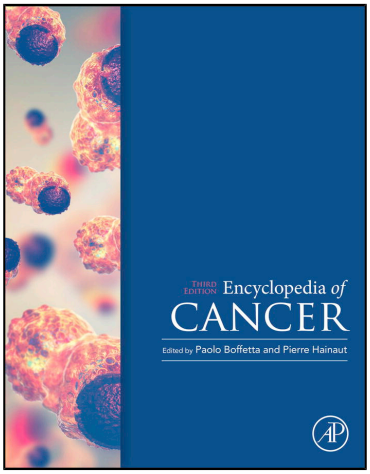

From Karlic, H., Varga, F., 2019. Mevalonate Pathway. In: Boffetta, P., Hainaut, P. (Eds.), Encyclopedia of Cancer, 3rd edition, vol. 2, Elsevier, Academic Press, pp. 445–457. http://dx.doi.org/10.1016/B978-0-12-801238-3.65000-6
ISBN: 9780128124840

Mevalonate Pathway

Heidrun Karlic, Ludwig Boltzmann Cluster Oncology, Hanusch Hospital, Vienna, Austria
Franz Varga, Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, Vienna, Austria

Glossary

Aceto(acetyl)-CoA Acetyl CoA is formed from pyruvate in the final stages of glycolysis and is also a metabolite from fatty acid catabolism (beta oxidation) and a precursor of ketone bodies, which are accumulated in a status of hunger. Addition of an acetyl-residue results in aceto(acetyl)-CoA, a substrate for enzymes of the upper mevalonate pathway.

Bisphosphonate Also named diphosphonates, group of chemical compounds which contain two phosphonate groups, this group of drugs is known to inhibit osteoclasts.

GTPases A large family of hydrolase enzymes that can bind to and hydrolyze guanosine triphosphate (GTP). The GTP binding and hydrolysis takes place in the highly conserved G domain, which is common to all GTPases.

NAD(P)H Nicotinamide adenine dinucleotide phosphate, abbreviated $NADP^+$, is a cofactor on anabolic reactions, which require NAD(P)H as a reducing agent. $NADP^+$ differs from NAD^+ in the presence of an additional phosphate group on the 2′ position of the ribose ring that carries the adenine moiety.

Prenylation The addition of hydrophobic molecules to a protein or to a chemical compound. It is assumed that prenyl groups (3-methyl-but-2-en-1-yl) facilitate attachment to cell membranes.

RHOA and RHOB A family of small (~21 kDa) signaling G proteins. The members of the Rho family have been shown to regulate many aspects of intracellular actin dynamics and metabolic processes.

Squalene A 30′carbon unsaturated oily liquid hydrocarbon and an important metabolite in cholesterol biosynthesis.

Sterol regulatory element-binding protein (SREBP) SREBPs are transcription factors that bind to the sterol regulatory element DNA sequence TCACNCCAC.

Statin A class of drugs that reduce the levels of lipids in the blood by altering the enzyme activity of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR).

Sterol isoprenoids Basically, isoprenoids are a class of organic compounds composed of two or more units of hydrocarbons, with each unit consisting of five carbon atoms arranged in a specific pattern. These compounds include certain sterols, oxysterols, farnesol, and geranylgeraniol, as well as the diphosphate derivatives of isopentenyl, geranyl, farnesyl, geranylgeranyl, and presqualene. They regulate transcriptional and posttranscriptional events that in turn affect lipid synthesis, meiosis, apoptosis, developmental patterning, protein cleavage, and protein degradation.

Introduction

The mevalonate pathway (MP) also known as the isoprenoid pathway or 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) pathway is an anabolic pathway providing metabolites for multiple cellular processes in eukaryotes, archaea, as well as some bacteria, thus underscoring its importance for nearly all living organisms including humans.

The mevalonate which is produced from acetoacetyl-CoA by HMGCR (Fig. 1) is further processed to sterol isoprenoids, such as cholesterol, which is an indispensable precursor of bile acids, lipoproteins, and steroid hormones, and to a number of hydrophobic molecules including nonsterol isoprenoids, such as dolichol, heme-A, isopentenyl tRNA, and ubiquinone. Intermediates of this network play important roles in the posttranslational modification of a multitude of proteins involved in inter- and intracellular signaling.

Besides its key role for cholesterol synthesis, the MP has become a challenging topic, when a large number of experimental and clinical studies suggested that inhibition of the MP might have valuable interest in human disease the management of multiple human diseases, besides cardiovascular diseases. Molecules arising from the MP are essential for cell growth and differentiation. They appear to be potential interesting therapeutic targets for many areas of ongoing research: oncology, autoimmune disorders, atherosclerosis, and Alzheimer disease. Also, considerable progress has been made in understanding the pathophysiology of two autoinflammatory disorders resulting from an inherited deficiency of mevalonate kinase (MK), the first committed enzyme of the MP.

Biochemistry of the MP

Upper MP

The mevalonate–isoprenoid pathway involves first the synthesis of 3-hydroxy-3-methylglutaryl-CoA (HMG)-CoA from acetyl-CoA through acetoacetylCoA. HMGCR, one of the best-regulated enzymes in nature, catalyzes the conversion of HMG-CoA to mevalonic acid. HMGCR is the rate-limiting enzyme of MP.

