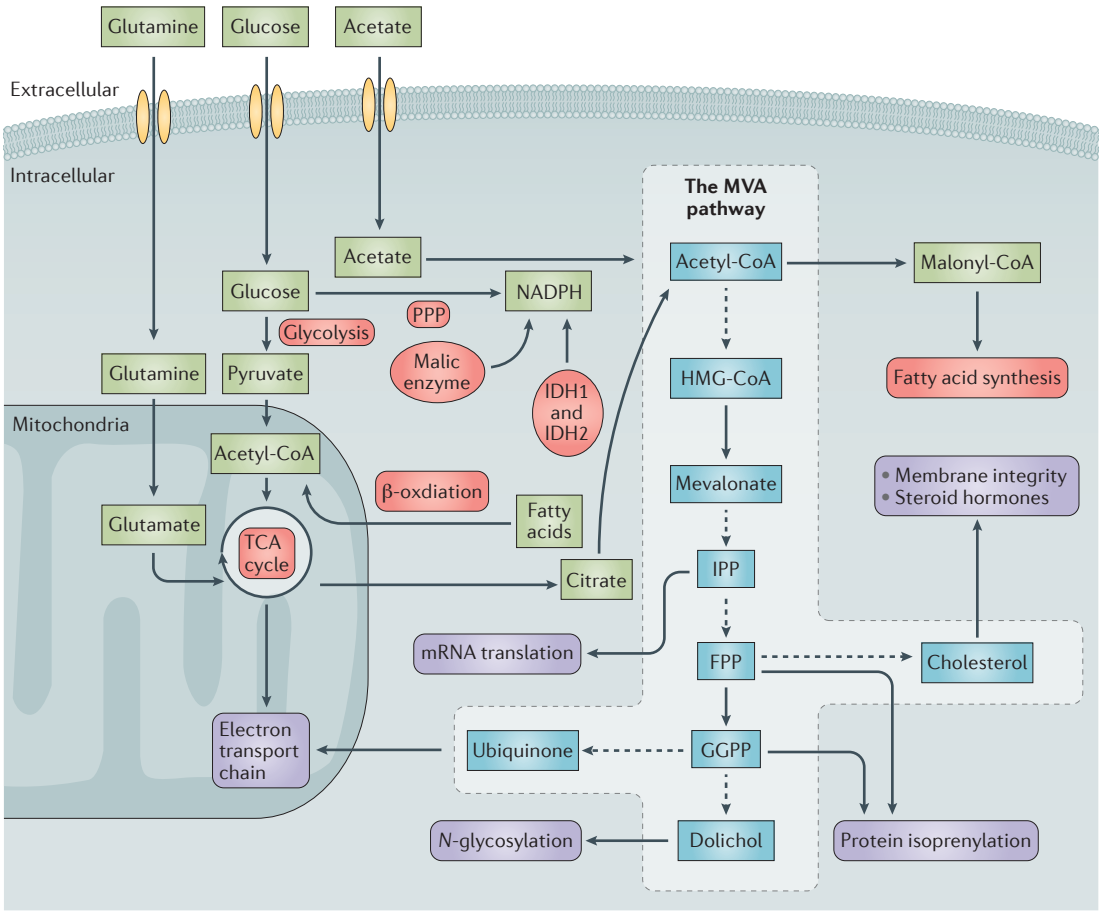


Figure 1 | **Overview of the MVA pathway.** The mevalonate (MVA) pathway is an essential anabolic pathway that uses acetyl-CoA, derived from glucose, glutamine and/or acetate metabolism, to produce sterols and isoprenoid metabolites that are essential for a variety of biological processes. MVA pathway metabolites are shown in blue boxes, other metabolites are shown in green boxes, and metabolic processes and enzymes are shown in pink. Processes discussed in this Review that require MVA-derived metabolites are shown in purple. Dashed arrows represent multiple steps in the MVA pathway. Many reactions, and their ability to be reversed, have been omitted for simplicity. FPP, farnesyl diphosphate; GGPP, geranylgeranyl-diphosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; IDH, isocitrate dehydrogenase; IPP, isopentenyl-diphosphate; PPP, pentose phosphate pathway; TCA cycle, tricarboxylic acid cycle.

SREBP cleavage-activating protein
(SCAP). Essential for sterol regulatory element-binding protein (SREBP) endoplasmic reticulum (ER)-to-Golgi translocation. SCAP contains a sterol-sensing domain and undergoes a conformational change when levels of ER membrane sterols are low. This change causes a dissociation of the SCAP–SREBP complex from insulin-induced genes (INSIGs).

Insulin-induced genes
(INSIGs). INSIG1 and INSIG2 interact with SREBP cleavage-activating protein (SCAP) under sterol-rich conditions. They prevent sterol regulatory element-binding protein (SREBP) activation by retaining the SCAP–SREBP complex in the endoplasmic reticulum (ER). They also promote the sterol-regulated degradation of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR).

Site-1 protease and site-2 protease
(S1P and S2P). Two proteases that cleave the sterol regulatory element-binding proteins (SREBPs), in the Golgi. S1P cleaves at the luminal loop of the SREBPs, whereas S2P is a hydrophobic protein that cleaves the SREBPs at a transmembrane residue.

Sterol regulatory elements
(SREs). Motifs found in the promoters of genes that are transcribed in response to sterol deprivation. SREs are necessary for the transcription of mevalonate (MVA) pathway genes by the sterol regulatory element-binding proteins (SREBPs).

Lipid rafts
Membrane domains that contain high concentrations of cholesterol, saturated fatty acids and sphingolipids. They are tightly packed and form the liquid ordered phase of membranes. One key role is to enable protein complexes to be pre-organized for efficient signal transduction.

