The interplay between cell signaling and the mevalonate pathway in cancer

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Biography

LZP focuses on two major areas of research: 1) understanding the regulation and function of the Myc oncogene; and 2) investigating the role of the mevalonate pathway in tumorigenesis, and how best to use statins to target this cancer vulnerability and impact cancer patient care. MCA is now Professor Emeritus and his research previously focused on the role of environmental factors and susceptibility genes in cancer development. Research trainees in the Penn lab include PJM (Post-doctoral Fellow), RY and JL (PhD students).

Abstract

The mevalonate pathway is an essential metabolic pathway that uses acetyl-CoA to produce sterols and isoprenoids integral to tumour growth and progression. In recent years, many oncogenic signaling pathways have been shown to increase the activity and/or expression of mevalonate pathway enzymes. This review summarizes recent advances and discusses the unique opportunities to immediately target this metabolic vulnerability with approved agents, such as the statin family of drugs, to impact patient care and outcome.

Key points

1. Mevalonate pathway metabolites are essential for cancer cell survival and growth.

2. Expression of mevalonate pathway enzymes is controlled by the SREBP family of transcription factors.

3. In cancer cells, oncogenic signaling pathways deregulate the activity of the SREBP transcription factors and mevalonate pathway enzymes.

4. Deregulated production of mevalonate pathway metabolites modulates multiple signaling pathways in cancer cells and contributes to transformation.

5. Clinical trials evaluating the utility of mevalonate pathway inhibitors as anti-cancer agents have shown responses in some, but not all, patients; discovering biomarkers to identify responders and developing combination therapies will further enhance their utility.

6. Inhibiting the SREBP transcription factors is a promising strategy to increase the efficacy of mevalonate pathway inhibitors as anticancer therapeutics, and also to potentially combat resistance.

Cancer cells reprogram their metabolism to provide energy and essential building blocks required to maintain their aberrant survival and growth[1-5]. This reprogramming may occur through mutations in metabolic enzymes (e.g. isocitrate dehydrogenase[6, 7]) or alterations in cell signaling due to oncogenic events and/or the remodeled tumour microenvironment. These activated signaling cascades in turn deregulate the expression[8, 9] and/or activity of enzymes in key metabolic pathways[10], including the mevalonate (MVA) pathway[11] (**Fig.1A, 1B**).

The MVA pathway uses acetyl-CoA, nicotinamide adenine dinucleotide phosphate (NADPH) and ATP to produce sterols and isoprenoids that are essential for tumour growth[12] (**Fig.1A, 1B**). 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 isocitrate dehydrogenases[15, 16]. Therefore, the MVA pathway is highly integrated into the overall metabolic state of cancer cells (**Fig.1A**). Transcription of MVA pathway genes is primarily controlled by the sterol regulatory element-binding protein (SREBP) family of transcription factors. When 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 SCAP (SREBP cleavage activating protein), dissociating from the INSIGs (insulin induced genes) and translocating from the ER to the Golgi (**Fig.2**). At the Golgi, the SREBPs are cleaved and 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[17].

The importance of MVA pathway metabolites to the survival of cancer cells is highlighted in 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 being a key dependency in tumours, and one that is readily targetable.

The MVA pathway has been suggested to be oncogenic in some studies. Early work in chronic lymphocytic leukemia (CLL) showed that MVA can stimulate replication in primary leukemic cells[26]. In an independent study, overexpressing the catalytic domain of HMGCR in primary mouse embryonic fibroblasts cooperated with RAS to promote foci formation, suggesting that HMGCR is a metabolic oncogene[27]. Also, 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 higher expression of MVA pathway genes correlating with poor prognosis in breast cancer[27]. Collectively, this evidence indicates that the MVA pathway plays a key role in cancer.

In this article, we review recent evidence demonstrating that the MVA pathway is deregulated in cancer through aberrant cell signaling, which in turn establishes a tumour vulnerability that can be therapeutically targeted to impact patient care and outcome.

Mevalonate-derived metabolites in cancer

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

Cholesterol. Cholesterol is an important component of most cellular membranes. Highly proliferative cancer cells need to rapidly produce membranes, and an increase in cholesterol synthesis contributes to this process. Cholesterol is also an integral component of lipid rafts, which are necessary to form signaling 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]. It is therefore possible that high cholesterol, produced by the MVA pathway, could play a role in protecting cancer cells from immune surveillance and immunotherapies[35, 36]. Cholesterol also serves as the precursor for downstream products, such as steroid hormones and oxysterols: steroid hormones drive the initiation and progression of cancers such as breast and prostate carcinomas[37]; increased oxysterol production can activate the liver X receptors (LXRs), which have been proposed to be a therapeutic target in multiple cancer types[38, 39].