Encyclopedia of Cancer, Third Edition, 2019, 445–457

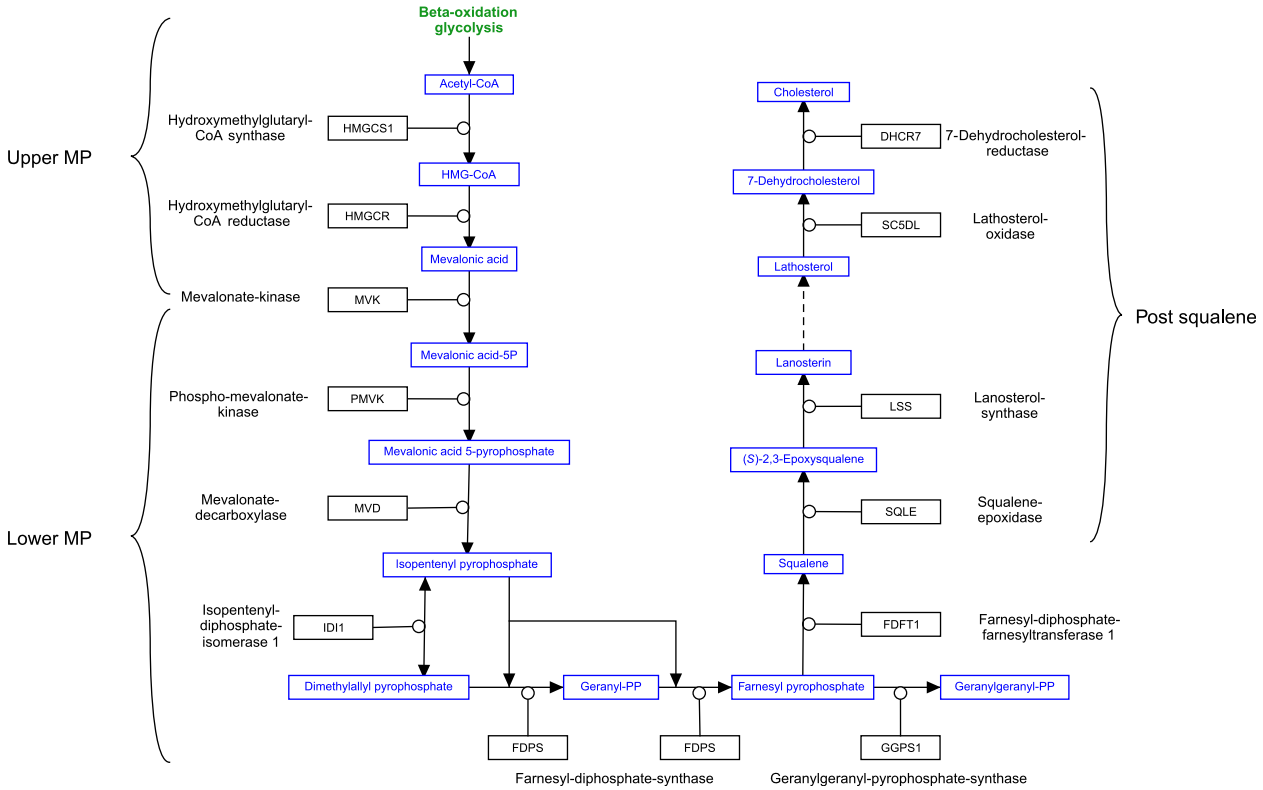

Fig. 1 Chemical reactions of the mevalonate pathway (MP). MP pathway enzymes condense three acetyl-CoA molecules in a two-step reaction to produce 3-hydroxy-3-methylglutaryl CoA (HMG-CoA). Both reactions are reversible and in equilibria, with the intracellular concentration of acetyl-CoA being the primary driver. HMG-CoA is then reduced by HMG-CoA reductase (HMGCR) to produce mevalonic acid (MA) via an irreversible reaction. MA is then converted into isopentenyl-diphosphate through a series of enzymatic steps, which serves as a monomeric unit for the consequent synthesis of all downstream metabolites. *Dashed arrows* indicate multiple steps.

In the absence of sterol isoprenoids in the cell, a family of transcription factors, named sterol regulatory element-binding proteins (SREBPs), directly activates HMGCR gene transcription. SREBPs regulate not only HMGCR gene transcription but also every step of cholesterol synthetic pathway by increasing gene expression of all the enzymes acting in the MP. In addition, other regulatory mechanisms can influence the activity of HMGR. The degradation rate of HMGR protein is influenced by cell's requirements for isoprenoids. Cell's requirements for isoprenoids will determine the rate of translation of HMGCR mRNA.

The investigation of yeast HMGCR accounted for mevalonate production following the reaction:

$$S - HMG - CoA + 2NADPH + H^{+} \rightarrow R - \text{mevalonate} + 2NADP + CoASH$$

The enzyme HMGCR is found in eukaryotes, archaea, and some eubacteria. The conversion of the thioesterified HMGCoA carboxyl to an alcohol represents a two-step reduction, accounting for the stoichiometry of NADPH in the reaction. The reaction thus proceeds through the successive reduction steps to first produce bound mevaldyl-CoA, collapse of the thiohemiacetal to release CoASH and form mevaldehyde; the second reduction step then forms product mevalonate.

The eukaryotic proteins (class I HMG-CoA reductases) are associated with the endoplasmic reticulum (ER) and interact through membrane spanning helices in the N-terminal domain. Consequently, the catalytic domain follows this membrane-anchoring sequence. These class I enzymes are potently inhibited by the class of statin drugs that effectively modulate sterol synthesis and, as a result, have been heavily investigated. There is no homologous sequence for membrane association at the N-terminus in the bacterial HMG-CoA reductases (class II), and a few of these (some in recombinant form) have been isolated as soluble proteins. The *Pseudomonas mevalonii* enzyme has a degradative function, allowing this microbe to grow on mevalonate as a carbon source. In contrast, the *Staphylococcus aureus* enzyme has a biosynthetic function and is encoded by a gene within a MP gene cluster.

Despite the low overall sequence homology (< 20%) and overall protein structure architecture between class I (eukaryotic) and class II (bacterial) HMGCR enzymes, there is considerable similarity between these enzymes in the positioning of active site residues important to catalytic function. Residues from two different subunits contribute to an active site. The histidine proposed to function in protonation of product Coenzyme A is appropriately positioned for this role. The aspartate implicated by mutagenesis is located within the active site and involved in a hydrogen bond network with the lysine and the glutamate that have been identified by functional studies. While both lysine and glutamate are in close proximity to the HMG-CoA thioester carbonyl that is reduced to form

mevalonate, there are different proposals concerning their precise roles in substrate carbonyl polarization and/or the proton transfers that accompany substrate reduction by NADPH.

Lower MP

The lower MP converts mevalonate into the relatively unreactive isopentenyl pyrophosphate (IPP), which further is converted to the more reactive electrophile dimethylallyl pyrophosphate. There exist (at least) three variants of the lower MP: In eukaryotes, mevalonate (MV) is phosphorylated twice in the 5-OH position and then decarboxylated to yield IPP. In some archaea such as *Haloferax volcanii*, mevalonate is phosphorylated once in the 5-OH position, decarboxylated to yield isopentenyl phosphate (IP), and finally phosphorylated again to yield IPP (Archaeal Mevalonate Pathway I). A third MP variant found in the archaeon *Thermoplasma acidophilum* phosphorylates mevalonate at the 3-OH position followed by phosphorylation at the 5-OH position. The resulting metabolite, mevalonate-3,5-bisphosphate, is decarboxylated to IP and finally phosphorylated to yield IPP (Archaeal Mevalonate Pathway II).