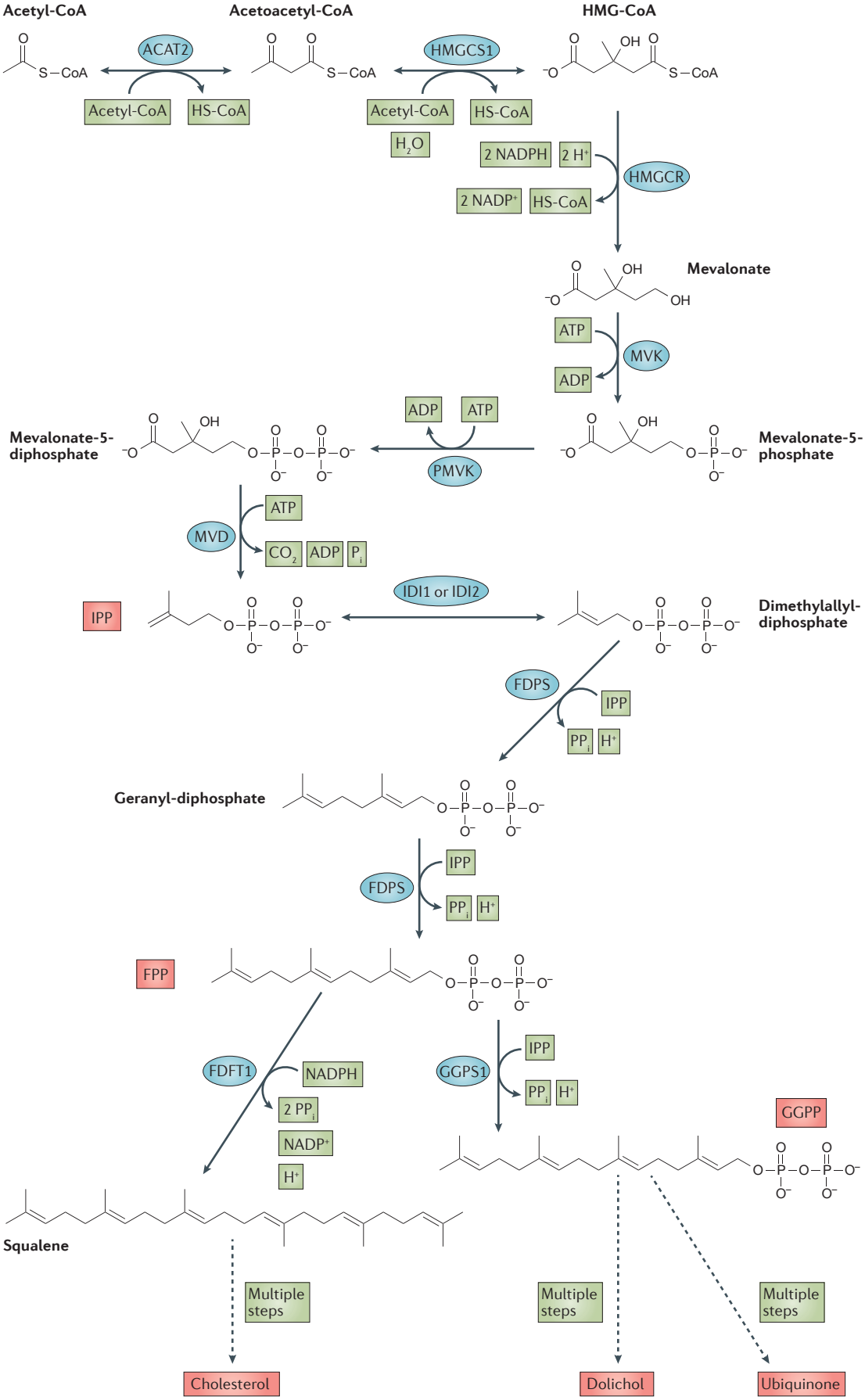
MVA-derived metabolites in cancer

Initially, the regulation and function of the MVA pathway and its metabolites were studied in the context of normal and hypercholesterolaemic tissues, which led to the Nobel prize-winning discoveries of Bloch and Lynen in 1964 (REF. 29), and Brown and Goldstein in 1985 (REFS 11,30). In recent years, the importance of MVA pathway-derived metabolites in cancer has become increasingly appreciated (discussed below).

Cholesterol. Cholesterol is an important component of most cellular membranes. Highly proliferative cancer cells need to produce membranes rapidly, and an increase in cholesterol synthesis contributes to this process. Cholesterol is also an integral component of lipid rafts, which are necessary to form signalling complexes[31–33]. The cholesterol content of the ER has recently been linked to the antiviral type I interferon (IFN) response, with low ER cholesterol triggering an IFN response in macrophages that protects mice from viral challenge[34]. Therefore, it is possible that high levels of cholesterol, produced by the MVA pathway, could have a role in protecting cancer cells from immune surveillance and various therapies[35,36]. Cholesterol also serves as the precursor of downstream products, such as steroid hormones and oxysterols: steroid hormones drive the initiation and progression of various cancers, including breast and prostate carcinomas[37]; and increased oxysterol production can activate the liver X receptors (LXRs), which have been proposed to be therapeutic targets in multiple cancer types[38,39].

Therefore, cancer cells require cholesterol for growth and survival, and decreasing intracellular cholesterol biosynthesis is a promising anticancer strategy.

Isopentenyl-diphosphate. In human cells, the MVA pathway is the sole intracellular source of isopentenyl-diphosphate (IPP)[40] (FIG. 2). Aberrant activation of the MVA pathway in cancer results in increased intracellular levels of IPP, which has been shown to activate host γδ T cells that subsequently kill the IPP-overexpressing cells[41,42]. These observations led to phase I clinical trials



γδ T cells
T cells with a T cell receptor that contains a γ- and a δ-chain instead of the more common α- and β-chains. They are known to recognize lipid antigens, are independent of major histocompatibility complex (MHC) class I presentation and are currently being investigated for their anticancer potential.

Isoprenylation
The attachment of a hydrophobic farnesol or geranygeraniol to the carboxyl terminus of proteins that contain a CAAX motif, which anchors the proteins to lipid membranes. Geranylgeraniol can also be attached to non-CAAX motif-containing proteins.

Quinone
A cyclic organic compound that contains two C = O groups. The quinone coenzyme Q is derived from the essential amino acid tyrosine.

that evaluated the *in vivo* expansion of γδ T cells in response to zoledronate, a bisphosphonate that inhibits farnesyl diphosphate synthase (FDPS) and leads to the accumulation of IPP (TABLE 1), in combination with interleukin-2 (IL-2) treatment in advanced-stage breast[43] cancer and prostate[44] cancer. In both studies, the therapy was well-tolerated and the number of sustained peripheral γδ T cells correlated with improved clinical outcome[43,44]. Future phase II clinical trials will reveal whether combined zoledronate and IL-2 therapy is an effective anticancer strategy.

Farnesyl-diphosphate and geranylgeranyl-diphosphate. Farnesyl-diphosphate (FPP) and geranylgeranyl-diphosphate (GGPP) are produced by sequential condensation reactions of dimethylallyl-diphosphate with two or three units of IPP, respectively. FPP and GGPP contain hydrophobic chains that are essential for the isoprenylation of proteins. This post-translational modification tethers proteins to cell membranes, enabling proper protein localization and function[45–48]. Most small GTPases — many of which are involved in tumorigenesis, such as RAS and RHO — are isoprenylated[49]; inhibition of the MVA pathway can reduce the isoprenylation of these small GTPases[50–52] and can induce the death of some cancer cells[52–56]. This cell death can be reversed by the addition of GGPP, and sometimes FPP, suggesting that these MVA pathway metabolites are essential for tumour cell viability[52–56]. Evidence suggests that it is unlikely that any one isoprenylated protein can be assigned functional responsibility for this cancer cell dependency on GGPP and FPP[52,57]; instead, it seems that this is a 'class effect', with the depletion of these isoprenoid pools potentially affecting the many proteins that are isoprenylated[58]. Despite this dependency, directly inhibiting the isoprenylation of proteins using geranylgeranyltransferase inhibitors (GGTIs) or farnesyltransferase inhibitors (FTIs) has not been a successful anticancer strategy to date[59]. The rationale behind these drug development programmes was that key isoprenylated oncoproteins, such as RAS, could be targeted. However, the efficacy of FTIs was impeded by alternative isoprenylation using GGPP, and GGTIs have been disappointingly toxic[60,61]. Further development of next-generation FTIs and GGTIs remains a fairly limited and focused area of research[59,62–66] (TABLE 1).