Cancer cells therefore require cholesterol for growth and survival, and lowering intracellular cholesterol biosynthesis is a promising anti-cancer strategy.

Isopentenyl-diphosphate. In human cells, the MVA pathway is the sole intracellular source for isopentenyl-diphosphate (IPP) (**Fig. 1B**)[40]. Aberrant activation of the MVA pathway in cancer results in elevated 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 that evaluated the *in vivo* expansion of γδ T cells in response to zoledronate, a bisphosphonate that inhibits the MVA pathway downstream of IPP (**Table 1**), in combination with IL-2 treatment in advanced-stage breast[43] and prostate[44] cancer. In both studies, the therapy was well-tolerated and the number of sustained peripheral γδ T cells was correlated with improved clinical outcome[41, 43, 44]. Future phase II clinical trials will reveal whether combined zoledronate and IL-2 therapy is an effective anti-cancer strategy.

Farnesyl- 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 are hydrophobic chains that are essential for the isoprenylation of proteins. This post-translational modification (PTM) tethers proteins to cell membranes, enabling proper protein localization and function[45-48]. Most small GTPases, like RAS and RHO, are isoprenylated[49], and many are involved in tumourigenesis. Inhibiting the MVA pathway can reduce the isoprenylation of RAS, RHO and other small GTPases[50-52], and leads to cell death in some cancer cells.

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 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 appears that this is a 'class effect', with depletion of these isoprenoid pools potentially affecting the many proteins that are isoprenylated[58]. Despite this dependency, directly inhibiting the isoprenylation of proteins by geranylgeranyl transferase inhibitors (GGTIs) or farnesyl transferase inhibitors (FTIs) has not been a successful anti-cancer strategy to date[59]. The rationale behind these drug development programs was that key isoprenylated onco-proteins, like RAS, could be targeted. However, the efficacy of FTIs was impeded by alternate isoprenylation using GGPP, and GGTIs have been disappointingly toxic[60, 61]. Further development of next generation FTIs and GGTIs remains a relatively limited and focused area of research[59, 62-66].

Dolichol. Dolichol is derived from an 18-20mer of IPP, and is an essential component for 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[69]). Glucose-derived *N*-acetylglucosamine has recently been shown to be necessary for the *N*-glycosylation of SCAP prior to ER-to-Golgi translocation. The SCAP/SREBP complex therefore remains inactive in the ER when glucose is absent, even in the presence of low sterols[70].

Coenzyme Q. Together with quinone groups, isoprenoids are also used to produce coenzyme Q (CoQ). The hydrophobic isoprenoid chain localizes CoQ to the inner membrane of the mitochondria, where the quinone group acts to transfer electrons from complex I or II to complex III of the electron transport chain, thus enabling ATP production[71]. CoQ is therefore critical for ATP production in those 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.2**). There are three SREBP proteins, 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 alternate 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 synthesis genes[74, 75]. ChIP-seq studies have indicated some overlap in the target genes of each SREBP, including MVA pathway genes, affording some redundancy[76, 77]. Most work also shows 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 due to antibody specificity. 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 therefore primed to upregulate the MVA pathway to provide essential building blocks for continued proliferation. The integration of cellular signaling 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 signaling 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 *PIK3CA* is the second most frequently mutated gene[78]. Inactivating mutations in its negative regulator PTEN, and/or hyperactivity of receptor tyrosine kinases are also frequent in cancer. Alterations in this pathway generally act to augment PI3K/AKT signaling, and consequently increase proliferation of cancer cells.