In eukaryotes, the above-mentioned phosphorylation is done by MK. MK also known as MVK or ATP: mevalonate 5-phosphotransferase catalyzes the transfer of ATP's c-phosphoryl to the C5 hydroxyl oxygen of mevalonic acid, resulting in formation of mevalonate 5-phosphate and ADP. The reaction was characterized in yeast; the protein is found in eukaryotes, archaea, and certain eubacteria. The enzyme was highly purified from porcine liver and was demonstrated to catalyze a sequential reaction with mevalonate substrate binding first and MgADP product released.

MK is the second essential enzyme of the isoprenoid/cholesterol biosynthesis pathway, after HMGCR, catalyzing the phosphorylation of mevalonic acid into phosphomevalonate. Although MK not has the rate-limiting properties of HMGCR, it is demonstrated that MK activity is regulated via feedback inhibition by intermediates in the isoprenoid/cholesterol pathway geranyl pyrophosphate, farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP).

High-affinity feedback inhibition has also been observed using a recombinant human protein and contrasted with lower-potency inhibition (10^{-5} M) of the *Staphylococcus aureus* enzyme.

Inherited human mevalonate kinase (MVK) mutations are correlated with two diseases, accounting for mevalonic aciduria (MVA) and Hyper-IgD syndrome.

Phosphomevalonate kinase (PMK) catalyzes the next step in isoprenoid/sterol biosynthesis, converting mevalonate 5-phosphate and ATP to mevalonate 5-diphosphate and ADP.

Activity of this enzyme was demonstrated in pig liver, and the pig liver enzyme has subsequently been isolated and more extensively characterized. PMK is found in eukaryotes and some eubacteria. The amino acid sequences for animal and low-homology invertebrate PMK proteins are not orthologous to those for PMK in plants, fungi, and bacteria. Thus, the proteins that catalyze the enzymatic reaction differ widely, depending on their source. Animal and invertebrate PMK proteins are known for a tertiary and quaternary structure, which is typical of the nucleoside monophosphate kinase family. The other PMK proteins are members of the galactokinase, homoserine kinase, MK, and phosphomevalonate kinase (GHMP) family. The tissue-isolated pig enzyme is reported to catalyze an ordered sequential reaction with mevalonate 5-phosphate assigned as the first substrate bound and ADP as the last product released. A recombinant form of *Streptococcus pneumoniae* has been characterized and reported to catalyze a random sequential bi-bi reaction. A recombinant form of *Enterococcus faecalis* PMK has also been isolated and characterized. The sequence of human PMK has been deduced.

Functional investigations of the recombinant human enzyme showed that the reaction catalyzed by PMK is a reversible reaction; kinetic constants of human PMK have been determined for both forward (formation of mevalonate 5-diphosphate) and reverse (formation of mevalonate 5-phosphate) reaction.

In the next step, mevalonate diphosphate decarboxylase; various abbreviations appear in the literature: MVD (in Fig. 1), also known as MDD, MPD, DPMDC, catalyzes the ATP-dependent decarboxylation of mevalonate 5-diphosphate to form isopentenyl 5-diphosphate (IPP), as indicated in the equation (Fig. 1).

This reaction is essential to the MP of polyisoprenoid and sterol synthesis. Activity has been measured in animals, plants, and yeast. Genetic complementation has implied activity in the *Staphylococcus aureus* and *Trypanosoma brucei* proteins. Highly purified active enzyme has been prepared from avian, porcine, and rat tissue and in recombinant form from bacteria expressing the yeast, human *Trypanosoma brucei*, and *Staphylococcus aureus* proteins. Characterization of the tissue-isolated protein implied the presence of an arginine that influences activity of the avian enzyme and documented the selectivity for divalent cation. The avian enzyme was also used to demonstrate that the transient phosphoryl transfer to the C3 oxygen proceeds with inversion of stereochemistry and, thus, no covalent E–P intermediate forms.

Farnesyl transferase (FTase) and geranylgeranyl transferases (GGTase) are two enzymes that carry out the process of prenylation in the cell. This process involves the covalent attachment of hydrophobic molecules (either the C-15 isoprene farnesyl or the C-20 isoprene geranylgeranyl groups) to the C-terminal end of some proteins including the γ-subunit of heterotrimeric G proteins, heme-A, nuclear lamins, and small GTP-binding proteins. Prenylation promotes the attachment of these proteins to internal cell membranes by means of a lipid anchors such as palmitate.

Such posttranslational modifications and activation GTP-binding proteins Rho, Rac, Rab, Rap, Ras play an important role in many important signaling cascades within the cell. Downstream from FPP the squalene synthase (FDFT1, also known as farnesyl-diphosphate farnesyl transferase 1) catalyzes the first committed step of the specific hepatic cholesterol biosynthesis at the final branch point of the cholesterol biosynthetic pathway, converting farnesyl-pyrophosphate into squalene. Squalene is then converted after a two-step cyclization into lanosterol, which is converted to cholesterol after a series of additional reactions.

Another downstream branch from FPP is catalyzed by the enzyme geranylgeranyl diphosphate synthase 1 (GGPS1) and leads to the synthesis of GGPP from farnesyl diphosphate and isopentenyl diphosphate. GGPP is an important molecule responsible for the C20-prenylation of proteins and for the regulation of a nuclear hormone receptor (Fig. 1).