◀ Figure 2 | **The chemical reactions of the MVA pathway.** Mevalonate (MVA) pathway enzymes condense three acetyl-CoA molecules in a two-step reaction to produce 3-hydroxy-3-methylglutaryl CoA (HMG-CoA). Both reactions are reversible and in equilibria, with the intracellular concentration of acetyl-CoA being the primary driver. HMG-CoA is then reduced by HMG-CoA reductase (HMGCR) to produce MVA via an irreversible reaction. MVA is then converted into isopentenyl-diphosphate (IPP) through a series of enzymatic steps, which serves as a monomeric unit for the consequent synthesis of all downstream metabolites (metabolites discussed in this Review are highlighted in pink). Dashed arrows indicate multiple steps. ACAT2, acetyl-CoA acetyltransferase 2; FDFT1, farnesyl-diphosphate farnesyltransferase 1; FDPS, farnesyl diphosphate synthase; FPP, farnesyl diphosphate; GGPP, geranylgeranyl-diphosphate; GGPS1, geranylgeranyl diphosphate synthase 1; HMGCS1, HMG-CoA synthase 1; IDI, isopentenyl diphosphate isomerase; MVD, mevalonate-diphosphate decarboxylase; MVK, mevalonate kinase; PMVK, phosphomevalonate kinase.

Dolichol. Dolichol is derived from 18–20 IPP molecules and is an essential component of the *N*-glycosylation of nascent polypeptides in the ER[67,68]. Protein *N*-glycosylation is frequently altered in cancer and can contribute to tumour formation, proliferation and metastasis[69]. Not all *N*-glycans are associated with tumour progression; the complex branching of *N*-glycans leads to tumour-suppressive properties in some cancers (reviewed in REF. 69). Glucose-derived *N*-acetylglucosamine has recently been shown to be necessary for the *N*-glycosylation of SCAP before ER-to-Golgi translocation. The SCAP–SREBP complex thus remains inactive in the ER when glucose is absent, even in the presence of low levels of sterols[70].

Coenzyme Q. Isoprenoids are also used to produce the quinone coenzyme Q (CoQ). The hydrophobic isoprenoid chain localizes CoQ to the inner membrane of the mitochondria, where the quinone group transfers electrons from complex I or II to complex III of the electron transport chain, thus enabling ATP production[71]. Therefore, CoQ is crucial for ATP production in cancer cells that rely on oxidative phosphorylation to produce energy[72,73].

Oncogenic regulation of the MVA pathway

Intracellular pools of MVA pathway metabolites are tightly regulated by modulating the expression and activity of the MVA pathway enzymes. MVA pathway gene expression is mainly controlled by the SREBP transcription factors (FIG. 3). There are three SREBP proteins, which are transcribed from two genes: SREBP2 is transcribed from the *SREBF2* gene, and is the main transcription factor for MVA pathway-associated genes; SREBP1a and SREBP1c are transcribed from alternative start sites in the *SREBF1* gene, with SREBP1a regulating the expression of both MVA and fatty acid metabolism genes, and SREBP1c predominantly regulating the expression of fatty acid metabolism genes[74–77]. Chromatin immunoprecipitation followed by sequencing (ChIP–seq) studies have indicated some overlap in the target genes of each SREBP, including MVA pathway genes, indicating some redundancy[78,79]. Most studies have also shown an overlap in the regulation of the SREBPs; however, the majority of studies limit full characterization to SREBP1, and most do not distinguish between SREBP1a and SREBP1c as available antibodies cannot differentiate between the two. Given the importance of the MVA pathway in cancer, a complete characterization of SREBP2 in transformed cells is needed.

In recent years, oncogenic and tumour-suppressive pathways have been shown to converge on the MVA pathway and its regulatory feedback loop. Cancer cells, with their aberrant growth and metabolism, are thus primed to upregulate the MVA pathway to provide essential building blocks for continued proliferation. The integration of cellular signalling from growth factors and essential metabolites, with the regulation of the MVA pathway and its SREBP-regulated feedback response, highlights the importance of this pathway in cancer cells.

PI3K–AKT. The PI3K–AKT signalling pathway is a major regulator of cell survival and proliferation in response to growth factors. It is the single most frequently altered pathway in cancer, and the second most frequently mutated gene is *PIK3CA*, which encodes PI3K catalytic subunit-α (REF. 80). Inactivating mutations in the PI3K–AKT pathway negative regulator PTEN and/or the hyperactivity of growth factor receptor tyrosine kinases are also common in cancer[81,82]. Alterations in the PI3K–AKT pathway generally act to augment signalling, and consequently increase the proliferation of cancer cells.

PI3K–AKT can activate the MVA pathway through various mechanisms (FIG. 4). For example, the stimulation of PI3K–AKT signalling by growth factors, such as insulin, platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), can increase the mRNA and protein expression of SREBP1 and SREBP2 (REFS 83–87). It should be noted that although PI3K–AKT signalling strongly and consistently increases the mRNA and protein levels of SREBP1a and SREBP1c, its effects on SREBP2 expression are context dependent[88–90]. AKT has also been suggested to increase the stability of nuclear SREBP1a, SREBP1c and SREBP2 by preventing their proteasomal degradation mediated by the F-box and WD repeat domain containing 7 (FBXW7) E3 ubiquitin ligase[91]. The importance of this degradation pathway is highlighted by an increase in cholesterol and fatty acid synthesis in FBXW7-deficient cells[91]. The residues that are recognized by FBXW7 are phosphorylated by glycogen synthase kinase-3β (GSK3β); AKT, which inhibits this phosphorylation, may prevent FBXW7-mediated degradation of the SREBPs (FIG. 4). Insulin also causes the dissociation of INSIG from SCAP–SREBP1c in a sterol-independent manner, leading to the increased transcription of MVA pathway genes[92–95]. These studies were further validated through genetic approaches, in which SREBP1 and SREBP2 expression and activity were increased with the expression of constitutively active PI3K or AKT, and abrogated by dominant-negative AKT[84,95,96]. The increase in lipid and cholesterol production that is mediated by the PI3K–AKT–SREBP axis promotes the proliferation of cancer cells and tumorigenesis *in vitro* and *in vivo*[90,97,98]. Increased MVA pathway activity is inconsequential without the availability of both acetyl-CoA and NADPH, and PI3K–AKT signalling meets this requirement by increasing glucose uptake and the rate of glycolysis in cancer cells[99]. Conversely, inhibition of the MVA pathway decreases PI3K activity[100], possibly through decreased RAS isoprenylation[100,101], thus demonstrating a two-way regulatory relationship between PI3K–AKT signalling and the MVA pathway.

mTORC1. Downstream of PI3K–AKT signalling, mTOR complex 1 (mTORC1) acts as a sensor of growth signals (such as insulin) and nutrients (such as amino acids) to regulate cellular growth[102]. mTORC1 is often deregulated in cancer, and this supports aberrant growth. mTORC1 increases mRNA translation by phosphorylating and activating ribosomal S6 kinase 1 (S6K1; also known as RPS6KB1)[103,104] and repressing the activity of the inhibitor of cap-dependent translation, eukaryotic translation