PI3K/AKT can activate the MVA pathway by a variety of mechanisms (**Fig.3**). For example, stimulation of PI3K/AKT signaling by growth factors, such as insulin, PDGF or VEGF, can increase the mRNA and protein expression of SREBP1 and SREBP2[79-83]. It should be noted that while PI3K/AKT signaling strongly and consistently increases the mRNA and protein levels of SREBP1a and 1c, its effects on SREBP2 expression are context-dependent. AKT, alternatively known as PKB, has also been suggested to increase the stability of nuclear SREBP1a, SREBP1c and SREBP2 by preventing their FBXW7-mediated degradation[84]. FBXW7 is an E3 ubiquitin ligase that binds to and ubiquitylates phosphorylated SREBPs, leading to their proteasomal degradation. The importance of this degradation pathway is highlighted by an increase in cholesterol and fatty acid synthesis in FBXW7-deficient cells[84]. The residues that are recognized by FBXW7 are phosphorylated by GSK-3β, and AKT has been suggested to inhibit this phosphorylation and prevent FBXW7-mediated degradation of the SREBPs (**Fig.3**). Insulin also causes the dissociation of INSIG from SCAP/SREBP1c in a sterol-independent manner, leading to increased transcription of MVA pathway genes[85-88]. These studies were further validated through genetic approaches, where SREBP1 and SREBP2 expression and activity were increased with expression of constitutively active PI3K or AKT, and abrogated by dominant-negative AKT[80, 88, 89]. The increase in lipid and cholesterol production mediated by the PI3K/AKT/SREBP axis promotes proliferation of cancer cells and tumorigenesis *in vitro* and *in vivo*[90-92]. Conversely, inhibiting the MVA pathway decreases PI3K activity[93], possibly through decreased RAS isoprenylation[93, 94], demonstrating a two-way regulatory relationship between PI3K/AKT signaling and the MVA pathway.

Increased MVA pathway activity is inconsequential without the availability of both acetyl-CoA and NADPH, and PI3K/AKT signaling meets this requirement by increasing glucose uptake and the rate of glycolysis in cancer cells[95]. This is important as acetyl-CoA is also used by other processes, such as fatty acid synthesis and protein acetylation[13]. Thus, PI3K/AKT signaling couples substrate availability with the activity of the MVA pathway in cancer.

mTORC1. Downstream of PI3K/AKT signaling, mTOR complex 1 (mTORC1) acts as a sensor of growth signals (such as insulin) and nutrients (such as amino acids) to regulate cellular growth[96]. It is often deregulated in cancer, and this supports aberrant growth. mTORC1 increases mRNA translation by phosphorylating and activating ribosomal S6 kinase 1 (S6K1)[97, 98] and repressing the activity of the inhibitor of cap-dependent translation, eIF4E-binding protein 1 (4E-BP1)[99]. SREBPs are major downstream effectors of mTORC1 signaling, as evidenced by increased lipogenesis in response to mTORC1 activation[100-102]. The observation that SREs are the most common regulatory elements in mTORC1-induced genes further strengthens the link between mTORC1 and the SREBPs[102]. This link is also evident in primary breast cancer patient samples, where patients with high levels of phosphorylated S6K1 had corresponding high expression of SREBP target genes such as *FASN*, *LDLR* and *MVK*[90]. This study also compared protein from tumour 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.3**). S6K1 has been shown to activate SREBP2 processing and increase expression of MVA pathway genes in a hepatocellular carcinoma cell line, although the mechanism remains unclear[103]. Greater understanding of the role of mTORC1 in SREBP activity came with the development of torins, which are catalytic site mTOR inhibitors[104]. The original allosteric mTOR inhibitor, rapamycin, prevents phosphorylation of S6K1 but does not inhibit 4E-BP1 phosphorylation equally in all systems. In contrast, catalytic site inhibitors, like torins, inhibit the phosphorylation of multiple mTOR targets, including S6K1 and 4E-BP1[104, 105]. Recent work comparing torin and rapamycin action implicated a role for LIPIN1 in mediating the effects of mTORC1 on the SREBPs[106]. LIPIN1 is a nuclear phosphatidic acid phosphatase that is inhibited by direct phosphorylation by mTORC1, independent of S6K1. Active, unphosphorylated LIPIN1 indirectly prevents the transcription of SREBP target genes, although the mechanism remains unclear. A further link between LIPIN1 and the MVA pathway was uncovered in studies using skeletal muscle, in which statins and LIPIN1 were shown to

increase autophagy[107]. Given the role of SREBP2 in transcribing numerous autophagy genes[77, 108], further work is needed to fully understand the interplay between mTORC1, LIPIN1 and the SREBPs.

The position of the SREBPs as key effectors of mTORC1 signaling 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[109]. A separate study showed that genetic knockdown of SREBPs reduced proliferation and increased cell death in mTORC1-activated breast cancer cell lines[90]. The observation that double knockdown of SREBP1 and SREBP2 showed the greatest pro-apoptotic effect suggests that small molecule inhibitors that target both SREBP1 and SREBP2 will have the greatest therapeutic benefit.