Prenylation of Small GTPases and Brain Function

As mentioned earlier, FPP and GGPP are substrates for protein prenylation, and small GTP-binding proteins (sGTPases) represent the largest and most extensive characterized group of prenylated proteins. This superfamily is classified according to sequence and function similarity, into Ras, Rho, Rab, Sar1/Arf, and Ran families. The regulation of the activity of sGTPases is somewhat complex, and it has been extensively reviewed in greater detail, nonetheless, in general terms, sGTPases cycle between a guanosine diphosphate (GDP)- and GTP-bound conformation. The GDP-bound conformation is generally considered an inactive state, while the GTP-bound sGTPase activates downstream pathways by binding to specific effectors. sGTPases associate with the cytoplasmic leaflet of cellular membranes, where they perform their respective function. However, sGTPases are not transmembrane proteins, and in order to anchor to membranes they have to be covalently attached to lipid groups, with the exception of the so-called Ran (RAs-related nuclear protein) also known as GTP-binding nuclear proteins, for which no lipid modification has been reported. All the members of Ras, Rho, and Rab families undergo the lipid posttranslational modification of prenylation as the first committed step toward membrane association, while ARF family members undergo myristoylation. Both FPP and GGPP can be used as moieties for protein prenylation, catalyzed by the enzymes FTase and GGTase-I and -II, respectively.

Rho GTPases are essential players in neuronal development and function. Their activity is critical for neuronal cell migration, axon growth and guidance, dendritic arborization, dendritic spine formation and stabilization, growth cone motility and collapse, and synapse formation. Therefore, it is not surprising that studies in which statins or inhibitors of protein prenyltransferases were used, a decrease in neurite outgrowth and dendritic spines density was observed. In fact, inhibiting GGTase-I in vivo induces deficits in memory and learning in mice. However, there are also a number of studies showing that statins can actually increase neurite outgrowth, which might be explained by the different cell models, dosages, and statins that were used. Interestingly, neuronal survival has also been observed to diminish in the presence of statins, which was shown to be independent of a reduction on cholesterol levels, since the reversal of this phenotype was obtained by FPP/GGPP supplementation, but not by cholesterol addition.

Studies have shown that increased expression of CYP46A1 (= cholesterol 24-hydroxylase) in neurons enhanced prenylation and activation of sGTPases of the Rho and Rab family, and that this effect was dependent on the activation of the MP and GGTase-I activity, which is in accordance to previously mentioned studies. Moreover, it has been reported that this increase of sGTPases prenylation induced by CYP46A1 leads to an increase in neuronal dendritic outgrowth and dendritic protrusion density and elicits an increase of synaptic proteins in crude synaptosomal fractions, further highlighting the importance of how the activation of the MP and sustained production of isoprenoids are essential for neuronal development and function.

It is noteworthy that FPP and GGPP levels are much higher in the brain than in other tissues. Accordingly, in mouse brain cytosol, FPP and GGPP synthase activities are higher than those in the corresponding fractions from the liver, perhaps reflecting a higher demand for protein prenylation in the brain. Furthermore, it has been reported that in aged mouse brain, there is a reduction of GGTase-I activity, leading to a reduction of Rho GTPases membrane association, which is suggested to be one of the mechanisms underlying age-related cognitive dysfunction. There is a great lack of studies comparing the levels of nonsteroid products from the MP between neurons and astrocytes. It is noteworthy that CoQ10, which derives from the decaprenoide intermediary, has actually been measured and compared between both cell types (unpublished data reported in a PhD dissertation). Neurons were found to possess CoQ10 levels at around 68 pmol/mg protein, while in astrocytes the levels were significantly lower, about 38 pmol/mg protein. These results suggest that although neurons might have a lower cholesterol synthesis than astrocytes, an opposite profile might exist for isoprenoid synthesis, which supports our hypothesis that neurons undergo a shift from the postsqualene to the nonsterol branch of the MP. Based on those results and data presented in literature we hypothesize that the postsqualene branch and cholesterol synthesis might be downregulated in neurons to redirect the intrinsic MP toward the nonsterol branch, producing important isoprenoid intermediaries for neuronal function, while relying on external supply of cholesterol. Nevertheless, many questions remain unanswered: What are the underlying regulatory mechanisms involved the differential expression patterns of the MP enzymatic machinery in distinct neural cell types? Is the synthesis rate of isoprenoids different in different neural cell types? Is there a shuttle of isoprenoids from astrocytes to neurons? Is there a soluble factor released by astrocytes that signals for the shutdown of the postsqualene pathways in neurons? Are specific neuronal signaling pathways being modulated by isoprenoids levels, in a prenylation independent manner? Is there a specific deregulation of the nonsterol branch in neurons in pathological contexts? In order to answer these questions, the challenges ahead are also dependent on the development of new tools that facilitate imaging and quantification of the prenylome in neurons and astrocytes and to the identification their cognate protein prenyltransferases or unknown prenyl-binding proteins.

Evidence for a Redirection of the MP in Neurons

A compelling body of evidence suggests that, although neurons retain an active MP and can synthesize cholesterol, the efficiency of cholesterol synthesis is markedly lower when compared to astrocytes, possibly due to lower expression levels of enzymes belonging

to the postsqualene branch. There exists a hypothesis indicating that the postsqualene branch and cholesterol synthesis might be downregulated in neurons, and with this downregulation there is a redirection of the intrinsic MP toward the nonsterol branch, while neurons still rely on external supply of cholesterol. This hypothesis seems to be corroborated by data from other laboratories that published a RNA-Seq transcriptome of 7 days postnatal (P7) mice cortical neurons, astrocytes, oligodendrocytes, and vascular cells that corroborated a previous analysis of an array dataset using RNA isolated from forebrain cells of P7 and P16–17 (= 16–17 days old) mice. Based on data present in these two distinct datasets, the expression levels of genes involved in nonsterol, pre-, and postsqualene pathways in neurons and astrocytes and also between P7 and P16 neurons were compared. Both datasets suggest that the pre- and postsqualene branches in P7 neurons are substantially downregulated compared to astrocytes (blue and red, respectively). Nonetheless, the genes related to the nonsterol pathway have a similar or a higher-expression level in neurons when compared to astrocytes, with the exception of prenyl (solanesyl) diphosphate synthase, subunit 1 (Pdss1). Furthermore, the array data also enabled to compare the expression levels of genes involved in the MP in P16 neurons when compared to P17 astrocytes. Although there are two genes upregulated in the presqualene pathway (blue), the majority of genes have decreased mRNA levels in neurons when compared to astrocytes. Similar to P7 cells, while P16 neurons exhibit a clear downregulation of postsqualene pathway, they maintain or even increase the mRNA levels of genes belonging to the nonsterol branch, when compared to astrocytes. These expression patterns may indicate that while maintaining the efficiency of the nonsterol branch some what intact or even upregulated, there is a robust downregulation of the pre- and postsqualene pathway in neurons compared to astrocytes, which is a profile that seems to be conserved between these two cell types throughout CNS maturation. The fact that the nonsterol branch, in contrast to the pre- and postsqualene pathways, is not downregulated and might actually be somehow induced in neurons, is in line with the idea that the neuronal MP might be favoring the production of isoprenoids in detriment of cholesterol, which is ought to be supplied by astrocytes.