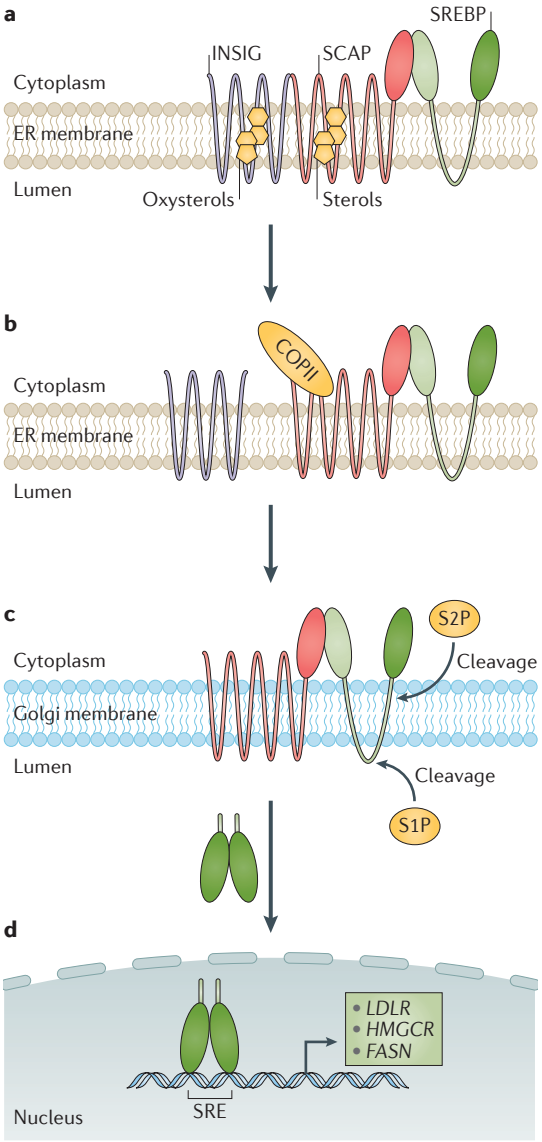


Figure 3 | **The SREBP-regulated sterol feedback response controls the transcription of MVA pathway (and other) genes. a** | When endoplasmic reticulum (ER) sterol concentrations are high, the full-length, precursor sterol regulatory element-binding proteins (SREBPs) are localized to the ER in a complex with SREBP cleavage-activating protein (SCAP) and insulin-induced gene (INSIG)[193]. This complex is maintained through the binding of sterols to SCAP and/or the binding of oxysterols to INSIG. **b** | When levels of sterols are low, SCAP undergoes a conformational change that causes the SCAP–SREBP complex to dissociate from INSIG. SCAP is then able to bind coat protein complex II (COPII) proteins and be transported in vesicles, with SREBP, to the Golgi. **c** | SREBP is sequentially cleaved by site-1 protease (S1P) and S2P at the Golgi. Although not shown, S1P and S2P are transmembrane proteins. **d** | The cleaved, mature SREBP homodimerizes and then translocates to the nucleus, where it binds to sterol-response elements (SREs) in the promoter regions of its target genes to activate transcription, such as of low-density lipoprotein receptor (*LDLR*), 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) and fatty acid synthase (*FASN*).

Table 1 | **Agents that target the MVA pathway and/or its SREBP-regulated feedback mechanism**

Drugs	Target	Stage of clinical development	Refs
MVA pathway inhibitors			
Statins	HMGCR	Approved as cholesterol-lowering agents and currently in phase I, II and III clinical trials for the treatment of various cancer types	171–175
Bisphosphonates	FDPS	Approved for the treatment of osteoporosis, multiple myeloma and solid tumour bone metastases, in combination with standard therapy	194–196
Isoprenylation inhibitors			
FTIs and GGTIs	Farnesyltransferases and geranylgeranyl-transferases	In phase I, II and III clinical trials for the treatment of various cancer types, as single agents or in combination with standard therapy	65,197,198
SREBP inhibitors			
Fatostatin	SCAP	In preclinical development	190–192
Betulin	SCAP	In preclinical development	199
Tocotrienols	Unknown	In preclinical development	188,189
Nelfinavir	S2P	Approved for the treatment of HIV infection and in phase I and II clinical trials for the treatment of various cancer types	200–202
Dipyridamole	Unknown	Approved for the prevention of cerebral ischaemia and in preclinical development as an inhibitor of SREBP	51

FDPS, farnesyl diphosphate synthase; FTI, farnesyltransferase inhibitor; GGTI, geranylgeranyltransferase inhibitor; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; MVA, mevalonate; S2P, site-2 protease; SCAP, SREBP cleavage-activating protein; SREBP, sterol regulatory element-binding protein.

initiation factor 4E-binding protein 1 (4EBP1; also known as EIF4EBP1)[105]. SREBPs are major downstream effectors of mTORC1 signalling, as evidenced by increased lipogenesis in response to mTORC1 activation[106–108]. The observation that SREs are the most common regulatory elements in mTORC1-induced genes further strengthens the link between mTORC1 and the SREBPs[108]. This link is also evident in samples from patients with primary breast cancer, as patients with high levels of phosphorylated S6K1 had correspondingly high expression of SREBP target genes, such as fatty acid synthase (*FASN*), low-density lipoprotein receptor (*LDLR*) and mevalonate kinase (*MVK*)[90]. This study also compared proteins from tumour samples and adjacent normal breast samples, and described an increase in FASN protein levels in the tumours that had higher levels of phosphorylated S6K1.

mTORC1 can regulate the SREBP transcription factors at multiple levels, although there are some cell- and tissue-type differences (FIG. 4). For example, S6K1 has been shown to activate SREBP2 processing and increase the expression of MVA pathway genes in a hepatocellular carcinoma (HCC) cell line, although the mechanism involved remains unclear[109]. Greater understanding of the role of mTORC1 in SREBP activity came with the development of torins, which are mTOR catalytic site inhibitors[110]. The original allosteric mTOR inhibitor, rapamycin, prevents the phosphorylation of S6K1 but does not inhibit 4EBP1 phosphorylation equally in all systems. By contrast, torins inhibit the phosphorylation of multiple mTOR targets, including S6K1 and 4EBP1 (REFS 110,111). Recent work comparing torin and rapamycin action implicated a role for lipin 1 (LPIN1) in mediating the effects of mTORC1 on the SREBPs[112]. LPIN1 is a nuclear phosphatidic acid phosphatase that is inhibited through direct phosphorylation by mTORC1, independently of S6K1. Active, unphosphorylated LPIN1 indirectly prevents the transcription of SREBP target genes by preventing the SREBPs from binding to chromatin, although the mechanism involved remains unclear[112]. A further link between LPIN1 and the MVA pathway was uncovered by studies using skeletal muscle, in which statins and LPIN1 were shown to increase autophagy[113]. Given the role of SREBP2 in transcribing numerous autophagy genes[79,114], further work is needed to fully understand the interplay between mTORC1, LPIN1 and the SREBPs.

The position of the SREBPs as key effectors of mTORC1 signalling presents a potential vulnerability in tumours that have deregulated mTORC1 activity. Previous studies have linked the loss of SREBPs in breast cancer to the induction of ER stress, which induced apoptosis through mTOR[115]. A separate study showed that genetic knockdown of *SREBF1* and/or *SREBF2* reduced proliferation and increased cell death in mTORC1-activated breast cancer cell lines[90]. The observation that double knockdown of *SREBF1* and *SREBF2* showed the greatest pro-apoptotic effect suggests that small-molecule inhibitors that target both SREBP1 and SREBP2 will have the greatest therapeutic benefit.