AMPK. Playing an opposing role to mTORC1, AMP-activated protein kinase (AMPK) acts to dampen anabolic pathways when intracellular ATP levels are low. This role as an energy sensor and central regulator of metabolism is critical in metabolic disorders such as type II diabetes and cancer[110]. AMPK was discovered through its ability to phosphorylate and reduce the activity of microsomal HMGCR in rat liver extracts[111, 112]. Further studies showed AMPK phosphorylates S872 within the catalytic domain of HMGCR, inhibiting its enzymatic activity in a manner that is independent of its feedback regulation by MVA pathway metabolites[113, 114]. The SREBPs are also direct targets of AMPK phosphorylation[115]. Activated AMPK specifically interacts with both the precursor and nuclear forms of the SREBP1c and SREBP2, and phosphorylation by AMPK inhibits SREBP proteolytic processing and transactivation activity[115]. Activation of AMPK in HepG2 cells by either polyphenols or metformin has been shown to stimulate this phosphorylation, which suppressed the accumulation of SREBPs in the nucleus under hyperglycemic and hyperinsulinemic conditions[115]. Moreover, activation of AMPK in the livers of insulin-resistant mice inhibited the transcription of enzymes involved in lipid and cholesterol biosynthesis, including the MVA pathway enzymes HMGCS1 and HMGCR, which consequently resulted in a decrease in hepatic triglyceride and cholesterol levels[115]. AMPK can therefore inhibit MVA pathway activity directly via phosphorylation of HMGCR, and indirectly through the phosphorylation and

repression of the SREBPs. However, the relevance of this regulation in the context of cancer is poorly understood.

The MVA pathway may also play a role in regulating AMPK activity, thereby forming a regulatory feedback loop. The tumour suppressor liver kinase B1 (LKB1), which phosphorylates and activates AMPK, is farnesylated at a highly conserved C-terminal CAAX motif[116, 117]. Knock-in mice expressing a mutant LKB1, which could not be farnesylated, exhibited reduced membrane-bound LKB1 and impaired AMPK activity[117]. This hints at a negative feedback loop, whereby 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.

p53 and pRB. The p53 tumour suppressor is one of the most frequently altered genes in cancer, and mutations within the coding region of this gene can confer oncogenic properties to the p53 protein product. Two gain-of-function mutations ($p53^{R273H}$ and $p53^{R280K}$) enable p53 to functionally interact with nuclear SREBP2 and increase transcription of MVA pathway genes (**Fig.4**). This MVA pathway gene activation was necessary and sufficient for mutant p53 to disrupt normal breast acinar morphology[118], and mutant p53 expression in primary breast cancer tissues was correlated with elevated expression of sterol biosynthesis genes. Conversely, wild type p53 can reduce lipid synthesis under conditions of glucose starvation[119] by inducing the expression of LIPIN1, which, as described above, can prevent the association of SREBPs with chromatin[106]. The interplay between p53 and the MVA pathway suggests that the MVA pathway may be a novel therapeutic target for tumours, particularly breast cancers that harbour p53 gain-of-function mutations.

The tumour suppressor protein retinoblastoma (pRB) has also been implicated as a regulator of the MVA pathway (**Fig.4**). In a mouse model of C-cell adenoma, *Rb* loss resulted in enhanced isoprenylation and activation of N-RAS[120]. Loss of pRB relieved suppression of the transcription factors E2F-1 and E2F-3,

which were shown to bind and activate the promoters of numerous prenyltransferase genes, farnesyl diphosphate synthase (*Fdps*) and *Srebf1*[120]. Moreover, pRB prevented the association of SREBP1 and SREBP2 with the *Fdps* gene promoter[120], suggesting that pRB negatively regulates the MVA pathway at both the transcriptional and post-translational level.