The decrease in mRNA levels from MP-associated genes in the presqualene pathway observed in neurons also favors the nonsterol pathway. Indeed, the affinity of GGPPS for FPP (*Km* value of 0.6 μM) is much higher than SQS (*Km* value of ~15 μM), thus under limited concentrations of FPP, isoprenoid synthesis will be favored. These kinetic findings together with the fact that during neuronal maturation the mRNA levels of Gggps increase while Fdft1 (the gene that codes for SQS) decrease argues that the nonsterol branch might be boosted in neurons in detriment of the postsqualene pathway. Accordingly, as previously mentioned, sGTPases activity is crucial during neuronal development and has been widely associated with neuronal dendritic development and synaptic regulation. Hence, as neurons mature, the demand for isoprenoids to prenylate sGTPases should increase, which is in agreement with the hypothesis of a redirection of the MP branch toward production of nonsterol intermediaries, while cholesterol needs are met by uptake from astrocytic-derived lipoproteins.

Role of the MP in Cancer

Cancer cells reprogram their metabolism to provide energy and the essential building blocks required to maintain their aberrant survival and growth. This reprogramming may occur through either mutations in metabolic enzymes (e.g., isocitrate dehydrogenases (IDHs)) or alterations in cell signaling owing to oncogenic events and/or the remodeled tumor microenvironment. These activated signaling cascades in turn deregulate the expression and/or the activity of enzymes in key metabolic pathways, including the MP.

The MP uses acetyl-CoA, NADPH, and ATP to produce sterols and isoprenoids that are essential for tumor growth (Fig. 1). The production of acetyl-CoA occurs following glucose, glutamine, or acetate consumption, which are often increased in cancer cells. NADPH is produced from a variety of sources, including the pentose phosphate pathway, malic enzyme, and IDHs. Therefore, the MP is highly integrated into the overall metabolic network of cancer cells. The transcription of genes encoding MP enzymes is primarily controlled by the SREBP family of basic helix–loop–helix leucine zipper transcription factors. When intracellular sterol levels are high, the SREBPs are maintained in an inactive state at the ER, where some MP 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. At the Golgi, the SREBPs are sequentially cleaved by site-1 protease and site-2 protease, 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 MP genes to restore sterol and isoprenoid levels. The importance of MP metabolites to the survival of cancer cells has been highlighted by recent studies that have identified a large number of MP enzymes as essential for the survival of several cancer cell lines. Additionally, numerous studies have shown that the statin family of drugs, which inhibit the initial flux-controlling enzyme of the MP, HMGCR, decrease growth and increase apoptosis in many cancer types in vitro and in vivo. These observations point to the MP as being a key dependency in tumors, and one that is readily targetable.

The MP has been suggested by some studies to be oncogenic. Early work in chronic lymphocytic leukemia showed that mevalonate can stimulate replication in primary leukemic cells. In another study, overexpression of the catalytic domain of HMGCR in primary mouse embryonic fibroblasts cooperated with HRASG12V to promote foci formation, suggesting that HMGCR is a metabolic oncogene. In addition, the direct infusion of MVA into mice harboring breast cancer cell xenografts caused an increase in tumor growth. Data from primary patient samples also suggest a role for the MP in promoting tumorigenesis, with a higher expression of MP genes correlating with poor prognosis in breast cancer. Collectively, this evidence indicates that the MP has a key role in cancer.

A series of evidences demonstrated that the MP is deregulated in cancer through aberrant cell signaling, which in turn establishes a tumor vulnerability that can be therapeutically targeted to improve outcomes for cancer patients.

MP-Derived Metabolites in Cancer

Initially, the regulation and function of the MP and its metabolites were studied in the context of normal and hypercholesterolemic tissues, which led to the Nobel prize-winning discoveries of Bloch and Lynen in 1964, and Brown and Goldstein in 1985. In recent years, the importance of MP-derived metabolites in cancer has become increasingly appreciated (discussed later).

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 signaling complexes. 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. Therefore, it is possible that high levels of cholesterol, produced by the MP, could have a role in protecting cancer cells from immune surveillance and various therapies. 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; increased oxysterol production can activate the liver X receptors, which have been proposed to be therapeutic targets in multiple cancer types. 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 MP is the sole intracellular source of isopentenyl-diphosphate (IPP) (**Fig. 1**). Aberrant activation of the MP 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. These observations led to phase I clinical trials that evaluated the in vivo expansion of γδ T cells in response to zoledronate, a bisphosphonate (BP) that inhibits farnesyl diphosphate synthase (FDPS) and leads to the accumulation of IPP, in combination with interleukin-2 (IL-2) treatment in advanced-stage breast cancer and prostate cancer. In both studies, the therapy was well-tolerated and the number of sustained peripheral γδ T cells correlated with improved clinical outcome. 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 posttranslational modification tethers proteins to cell membranes, enabling proper protein localization and function. Most small GTPases—many of which are involved in tumorigenesis, such as RAS and RHO—are isoprenylated; inhibition of the MP can reduce the isoprenylation of these small GTPases and can induce the death of some cancer cells. This cell death can be reversed by the addition of GGPP, and sometimes FPP, suggesting that these MP metabolites are essential for tumor cell viability. 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; instead, it seems that this is a "class effect," with the depletion of these isoprenoid pools potentially affecting the many proteins that are isoprenylated. Despite this dependency, directly inhibiting the isoprenylation of proteins using geranylgeranyl transferase inhibitors (GGTIs) or farnesyl transferase inhibitors (FTIs) has not been a successful anticancer strategy to date. The rationale behind these drug development programs 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. Further development of next-generation FTIs and GGTIs remains a fairly limited and focused area of research.

Dolichol

Dolichol is derived from 18 to 20 IPP molecules and is an essential component of the *N*-glycosylation of nascent polypeptides in the ER. Protein *N*-glycosylation is frequently altered in cancer and can contribute to tumor formation, proliferation, and metastasis. Not all *N*-glycans are associated with tumor progression; the complex branching of *N*-glycans leads to tumor-suppressive properties in some cancers. 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.