AMPK. With an opposing role to that of mTORC1, AMP-activated protein kinase (AMPK) acts to dampen anabolic pathways when intracellular ATP levels are low. This role of AMPK as an energy sensor and central regulator of metabolism is crucial in metabolic disorders such as type 2 diabetes and cancer[116]. AMPK was discovered through its ability to phosphorylate and reduce the

C-Cell adenoma
C-Cells (also known as parafollicular cells) are found in the thyroid and produce the hormone calcitonin. Tumours originating from the C-cells include medullary thyroid cancer, and mutations in the RET proto-oncogene are often found in patients.

activity of microsomal HMGCR in rat liver extracts[117,118]. Further studies showed that AMPK phosphorylates Ser872 within the catalytic domain of HMGCR, inhibiting its enzymatic activity in a manner that is independent of its feedback regulation by MVA pathway metabolites[119,120]. The SREBPs are also direct targets of AMPK phosphorylation[121]. Activated AMPK specifically interacts with both the precursor and the nuclear forms of SREBP1c and SREBP2, and phosphorylation by AMPK inhibits SREBP proteolytic processing and transactivation activity[121]. Activation of AMPK in HepG2 liver cancer cells by either polyphenols or metformin stimulates this phosphorylation, which suppresses the accumulation of SREBPs in the nucleus under hyperglycaemic and hyperinsulinaemic conditions[121]. Moreover, activation of AMPK in the livers of insulin-resistant mice inhibited the transcription of enzymes that are involved in lipid and cholesterol biosynthesis, including the MVA pathway enzymes 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) and HMGCR, which consequently resulted in a decrease in hepatic triglyceride and cholesterol levels[121]. AMPK can thus inhibit MVA pathway activity both directly via the phosphorylation of HMGCR and indirectly through the phosphorylation and repression of SREBPs. However, the relevance of this regulation in the context of cancer is poorly understood.

The MVA pathway may also regulate AMPK activity, thereby forming a feedback loop. The tumour suppressor liver kinase B1 (LKB1; also known as STK11), which phosphorylates and activates AMPK, is farnesylated at a highly conserved carboxy-terminal CAAX motif[122,123]. Knock-in mice expressing a mutant form of LKB1, which could not be farnesylated, exhibited reduced membrane-bound LKB1 and impaired AMPK activity[123]. This hints at a negative feedback loop, in which the activation of AMPK in response to decreased cellular energy results in the inhibition of the MVA pathway via the phosphorylation of HMGCR and the SREBPs. This in turn reduces the FPP pool within the cell, thereby hindering LKB1 farnesylation and inhibiting AMPK activation.

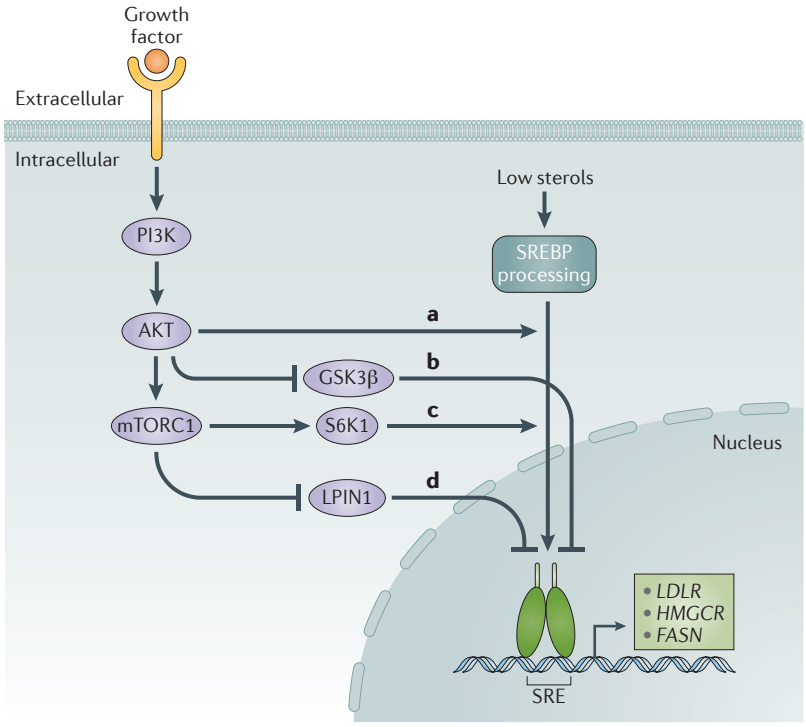


Figure 4 | **SREBP processing and activity are regulated by PI3K signalling at multiple levels.** AKT can increase sterol regulatory element-binding protein (SREBP) expression and activity (part **a**), partly through the inhibition of glycogen synthase kinase-3β (GSK3β; part **b**). mTOR complex 1 (mTORC1) increases SREBP processing and transcriptional activity through multiple substrates. mTORC1 activates ribosomal S6 kinase 1 (S6K1) through phosphorylation to increase SREBP translocation and, potentially, SREBP processing (part **c**). The negative regulator of SREBP, lipin 1 (LPIN1), is also phosphorylated and inactivated by mTORC1 (part **d**). Despite the multiple levels of regulation of the SREBPs by PI3K signalling, the mechanisms involved remain to be elucidated and may be context dependent. *FASN*, fatty acid synthase; *HMGCR*, 3-hydroxy-3-methylglutaryl-CoA reductase; *LDLR*, low-density lipoprotein receptor; SRE, sterol-response element.

p53 and RB. *TP53*, which encodes the p53 tumour suppressor, is one of the most frequently altered genes in cancer, and mutations within the coding region of *TP53* can confer oncogenic properties to p53 (REFS 124,125). Two gain-of-function mutations ($TP53^{R273H}$ and $TP53^{R280K}$) enable p53 to functionally interact with nuclear SREBP2 and increase the transcription of MVA pathway genes[126] (FIG. 5). This MVA pathway gene activation was necessary and sufficient for mutant p53 to disrupt normal breast acinar morphology[126], and mutant p53 expression in primary breast cancer tissues was correlated with the increased expression of sterol biosynthesis genes[126]. Conversely, wild-type p53 can reduce lipid synthesis under conditions of glucose starvation by inducing the expression of LPIN1 (REF. 127), which, as described above, can prevent the association of SREBPs with chromatin[112]. $TP53^{R273H}$ and $TP53^{R280K}$ mutations are also found in tumours from tissues other than the breast, for example, the ovaries[128], prostate[129] and lung[130]. The interplay between p53 and the MVA pathway suggests that the MVA pathway may be a novel therapeutic target for tumours that harbour these specific p53 gain-of-function mutations.

The tumour suppressor protein RB has also been implicated as a regulator of the MVA pathway (FIG. 5). In a mouse model of C-cell adenoma, loss of *Rb1* (which encodes RB) enhanced isoprenylation and activation of NRAS[131]. Loss of RB relieved the suppression of the transcription factors E2F1 and E2F3, which were shown to bind and activate the promoters of numerous prenyltransferase genes, *Fdps* and *Srebf1* (REF. 131). Moreover, RB prevented the association of SREBP1 and SREBP2 with the *Fdps* promoter[131], suggesting that RB negatively regulates the MVA pathway at both the transcriptional and the post-translational levels.