MYC. The MYC transcription factor is a potent oncogene that can drive transformation in multiple cancer types. It is deregulated in over 50% of cancers, and can reprogram cancer cell metabolism to enable proliferation and survival of cancer cells[121-124]. Like the SREBPs, it is a bHLH-LZ protein, and has been shown to bind to SREBP1 to drive somatic cell reprogramming into induced pluripotent stem cells[125]. Analysis of data from the ENCODE project[126] also shows that MYC binds to promoters of MVA pathway genes, in close proximity to SREBP1 and SREBP2 binding regions, suggesting that MYC can contribute to the expression of MVA pathway enzymes (**Fig.4**). As the MVA pathway is essential for cancer cells, and MYC has a major role in metabolic regulation, 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 hepatocellular carcinoma. In that study, atorvastatin reduced tumour initiation and growth, possibly through reduced isoprenylation of RAC1 leading to activation of PP2A, a negative regulator of MYC[127]. More recently, *Myc* haploinsufficient mice were shown to have an increased lifespan, which was associated with decreased expression of MVA pathway genes, including *Hmgcr* and *Srebf2*[128]. Given the importance of MYC in driving cancer, and the difficulty in targeting it therapeutically, further work is warranted to uncover the relationship between MYC and the MVA pathway.

Signaling from the MVA pathway

Altered metabolism in tumours not only fulfills the energetic and biosynthetic needs of a dividing cell, but also produces metabolites important for downstream signaling. 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 signaling pathways that are important for tumour progression.

YAP/TAZ. It was recently shown that the oncogenes YAP and TAZ require the MVA pathway to be fully functional[129]. YAP and TAZ are transcriptional co-activators that facilitate the transcriptional activation of pro-growth genes and repression of pro-apoptotic genes. The nuclear localization of YAP/TAZ is negatively regulated, in part, by activation of the tumour-suppressive Hippo signaling pathway. Activation of the Hippo cascade results in the phosphorylation and activation of the LATS1/2 kinases, which phosphorylate YAP and TAZ and retain them in the cytoplasm. YAP and TAZ nuclear localization requires the MVA pathway[129] (**Fig.5**). Concurrent knockdown of *SREBF1* and *SREBF2* reduced nuclear localization of YAP and TAZ[129]. These effects were mimicked by GGTIs, and prevented by a RHOA mutant that does not require geranylgeranylation[129]. This suggests that SREBP-mediated induction of the MVA pathway maintains intracellular GGPP pools, which is necessary for RHOA activity and YAP/TAZ nuclear localization. However, it is unclear whether these effects are dependent on Hippo signaling. While some studies showed that MVA pathway-mediated YAP/TAZ signaling is independent of LATS1/2 via RNAi-knockdown experiments[129, 130], one study demonstrated that atorvastatin or GGTI treatment increases phosphorylation of LATS1/2, suggesting that geranylgeranylation regulates Hippo signaling[131]. A separate study reported constitutive SREBP activation in the livers of mice with a liver-specific LATS2 deletion, which corresponded to an increase in liver free cholesterol and protection from p53-mediated apoptosis[132].

Activation of the MVA pathway and YAP/TAZ are correlated with mutant p53 expression in primary tumours, suggesting a dysfunctional mutant p53/SREBP/YAP/TAZ axis in cancer[129]. Overexpression of $p53^{R280K}$ in a p53-null cell line activated YAP/TAZ only when the MVA pathway was active, placing the MVA pathway as a critical intermediate in the oncogenic activation of YAP/TAZ by mutant p53[129].

Hedgehog. Cholesterol plays a multifaceted role in regulating cell signaling. For example, the Hedgehog (Hh) signaling pathway, which plays important roles in vertebrate development and tumorigenesis, is regulated by sterols at multiple levels[133]. Cholesterol itself can serve as a substrate for the post-translational modification of Hh ligands, which is required for their proper trafficking[134]. Cholesterol and cholesterol-derived oxysterols can also activate Hh signal transduction in medulloblastoma, whereas inhibiting the MVA pathway or downstream sterol biosynthesis decreased Hh signaling and reduced cell proliferation[135] (**Fig.5**).

Steroid hormone signaling. Cholesterol also serves as the precursor for steroid hormones, which drive the initiation and progression of cancers such as hormone-dependent breast and prostate cancer. In breast cancer, patients with oestrogen receptor alpha (ERα)-positive disease are commonly treated with aromatase inhibitors. Recent work demonstrated that long-term oestrogen deprivation of ERα-positive breast cancers led to stable epigenetic activation of the MVA pathway and cholesterol biosynthesis, coupled with increased SREBP occupancy on open chromatin[136]. The resulting elevated levels of 27-hydroxycholesterol was sufficient to activate ERα signaling in the absence of exogenous oestrogen, driving the activation of genes that promote an invasive cell phenotype[136]. Similarly, in prostate cancer, the *de novo* synthesis of androgens from cholesterol drives androgen receptor (AR) activity in castration-resistant disease[137] (**Fig.5**). This, coupled with the observations that SREBP expression is elevated in advanced-stage prostate cancer[138, 139], 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 for the treatment of hormone-driven cancers.