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. Therefore, CoQ is crucial for ATP production in cancer cells that rely on oxidative phosphorylation to produce energy.

Oncogenic Regulation of the MP

Intracellular pools of MP metabolites are tightly regulated by modulating the expression and activity of the MP enzymes. MP gene expression is mainly controlled by the SREBP transcription factors (Fig. 2). 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 MP-associated genes; SREBP1a and SREBP1c are transcribed from alternative start sites in the *SREBF1* gene, with SREBP1a regulating the expression of both MP and fatty acid metabolism genes, and SREBP1c predominantly regulating the expression of fatty acid metabolism genes.

Chromatin immunoprecipitation followed by sequencing (ChIP-seq) studies have indicated some overlap in the target genes of each SREBP, including MP genes, indicating some redundancy. 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 MP in cancer, a complete characterization of SREBP2 in transformed cells is needed.

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

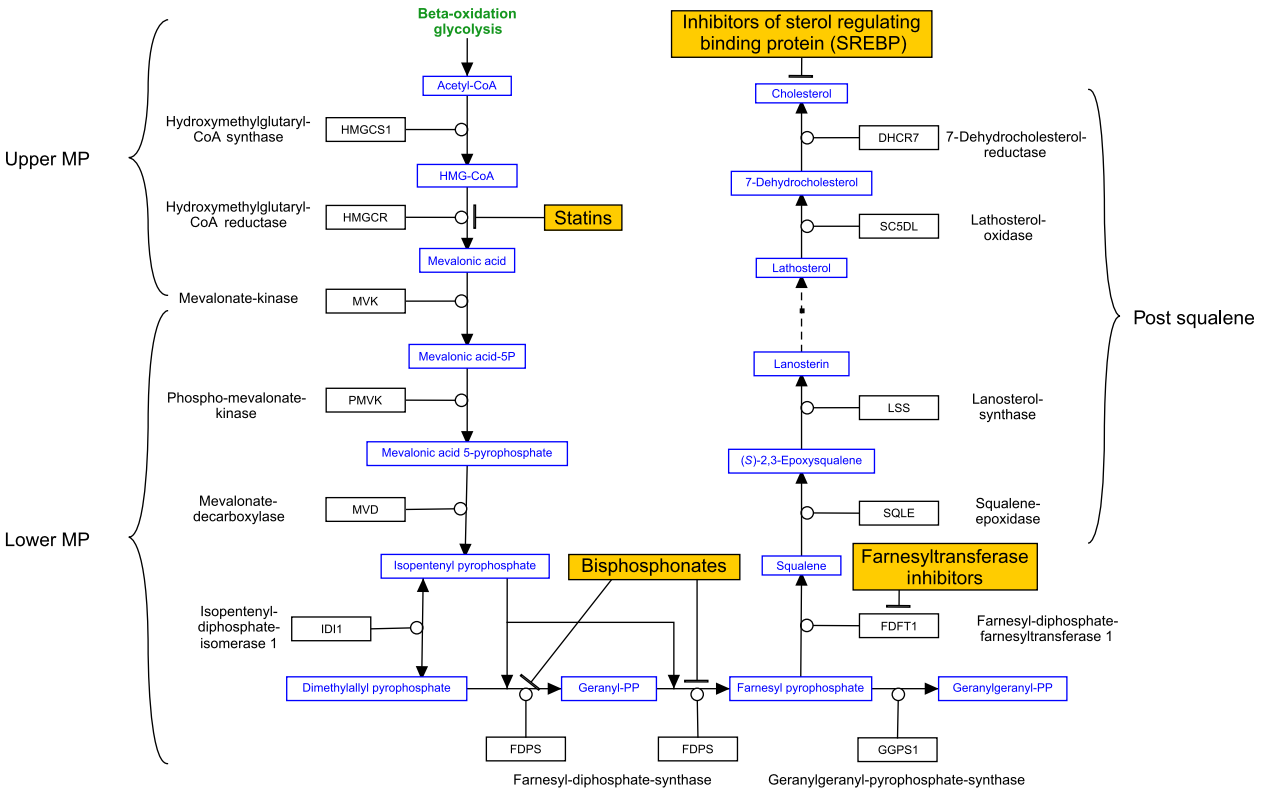

Fig. 2 Targets of inhibitors of the MP. *Statins* inhibit HMGCR, thereby reducing MP metabolites that are also essential for cancer cell growth and survival. This triggers sterol regulatory element-binding protein (SREBP) activation and the transcription of MP genes, thus restoring MP activity. Dipyridamole 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. *Bisphosphonates* act downstream of statins and inhibit farnesyl pyrophosphate synthase, a key enzyme in the MP, with consecutive decrease of the formation of isoprenoid lipids such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate. This part of the MP is also targeted by *farnesyl transferase inhibitors*.

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 the second most frequently mutated gene is *PIK3CA*, which encodes PI3K catalytic subunit alpha. 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. Alterations in the PI3K–AKT pathway generally act to augment signaling and consequently increase the proliferation of cancer cells. PI3K–AKT can activate the MP through various mechanisms. For example, the stimulation of PI3K–AKT signaling by growth factors, such as insulin, platelet-derived growth factor, and vascular endothelial growth factor, can increase the mRNA and protein expression of SREBP1 and SREBP. It should be noted that although PI3K–AKT signaling strongly and consistently increases the mRNA and protein levels of SREBP1a and SREBP1c, its effects on SREBP2 expression are context dependent. 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. The importance of this degradation pathway is highlighted by an increase in cholesterol and fatty acid synthesis in FBXW7-deficient cells. The residues that are recognized by FBXW7 are phosphorylated by glycogen synthase kinase-3β; AKT, which inhibits this phosphorylation, may prevent FBXW7-mediated degradation of the SREBPs. Insulin also causes the dissociation of INSIG from SCAP–SREBP1c in a sterol-independent manner, leading to the increased transcription of MP genes. 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. 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. Increased MP 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. Conversely, inhibition of the MP decreases PI3K activity, possibly through decreased RAS isoprenylation, thus demonstrating a two-way regulatory relationship between PI3K–AKT signaling and the MP.