MYC. The MYC transcription factor is a potent oncogene that can drive transformation in multiple cancer types. It is deregulated in more than 50% of cancers, and can reprogramme cancer cell metabolism to enable the proliferation and survival of cancer cells[132–135]. Like the SREBPs, MYC is a bHLH-LZ protein and it has been shown to bind to SREBP1 to drive somatic cell

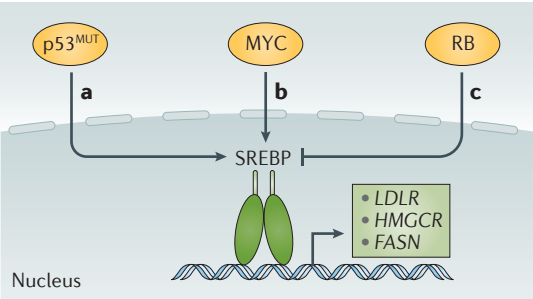


Figure 5 | **Transcriptional control of MVA pathway gene transcription by oncogenes and tumour suppressors.** **a** | Specific gain-of-function p53 mutants ($p53^{MUT}$) functionally interact with sterol regulatory element-binding protein (SREBP) to drive increased expression of mevalonate (MVA) pathway genes, such as low-density lipoprotein receptor (*LDLR*), 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) and other target genes, such as fatty acid synthase (*FASN*). **b** | MYC can bind to SREBP to increase the expression of SREBP target genes, and analysis of the Encyclopedia of DNA Elements (ENCODE) database shows that MYC and its binding partner, MAX, bind to the promoters of MVA pathway genes. **c** | The RB tumour suppressor can interact with SREBP and reduce its binding at target genes. Loss of RB in cancer removes this inhibition, leading to the increased transcription of specific MVA pathway genes.

reprogramming into induced pluripotent stem cells[136]. Analysis of data from the Encyclopedia of DNA Elements (ENCODE) project[137] also shows that MYC binds to promoters of MVA pathway genes in close proximity to SREBP1 and SREBP2 binding regions (P.J.M., W. B. Tu and L.Z.P., unpublished observations; analysis follows previous work (see REF. 138)), suggesting that MYC can contribute to the expression of MVA pathway enzymes (FIG. 5). As the MVA pathway is essential for cancer cells, and because MYC has a major role in metabolic regulation, deregulated MYC may ensure that MVA pathway metabolites are not limiting for tumorigenesis. The MVA pathway was also shown to be important in a MYC-driven transgenic model of HCC[139]. In that study, atorvastatin reduced tumour initiation and growth, possibly through reduced isoprenylation of the RHO-family GTPase RAC1, leading to the activation of serine/threonine-protein phosphatase 2A (PP2A), which is a negative regulator of MYC[139]. More recently, $Myc^{+/-}$ mice (which are haploinsufficient) were shown to have an increased lifespan, which was associated with the decreased expression of MVA pathway genes, including *Hmgcr* and *Srebf2* (REF. 140). Given the importance of MYC in driving cancer, and the difficulty of targeting it therapeutically, further work is warranted to uncover the relationship between MYC and the MVA pathway.

Signalling from the MVA pathway

Altered metabolism in tumours not only fulfils the energetic and biosynthetic needs of a dividing cell, but also produces metabolites that are important for downstream signalling. This is particularly true of the isoprenoid and sterol metabolites produced by the MVA pathway, which are also used by cancer cells to modulate multiple downstream signalling pathways that are important for tumour progression.

YAP and TAZ. It was recently shown that the oncogenes Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ; also known as WWTR1) require the MVA pathway to be fully functional[141]. YAP and TAZ are transcriptional co-activators that facilitate the transcriptional activation of pro-growth genes and the repression of pro-apoptotic genes[142,143]. The nuclear localization of YAP and TAZ is negatively regulated, partly by the activation of the tumour-suppressive Hippo signalling pathway[142,143]. Activation of the Hippo cascade results in the phosphorylation and activation of large tumour suppressor kinase 1 (LATS1) and LATS2, which phosphorylate YAP and TAZ and retain them in the cytoplasm[142,143]. YAP and TAZ nuclear localization requires the MVA pathway (FIG. 6), as concurrent knockdown of *SREBF1* and *SREBF2* reduces nuclear localization of YAP and TAZ[141]. These effects were mimicked by GGTIs and were prevented by a RHOA mutant that does not require geranylgeranylation[141]. This suggests that SREBP-mediated induction of the MVA pathway maintains intracellular GGPP pools, which is necessary for RHOA activity, as well as YAP and TAZ nuclear localization. However, it is unclear whether these effects are dependent on Hippo signalling. Although some studies showed that MVA pathway-mediated YAP and TAZ signalling is independent of LATS1 and LATS2 via RNA interference (RNAi)-knockdown experiments[141,144], one study demonstrated that both atorvastatin treatment and GGTI treatment increase the phosphorylation of LATS1 and LATS2, suggesting that geranylgeranylation regulates Hippo signalling[145]. A separate study reported constitutive SREBP activation in the livers of mice with a liver-specific *Lats2* deletion, which corresponded to an increase in free cholesterol in the liver and protection from p53-mediated apoptosis[146].

Activation of the MVA pathway and activation of YAP and TAZ are correlated with mutant p53 expression in primary tumours, suggesting a dysfunctional mutant p53–SREBP–YAP–TAZ axis in cancer[141]. Overexpression of $TP53^{R280K}$ in a *TP53*-null cell line activated YAP and TAZ only when the MVA pathway was active, suggesting that the MVA pathway is a crucial intermediate in the oncogenic activation of YAP and TAZ by mutant p53 (REF. 141).

Hedgehog. Cholesterol has a multifaceted role in the regulation of cell signalling. For example, the Hedgehog (HH) signalling pathway, which has important roles in vertebrate development and tumorigenesis, is regulated by sterols at multiple levels[147]. Cholesterol itself can serve as a substrate for the post-translational modification of HH ligands, which is required for their proper trafficking[148]. Cholesterol and cholesterol-derived oxysterols can also activate HH signal transduction in medulloblastoma, whereas inhibition of the MVA pathway or downstream sterol biosynthesis decreased HH signalling and reduced cell proliferation[149] (FIG. 6)

Aromatase inhibitors
Inhibitors of oestrogen production and a common treatment option for patients with oestrogen receptor-positive breast cancer.

Ki67 index
The fraction of Ki67-positive tumour cells as determined using immunohistochemistry. The expression of Ki67 is associated with cell proliferation.

Steroid hormone signalling. Cholesterol also serves as the precursor of steroid hormones, which drive the initiation and progression of cancers such as hormone-dependent breast cancer and prostate cancer. In breast cancer, patients with oestrogen receptor-α (ERα)-positive disease are commonly treated with aromatase inhibitors to deprive the tumours of oestrogen. Recent work demonstrated that long-term oestrogen deprivation of ERα-positive breast cancers leads to the stable epigenetic activation of the MVA pathway and cholesterol biosynthesis. This is coupled with an enrichment of SREBP1 and SREBP2 DNA-binding motifs, as determined by DNase I footprinting analyses, suggesting that there is increased SREBP occupancy on open chromatin[150]. The resulting increased levels of 27-hydroxycholesterol were sufficient to activate ERα signalling in the absence of exogenous oestrogen, driving the activation of genes that promote an invasive cell phenotype[150]. Similarly, in prostate cancer, the *de novo* synthesis of androgens from cholesterol drives androgen receptor (AR) activity in castration-resistant disease[151] (FIG. 6). This finding, coupled with the observations that SREBP expression is increased in advanced-stage prostate cancer[152,153], suggests a role for the MVA pathway in prostate cancer progression. These findings warrant further investigation into the utility of inhibitors of the MVA pathway and/or SREBPs in the treatment of hormone-driven cancers.