Targeting the MVA pathway in cancer.

As outlined above, multiple oncogenic signaling 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. These, coupled with the essentiality of several MVA pathway genes in

cancer cells, suggest that the MVA pathway is a tumour vulnerability that can be targeted as part of a therapeutic strategy to treat cancer. The most promising way to block this pathway in tumours is to inhibit HMGCR using statins, although inhibiting other flux-control points may also have anti-cancer benefits[17]. Statins have been safely used for decades to treat patients with hypercholesterolaemia[140], and although epidemiological evidence has been mixed, the majority of reports indicate that statin use is correlated with reduced mortality in multiple cancer types[141-143]. Evidence also suggests that certain stages of cancer progression, such as breast cancer recurrence, are particularly sensitive to the anti-cancer activities of statins[141, 144-146]. Although the cholesterol-lowering effects of statins are due to inhibition of MVA pathway activity in the liver, lipophilic statins such as atorvastatin, simvastatin and lovastatin have been detected in extra-hepatic tissues such as the brain, in both the active acid and inactive lactone forms[147]. In contrast, the hydrophilic pravastatin could only be detected in the liver[147], suggesting that hydrophilic statins may 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 [148]). Efforts are underway to directly address this issue, and to determine the clinical utility and recommended dose of statins when used as anti-cancer therapeutics.

Many studies have shown that statins can directly and specifically trigger apoptosis of tumour cells[53, 149-152]. For example, statins trigger apoptosis of cells derived from acute myelogenous leukemia (AML), while normal myeloid progenitors do not undergo apoptosis and retain full proliferative potential[25]. This tumour-normal index may be due to the altered metabolic reprogramming of tumour 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. Side-effects are regularly treated by switching to a different statin or potentially by co-treating with CoQ, although the latter is controversial due to conflicting clinical evidence[153, 154].

This suggests that statins possess 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[155] or fluvastatin[156], showed reductions in the Ki67 index in a subset of patients administered cholesterol-management doses of statins between 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 in hepatocellular carcinoma and AML, resulting in significantly longer median survival[157] and complete or partial response in 60% of patients[158], respectively. 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[159].

Despite evidence of patient response to statins as anti-cancer agents, many other patients remained non-responsive to statin treatment in other cancer clinical trials[160]. This is consistent with the current paradigm of tumour heterogeneity. This lack of response might also be expected considering the evidence we have laid out above showing that the MVA pathway is regulated by many key oncogenic signals. Like many anti-cancer agents, a personalized medicine approach is needed to implement statins, and/or other inhibitors of the MVA pathway, as a successful class of therapeutics. To this end, a molecular signature of basal mRNA expression has been developed for breast cancer[22] and deregulated MYC expression has been a proposed indicator of statin response in specific tumour-types[161]; however, essential follow-through validation is required. At this time, it is difficult to predict which cancers will be particularly sensitive to statin therapy. In addition to AML and MM (**Table 1**), encouraging results from both clinical trials[155, 156] and epidemiological[162, 163] studies suggest patients with hormone-dependent cancers, such as breast and prostate, may benefit from the addition of statins to their treatment regimen. This may be in part because the MVA pathway end-product cholesterol is the precursor for hormones such as oestrogen and androgens, which play a major role in the development of these types of cancers. Hepatocellular carcinoma also appears particularly responsive to statins[157], 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.

Critical to the regulation of the MVA pathway is the tightly-controlled, SREBP-mediated feedback mechanism, where 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 low-density lipoprotein receptor (LDLR), which leads to increased uptake of exogenous, lipoprotein-derived, cholesterol; an effect that has been shown to be important in cancer cells[164-167]. The SREBPs therefore function to replenish MVA pathway metabolites, which can dampen the apoptotic response following statin treatment. This would be a classic resistance mechanism, similar to what is seen with other anti-cancer 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 (e.g. MAP2K1) or increase in expression of receptor tyrosine kinases (e.g. EGFR), bypassing the need for BRAF activity[168]. These studies demonstrate that inhibiting both the cancer vulnerability and the resistance/feedback mechanism is crucial for maximum efficacy[169]. Hence, inhibiting the SREBP-regulated feedback response in conjunction with statin therapy could prevent resistance, thereby increasing the efficacy of statins as anti-cancer agents and the number of responsive patients (**Fig.6**).