mTOR Complex 1

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. 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) and repressing the activity of the inhibitor of cap-dependent translation, eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1; also known as EIF4EBP1). SREBPs are major downstream effectors of mTORC1 signaling, as evidenced by increased lipogenesis in response to mTORC1 activation. The observation that SREs are the most common regulatory elements in mTORC1-induced genes further strengthens the link between mTORC1 and the SREBPs. 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*). This study also compared proteins from tumor samples and adjacent normal breast samples and described an increase in FASN protein levels in the tumors 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. For example, S6K1 has been shown to activate SREBP2 processing and increase the expression of MP genes in a hepatocellular carcinoma (HCC) cell line although the mechanism involved remains unclear. Greater understanding of the role of mTORC1 in SREBP activity came with the development of torins, which are mTOR catalytic site inhibitors. 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. Recent work comparing torin and rapamycin action implicated a role for lipin 1 (LPIN1) in mediating the effects of mTORC1 on the SREBPs. 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. A further link between LPIN1 and the MP was uncovered by studies using skeletal muscle, in which statins and LPIN1 were shown to increase autophagy. Given the role of SREBP2 in transcribing numerous autophagy genes, 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 signaling presents a potential vulnerability in tumors 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. 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. The observation that double knockdown of *SREBF1* and *SREBF2* showed the greatest proapoptotic effect suggests that small-molecule inhibitors that target both SREBP1 and SREBP2 may have the greatest therapeutic benefit.

AMP-Activated Protein Kinase

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. AMPK was discovered through its ability to phosphorylate and reduce the activity of microsomal HMGCR in rat liver extracts. 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 MP metabolites. The SREBPs are also direct targets of AMPK phosphorylation. 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. 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 hyperglycemic and hyperinsulinemic conditions. 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 MP enzymes 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) and HMGCR, which consequently resulted in a decrease in hepatic triglyceride and cholesterol levels. AMPK can thus inhibit MP 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 MP may also regulate AMPK activity, thereby forming a feedback loop. The tumor suppressor liver kinase B1 (LKB1; also known as STK11), which phosphorylates and activates AMPK, is farnesylated at a highly conserved carboxyterminal CAAX motif. Knock-in mice expressing a mutant form of LKB1, which could not be farnesylated, exhibited reduced membrane-bound LKB1 and impaired AMPK activity. 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 MP 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 RB

TP53, which encodes the p53 tumor 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. Two gain-of-function mutations (*TP53*R273H and *TP53*R280K) enable p53 to functionally interact with nuclear SREBP2 and increase the transcription of MP genes. This MP gene activation was necessary and sufficient for mutant p53 to disrupt normal breast acinar morphology, and mutant TP53 expression in primary breast cancer tissues was correlated with the increased expression of sterol biosynthesis genes. Conversely, wild-type TP53 can reduce lipid synthesis under conditions of glucose starvation by inducing the expression of LPIN1, which, as described earlier, can prevent the association of SREBPs with chromatin. *TP53*R273H and *TP53*R280K mutations are also found in tumors from tissues other than the breast, for example, the ovaries, prostate, and lung.

The interplay between TP53 and the MP suggests that the MP may be a novel therapeutic target for tumors that harbor these specific p53 gain-of-function mutations.

The tumor suppressor protein RB has also been implicated as a regulator of the MP. In a mouse model of adenoma, loss of *Rb1* (which encodes RB) enhanced isoprenylation and activation of NRAS. 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*. Moreover, RB prevented the association of SREBP1 and SREBP2 with the *FDPS* promoter, suggesting that RB negatively regulates the MP at both the transcriptional and the posttranslational 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 reprogram cancer cell metabolism to enable the proliferation and survival of cancer cells. Like the SREBPs, MYC is a basic helix-loop-helix dimerizing protein and it has been shown to bind to SREBP1 to drive somatic cell reprogramming into induced pluripotent stem cells. Analysis of data from the Encyclopedia of DNA Elements (ENCODE) project also shows that MYC binds to promoters of MP genes in close proximity to SREBP1 and SREBP2 binding regions, suggesting that MYC can contribute to the expression of MP enzymes. As the MP is essential for cancer cells, and because MYC has a major role in metabolic regulation, deregulated MYC may ensure that MP metabolites are not limiting for tumorigenesis. The MP was also shown to be important in a MYC-driven transgenic model of HCC. In that study, atorvastatin reduced tumor initiation and growth, possibly through reduced isoprenylation of the RHO-family GTPase RAC1, leading to the activation of serine/threonine-protein phosphatase 2A, which is a negative regulator of MYC. More recently, *Myc*+/− mice (which are haploinsufficient) were shown to have an increased lifespan, which was associated with the decreased expression of MP genes, including *Hmgcr* and *Srebf2*. 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 MP.

Signaling From the MP

Altered metabolism in tumors not only fulfills the energetic and biosynthetic needs of a dividing cell but also produces metabolites that are important for downstream signaling. This is particularly true of the isoprenoid and sterol metabolites produced by the MP, which are also used by cancer cells to modulate multiple downstream signaling pathways that are important for tumor progression.

Yes-Associated Protein and TAZ

It was recently shown that the oncogenes Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ; also known as WWTR1) require the MP to be fully functional. YAP and TAZ are transcriptional coactivators that facilitate the transcriptional activation of progrowth genes and the repression of proapoptotic genes. The nuclear localization of YAP and TAZ is negatively regulated, partly by the activation of the tumor-suppressive Hippo signaling pathway. Activation of the Hippo cascade results in the phosphorylation and activation of large tumor suppressor kinase 1 (LATS1) and LATS2, which phosphorylate YAP and TAZ and retain them in the cytoplasm. YAP and TAZ nuclear localization requires the MP, as concurrent knockdown of *SREBF1* and *SREBF2* reduces nuclear localization of YAP and TAZ. These effects were mimicked by GGTIs (geranylgeranyl transferase inhibitors) and were prevented by a RHOA mutant that does not require geranylgeranylation. This suggests that SREBP-mediated induction of the MP maintains intracellular GGPP pools, which is necessary for RHOA upregulation and for downregulation of RHOB (which is essential for initiating protein degradation and recycling through an endolysosomal pathway), as well as YAP and TAZ nuclear localization. Although some studies showed that MP-mediated YAP and TAZ signaling is independent of LATS1 and LATS2 via RNA interference-knockdown experiments, one study demonstrated that both atorvastatin treatment and GGTI treatment increase the phosphorylation of LATS1 and LATS2, suggesting that geranylgeranylation regulates Hippo signaling. 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. Activation of the MP and activation of YAP and TAZ are correlated with mutant TP53 expression in primary tumors, suggesting a dysfunctional mutant p53–SREBP–YAP–TAZ axis in cancer. Overexpression of *TP53*R280K in a *TP53*-null cell line activated YAP and TAZ only when the MP was active, suggesting that the MP is a crucial intermediate in the oncogenic activation of YAP and TAZ by mutant TP53.