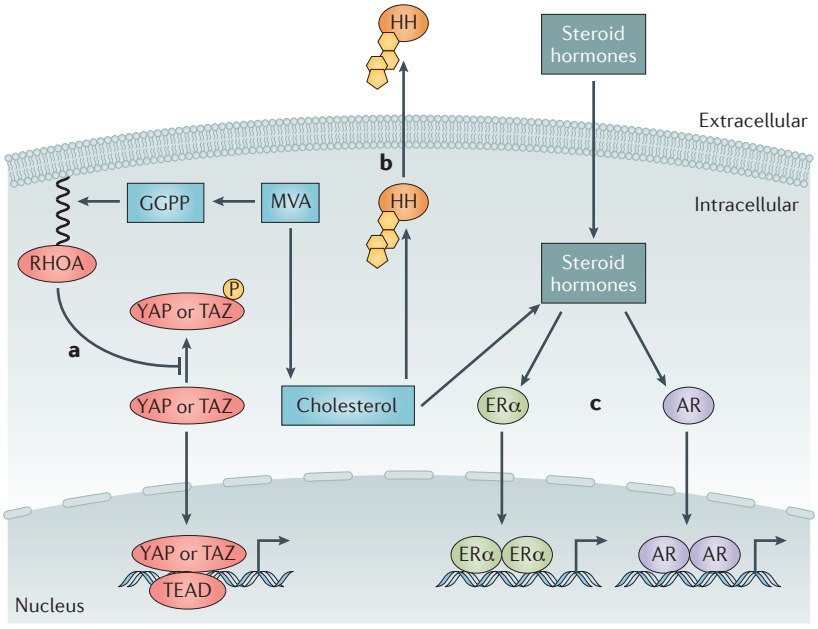


Figure 6 | **Activation of the MVA pathway drives oncogenic signalling pathways.** **a** | RHOA is required for the nuclear localization and activity of the Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) oncogenes. The activity of RHOA is dependent on geranylgeranylation, which localizes RHOA to the plasma membrane. Geranylgeranylation requires geranylgeranyl-diphosphate (GGPP) produced exclusively via the mevalonate (MVA) pathway, thus linking the MVA pathway to YAP and TAZ activity. **b** | Hedgehog (HH) signalling is involved in tumorigenesis in multiple cancer types, and HH ligands require the covalent attachment of cholesterol for proper processing and activity. **c** | Cholesterol is the precursor for steroid hormones such as oestrogen and androgen. These hormones are involved in hormone-driven breast cancers and prostate cancers via the activation of oestrogen receptor-α (ERα) and androgen receptor (AR), respectively. TEAD, TEA domain transcription factor.

Targeting the MVA pathway in cancer

As outlined above, multiple oncogenic signalling pathways can deregulate the MVA pathway for enhanced cell survival and growth. In turn, MVA pathway activity is required to regulate the downstream propagation of many cell signals. Coupled with the essentiality of several MVA pathway genes in cancer cells, this suggests that the MVA pathway is a tumour vulnerability that can be targeted as part of a therapeutic strategy.

Statins. The most promising method of blocking the MVA pathway in tumours is to inhibit HMGCR using statins, although inhibiting other flux-control points may also have anticancer benefits[17]. Statins have been safely used for decades to treat patients with hypercholesterolaemia[154], and although epidemiological evidence has been mixed, most reports indicate that statin use is correlated with reduced mortality in multiple cancer types[155–159]. Evidence also suggests that certain stages of cancer progression, such as breast cancer recurrence, are particularly sensitive to the anticancer activities of statins[155,160–162]. Although the cholesterol-lowering effects of statins are due to the inhibition of MVA pathway activity in the liver, lipophilic statins such as atorvastatin, simvastatin and lovastatin have been detected in extra-hepatic tissues, including the brain, in both the active acid form and the inactive lactone form[163]. By contrast, the hydrophilic pravastatin could only be detected in the liver[163], suggesting that hydrophilic statins might be clinically limited as anticancer agents. It is currently unknown whether lipophilic statins accumulate in tumour tissues at concentrations that are cytotoxic to cancer cells (reviewed in REF. 164). Efforts are underway to directly address this issue, and to determine the clinical utility and recommended dose of statins that could potentially be used as anticancer therapeutics.

Many studies have shown that statins can directly and specifically trigger the apoptosis of tumour cells[56,165–168]. For example, statins trigger the apoptosis of cells derived from acute myeloid leukaemia (AML), while normal myeloid progenitors do not undergo apoptosis and retain full proliferative potential[25]. This tumour-normal therapeutic index may be due to the altered metabolic reprogramming of AML cells leading to an increased dependence on MVA pathway metabolites for growth and survival. The widespread use of statins for cholesterol management also demonstrates that these drugs cause minimal damage to normal cells. The side effects of these drugs are regularly treated by switching to a different statin or potentially by co-treating with CoQ, although this co-treatment method is controversial owing to conflicting clinical evidence[169,170].

The data discussed above suggest that statins have a high therapeutic index to target tumours *in vivo*, despite the ubiquitous expression of the MVA pathway. This rationale has led to multiple clinical trials investigating the efficacy of various statins as a therapeutic option in a variety of tumour types. Two recent breast cancer window-of-opportunity clinical trials, using atorvastatin[171] and fluvastatin[172], showed reductions in the Ki67 index in a subset of patients who were administered with cholesterol-management doses of statins between cancer