Evidence that targeting the SREBPs in combination with statin therapy is a viable strategy has been provided by several recent studies. Firstly, a study looking at breast and lung cancer cell lines performed an shRNA screen to uncover genes that, when knocked down, potentiated the pro-apoptotic effects of statins[170]. The MVA pathway genes *HMGCS1*, *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 approved agent, dipyridamole[51]. Mechanistically, 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[171], an effect that may be associated with their ability to degrade nuclear SREBP2 and inhibit its transcriptional activity[172]. Although a number of 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 impact cancer patient care[173-175]. Therefore, at this time, clinical investigation into the utility of combined statins and SREBP inhibitors for the treatment of cancer is 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 signaling pathways that we have described in this review are important drivers in a majority of cancers, and they all have the ability to deregulate the MVA pathway, making those cancers potentially vulnerable to MVA pathway inhibition. Whether this occurs in every patient that 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, which may identify patients who will respond to MVA pathway inhibition. Designing clinical trials that will identify potential responders prior to treatment is needed 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 it and its feedback response, provides a strong rationale to continue exploring this key metabolic pathway in cancer.

Glossary.

Acetyl-CoA.

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

INSIG.

INSIG1 and INSIG2 interact with SCAP under sterol-rich conditions. They prevent SREBP activation by retaining the SCAP/SREBP complex in the ER. They also promote the sterol-regulated degradation of HMGCR.

SCAP.

Essential for SREBP ER-to-Golgi translocation. SCAP contains a sterol-sensing domain, and undergoes a conformational change when sterols are low. This change causes a dissociation of the SREBP/SCAP complex from INSIG.

S1P/S2P.

Two proteases that cleave the SREBPs, and other proteins such as ATF6, 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 response element (SRE).

Motifs found in the promoters of genes that are transcribed in response to sterol deprivation. SREs are necessary for the transcription of MVA pathway genes by the SREBPs.

Isoprenylation.

The attachment of a hydrophobic farnesol or geranygeraniol to the C-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.

Dipyridamole.

A clinically-approved drug used to prevent platelet aggregation. A recent study showed that it also prevents cleavage of SREBP2, potentiating the anti-cancer effects of statins, although the mechanism is not yet known.

Acknowledgements.

We thank Jenna van Leeuwen and William Tu for helping to prepare this review. We also thank other current and former members of the Penn lab for their helpful comments, including Alex Pandyra, Emily Chamberlain, Jason DeMelo, Dharmesh Dingar, Ashley Hickman, Manpreet Kalkat, Corey Lorenco, Diana Resetca and Aaliya Tamachi. We also acknowledge the many important contributions by our colleagues that could not be cited here owing to space and reference constraints. The funding agencies that enable our research include the Ontario Institute for Cancer Research through funding provided by the Province of Ontario, the Canadian Institute for Health Research, the Department of Defense Breast

Cancer Research Program, the Princess Margaret Cancer Foundation Hold'em for Life Prostate Cancer Research Fund, and the Terry Fox Foundation Canada.

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Figure legends

Fig.1A. The mevalonate (MVA) pathway. The 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. **B.** MVA pathway enzymes condense three acetyl-CoA molecules in a two-step reaction to produce 3-hydroxy-3-methylglutaryl coenzyme A (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 to isopentenyl diphosphate (IPP) through a series of enzymatic steps, which serves as a monomeric unit for the sequent synthesis of all downstream metabolites (highlighted in purple). Abbreviations: PPP = pentose phosphate pathway, IDH = isocitrate dehydrogenase, ACAT2 = acetyl-CoA acetyltransferase 2, HMGCS1 = HMG-CoA synthase 1, MVK = mevalonate kinase, PMVK = phosphomevalonate kinase, MVD = mevalonate-diphosphate decarboxylase, IDI1/2 = isopentenyl diphosphate isomerase, FDPS = farnesyl diphosphate synthase, FDFT1 = farnesyl-diphosphate farnesyltransferase 1, GGPS1 = geranylgeranyl diphosphate synthase 1. Dashed lines indicate multiple steps.

Fig.2. The SREBP-regulated sterol feedback response controls the transcription of MVA pathway genes[176]. (**i**) When ER sterol concentrations are high, the full-length, precursor SREBPs are localized to the ER in a complex with SCAP and INSIG. This complex is maintained through the binding of sterols to SCAP and/or the binding of oxysterols to INSIG. (**ii**) When sterols are low, SCAP undergoes a conformational change that causes the SCAP/SREBP complex to dissociate from INSIG. SCAP is then able to bind COPII proteins and be transported in vesicles, with SREBP, to the Golgi. (**iii**) SREBP is sequentially cleaved by site-1 protease (S1P) and site-2 protease (S2P) at the Golgi. Although not indicated, S1P and S2P are transmembrane proteins (**iv**) The cleaved, mature SREBP can then translocate to the nucleus, where it homodimerizes and binds to sterol-response elements (SRE) in the promoter regions of its target genes to activate transcription.

Fig.3. SREBP processing and activity are regulated by PI3K signaling at multiple levels. (**i, ii**) AKT can increase SREBP expression and activity, in part via the inhibition of GSK3β. (**iii**) mTORC1 increases SREBP processing and transcriptional activity through multiple substrates. mTORC1 activates S6K via phosphorylation to increase SREBP translocation, and potentially SREBP processing. (**iv**) The negative regulator of SREBP, LIPIN1, is also phosphorylated and inactivated by mTORC1. Despite the multiple levels of regulation of the SREBPs by PI3K signaling, the mechanisms remain to be elucidated and may be context-dependent.

Fig.4. Transcriptional control of MVA pathway gene transcription by oncogenes and tumour suppressors. (**i**) Specific gain-of-function p53 mutants functionally interact with SREBP to drive increased expression of MVA pathway genes. (**ii**) MYC can bind to SREBP to increase the expression of SREBP target genes and analysis of the ENCODE database shows that MYC and its binding partner, MAX, bind to the promoters of MVA pathway genes. (**iii**) The pRB tumour suppressor can interact with SREBP and reduce its binding at target genes. Loss of pRB in cancer removes this inhibition, leading to increased transcription of specific MVA pathway genes.

Fig.5. Activation of the MVA pathway drives oncogenic signaling pathways. (**i**) RhoA is required for the nuclear localization and activity of the YAP/TAZ oncogenes. The activity of RhoA is dependent on geranylgeranylation, which localizes RhoA to the plasma membrane. Geranylgeranylation requires GGPP produced exclusively via the MVA pathway, thus linking the MVA pathway to YAP/TAZ activity. (**ii**) Hedgehog (Hh) signaling is involved in tumorigenesis in multiple cancer types, and Hh ligands require the covalent attachment of cholesterol for proper processing and activity. (**iii**) Cholesterol is the precursor for steroid hormones such as oestrogen and androgen. These hormones are involved in hormone-driven breast and prostate cancers.

Fig.6. Inhibiting both the MVA pathway and the SREBP transcription factors is a viable cancer therapeutic. Statins have potent anti-cancer properties. They inhibit HMGCR, thereby reducing MVA pathway metabolites that are essential for cancer cell growth and survival (top panel). This triggers SREBP activation and 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 anti-cancer statin therapy. Dipyridamole is one example of an approved agent that inhibits SREBP cleavage, preventing the restorative feedback response and increasing apoptosis in multiple cancer cells. Combining these two approved drugs may increase the therapeutic response compared to statins alone.

Table 1: Available agents, both experimental and clinically-approved, that target the MVA pathway, production of its metabolites and/or its SREBP-regulated feedback mechanism.

Drug class		Target	Stage of clinical development	Refs
MVA pathway inhibitors	Statins	HMGCR	FDA-approved as cholesterol-lowering agents and currently in phase I-III clinical trials for the treatment of various cancer types	155-159
	Bisphosphonates	FDPS	FDA-approved for the treatment of osteoporosis, patients with multiple myeloma or solid tumour bone metastases, in combination with standard therapy	177-179
Prenylation inhibitors	FTIs/GGTIs	Farnesyl- and geranylgeranyl -transferases	In phase I-III clinical trials for the treatment of various cancer types, as single agents or in combination with standard therapy	65, 180, 181
SREBP inhibitors	Fatostatin	SCAP	In pre-clinical development	173-175
	Betulin	SCAP	In pre-clinical development	182
	Tocotrienols	Unknown	In pre-clinical development	171, 172
	Nelfinavir	S2P	FDA-approved for the treatment of HIV infection and in phase I-II clinical trials for the treatment of various cancer types	183-185
	Dipyridamole	Unknown	FDA-approved for the prevention of cerebral ischemia and in pre-clinical development as an inhibitor of SREBP	51