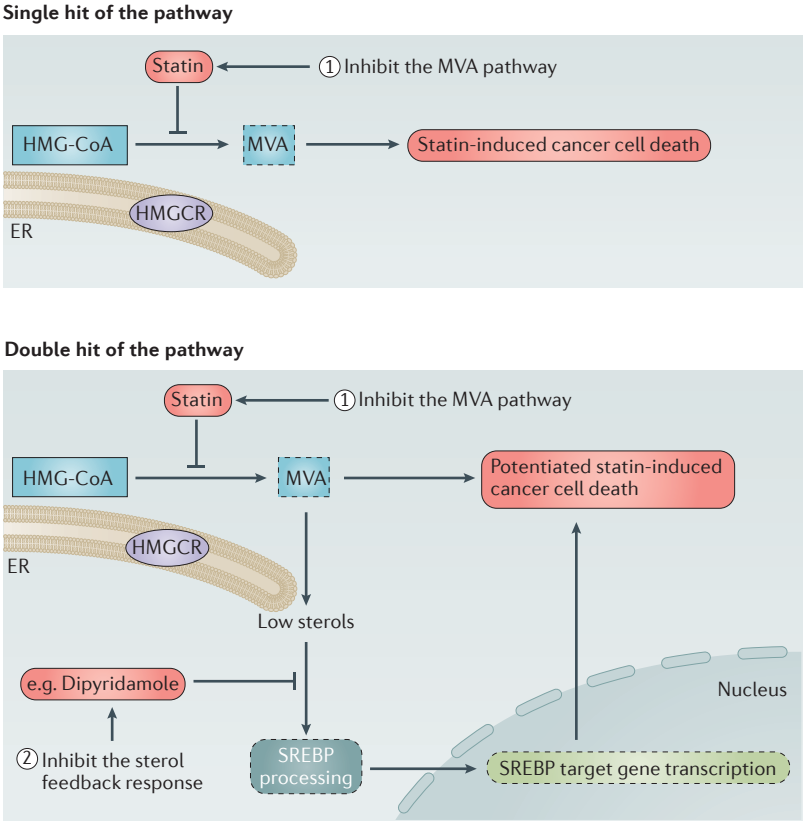


Figure 7 | **Inhibition of both the MVA pathway and the SREBP transcription factors is a viable cancer therapeutic strategy.** Statins have potential anticancer properties. They inhibit 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), thereby reducing mevalonate (MVA) pathway metabolites that are essential for cancer cell growth and survival (top panel). This triggers sterol regulatory element-binding protein (SREBP) activation and the transcription of MVA pathway genes, thus restoring MVA pathway activity (bottom panel). This is a classic resistance mechanism and may explain why not all patients respond to anticancer statin therapy. Dipyridamole is one example of an agent that inhibits SREBP cleavage, preventing the restorative feedback response and increasing apoptosis in multiple cancer types. Combining SREBP cleavage inhibitors with statins may increase the therapeutic response compared with the use of statins alone. Dashed boxes represent metabolites or steps that are reduced by the indicated treatments. ER, endoplasmic reticulum; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA.

diagnosis and surgery. Statins have also been safely used in combination with other agents to increase efficacy. For example, pravastatin was combined with standard-of-care treatment in HCC and AML, resulting in significantly longer median survival in HCC[173] and resulting in complete or partial response in 60% of patients with AML[174]. In another study, combining lovastatin with thalidomide and dexamethasone in patients with relapsed or refractory multiple myeloma (MM) led to prolonged overall survival and progression-free survival[175].

Despite evidence of patient response to statins as anticancer agents, many patients remained non-responsive to statin treatment in other cancer clinical trials[176]. This is consistent with the current paradigm of inter-patient tumour heterogeneity. This lack of response might also be expected considering the evidence that we discuss above showing that the MVA pathway is regulated by many key oncogenic signals. Similar to many anticancer agents, a personalized medicine approach is needed to implement statins, and/or other inhibitors of the MVA pathway, as a successful class of cancer therapeutics. To this end, a molecular signature of basal mRNA expression has been developed to predict statin response in breast cancer *in vitro*[22], and deregulated MYC expression has been a proposed indicator of statin response in specific tumour types[177]; however, essential follow-up validation is required before these biomarkers can be used clinically. It is currently difficult to predict which cancers will be particularly sensitive to statin therapy. In addition to AML and MM, encouraging results from both clinical trials[171,172] and epidemiological studies[178,179] suggest that patients with hormone-dependent cancers, such as breast cancer and prostate cancer, may benefit from the addition of statins to their treatment regimen. This may be partly because the MVA pathway end-product cholesterol is the precursor of hormones such as oestrogen and androgens, which have a major role in the development of these types of cancers. HCC also seems to be particularly responsive to statins[173], perhaps because of the hepatotropic pharmacology of this family of drugs. Clinical trials are required in these and other cancers to further define the subset of cancers that are particularly statin-sensitive[180].

Targeting the SREBP-regulated feedback response. Crucial to the regulation of the MVA pathway is the tightly controlled, SREBP-mediated feedback mechanism, in which inhibition of the MVA pathway results in the activation of the SREBPs and an increase in the expression of MVA pathway genes, an effect that may be amplified in cancer cells. SREBP activation also increases the expression of the LDLR, which leads to the increased uptake of exogenous, lipoprotein-derived cholesterol: an effect that has been shown to be important in cancer cells[181–184]. The SREBPs thus function to replenish MVA pathway metabolites, which can dampen the apoptotic response following statin treatment[51,52,185]. This would be a classic resistance mechanism, similar to that seen with other anticancer therapeutics such as BRAF inhibitors in BRAF-mutant melanoma. Cells treated with BRAF inhibitors, such as vemurafenib, can acquire an activating mutation in downstream kinases (for example, MAP2K1 (also known as MEK1)) or can have an increase in expression of receptor tyrosine kinases (for example, epidermal growth factor receptor (EGFR)), bypassing the need for BRAF activity[186]. These studies demonstrate that inhibiting both the cancer vulnerability and the resistance or feedback mechanism is crucial for maximum efficacy[187]. Therefore, inhibiting the SREBP-regulated feedback response in conjunction with statin therapy could prevent resistance, thereby increasing the efficacy of statins as anticancer agents and the number of responsive patients (FIG. 7).

Evidence that targeting the SREBPs in combination with statin therapy is a viable strategy has been provided by several recent studies. First, a study looking at breast and lung cancer cell lines used a short hairpin RNA (shRNA) screen to uncover genes that, when knocked down, potentiated the pro-apoptotic effects of statins[185]. The MVA pathway genes *HMGCS1*, geranylgeranyl

Dipyridamole
A clinically approved drug used to prevent platelet aggregation.

diphosphate synthase 1 (*GGPS1*), *SCAP* and *SREBF2* all scored highly, adding credence to either inhibiting other enzymes in the MVA pathway or inhibiting the SREBP-mediated feedback response in combination with statin therapy. A second study showed that statin-induced SREBP processing can be blocked by another agent that has been approved for a non-cancer indication, dipyridamole[51]. Dipyridamole reduced the transcription of SREBP target genes such as *HMGCS1* and *HMGCR*, and synergized with statins to increase apoptosis in AML and MM cell lines and patient samples. Other compounds, such as tocotrienols, have also been demonstrated to synergize with statins to induce cancer cell apoptosis[188], which is an effect that may be associated with their ability to degrade nuclear SREBP2 and inhibit its transcriptional activity[189]. Although several other small molecules, including fatostatin, have been shown to inhibit SREBP processing, their lack of approval for use in patients limits their potential to immediately have an impact on cancer patient care[190–192]. Therefore, clinical investigation into the utility of combined statins and SREBP inhibitors for the treatment of cancer is currently warranted (TABLE 1).

Outlook

Understanding tumour metabolism in the context of oncogenic signals has the potential to drive the development of targeted personalized therapies. The various signalling pathways that we describe in this Review are important drivers in many cancers, and they all have the ability to deregulate the MVA pathway, making these cancers potentially vulnerable to MVA pathway inhibition. Whether this occurs in every patient who presents with these lesions remains unclear. More work is needed to understand the extent to which driver mutations increase flux through the MVA pathway in patients. Rapidly developing technologies for the comprehensive flux-based analysis of MVA pathway metabolites will provide further advances in understanding how the MVA pathway receives and responds to oncogenic signals. In patients, it may be more feasible to determine pathway activity by mapping their oncogenic lesions to their sterol feedback response at the protein level (via SREBP localization) or mRNA expression level of MVA pathway genes, which may identify patients who will respond to MVA pathway inhibition. Designing clinical trials that will identify potential responders before treatment is required to prevent expensive failures of therapies that may still have benefits to a subset of patients. Improving reagents, particularly antibodies to HMGCR and SREBP2, will also aid trial design and interpretation.

The essentiality of the MVA pathway in many cancers, coupled with affordable and safe drugs that can target this pathway and its feedback response, provides a strong rationale for continuing to explore this key metabolic pathway in cancer.

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Competing interests statement
The authors declare no competing interests.