Hedgehog

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

Steroid Hormone Signaling

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 estrogen receptor-α (ERα)-positive disease are commonly treated with aromatase inhibitors to deprive the tumors of estrogen. Recent work demonstrated that long-term estrogen deprivation of ERα-positive breast cancers leads to the stable epigenetic activation of the MP 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. The resulting increased levels of 27-hydroxycholesterol were sufficient to activate ER1 (estrogen receptor alpha) signaling in the absence of exogenous estrogen, driving the activation of genes that promote an invasive cell phenotype. Similarly, in prostate cancer, the de novo synthesis of androgens from cholesterol drives androgen receptor activity in castration-resistant disease. This finding, coupled with the observations that SREBP expression is increased in advanced-stage prostate cancer, suggests a role for the MP in prostate cancer progression. These findings warrant further investigation into the utility of inhibitors of the MP and/or SREBPs in the treatment of hormone-driven cancers.

Inhibitors

Statins

Statins competitively inhibit HMGCR (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase), the first committed enzyme of the MP, blocking the conversion of HMG-CoA to mevalonic acid (Fig. 2). The molecular mechanism of inhibition of HMGR by statins is a catalytic mechanism. The statins molecules occupy the catalytic portion of HMGCR, specifically the binding site of HMG-CoA, thus blocking access of this substrate to the active site. The structures of the catalytic domains of the enzyme in complex with statin molecules have been identified.

Efficacy of HMGCR inhibition by various compounds in the family of statin inhibitors is markedly dependent (e.g., nanomolar versus millimolar inhibitor affinity) on whether class I or class II enzyme is used. The inhibitors are characterized by hydroxymethylglutaryl (HMG)-like moieties linked to an extensive hydrophobic scaffold (e.g., a decalin ring in the case of mevastatin and simvastatin). Complexes of human enzyme catalytic domain with a variety of statins have been crystallized, and X-ray structures have been published. The structural results indicated binding of the HMG moiety in the active site pocket where the catalytic glutamate and lysine residues are located. In contrast, the NADPH substrate site is not occupied upon inhibitor binding. The structural results indicating that access to the substrate HMG-CoA is blocked by inhibitor binding are in accord with the observation of competitive inhibition with respect to HMG-CoA. A variety of additional polar interactions with the HMG moiety have also been documented.

The hydrophobic portion of the inhibitors is bound in a shallow hydrophobic groove of the human protein. A large number of van der Waals contacts between nonpolar amino acids in this groove and the diverse hydrophobic substituents that are a common feature of the various statins are proposed to represent the dominant contribution to high-affinity binding. A structure of lovastatin bound to the class II *P. mevalonii* enzyme has also been reported. As in the case of the class I enzyme, the structure indicates interactions with residues (e.g., lys, glu) identified in catalysis as well other polar residues in this pocket, with some hydrogen bonds mediated by water molecules. The hydrophobic decalin ring component of the inhibitor blocks a closure of the C-terminal flap domain of the protein, which includes the histidine residue that has been implicated in catalysis. Thus, inhibitor binding both blocks the active site and makes correct orientation of active site amino acids impossible. A large difference between class I and class II enzyme interactions with the statin inhibitor is suggested to involve a pocket formed, in part, by the alpha helix of the *P. mevalonii* enzyme. The structural results could expedite potential design of HMGCR inhibitors that can discriminate between class I and class II enzymes. Additionally, an approach proposed to increase inhibitor affinity involves derivatization of the parent inhibitory compound to incorporate chemical substituents effective in interaction with the NADPH site.

By interrupting cholesterol synthesis in the liver, statins activate the production of microsomal HMGCR and cell surface low-density lipoprotein (LDL) receptors. This results in a predictable increased clearance of LDL from the bloodstream and a decrease in blood LDL cholesterol levels that may range from 20% to 55%. Statins have shown strong evidence-based proved capacity of decreasing the cardiovascular morbidity and mortality in both primary and secondary prevention settings. Because of these properties, statins are among the most widely used pharmaceutical agents in the world, their role in the global battle against cardiovascular disease being compared with the crucial role of antibiotics in decreasing the mortality of infections. Subgroup analyses of several large clinical trials have suggested that changes in lipid levels alone may not explain all the beneficial effects of statins in human pathology. Evidence has emerged from both basic research and clinical trials that statins have many cholesterol-independent or so-called pleiotropic effects: improving endothelial function, atherosclerotic plaque-stabilizing effects, antiinflammatory and immunomodulatory effects, antithrombotic properties, effects on bone metabolism, on risk of dementia, induction of apoptosis, and antiproliferative effects.

Most of these pleiotropic effects result from inhibition of the synthesis of important isoprenoid intermediates of the MP, such of FPP and GGPP. Mevalonate-derived prenyl groups have been shown to have essential roles in many cellular functions including cell signaling, cell differentiation and proliferation, myelination, cytoskeleton dynamics, and endo−/exocytotic transport. In the last decade, substantial progress has been made in understanding the nonlipid-related pharmacological properties of statins and a growing interest in the potential therapeutical implications of these pleiotropic effects of statins has emerged. Recent experimental and clinical data indicate that statins may be of potential therapeutic use in a variety of nonvascular diseases, including autoimmune diseases, multiple sclerosis, rheumatoid arthritis, sepsis, dementia, and different types of cancer.

Many studies have shown that statins can directly and specifically inhibit the proliferation of tumor cells. For example, statins promote the apoptosis of cells derived from acute myeloid leukemia (AML), while normal myeloid progenitors do not undergo apoptosis and retain full proliferative potential. This tumor-normal therapeutic index may be due to the altered metabolic reprogramming of AML cells leading to an increased dependence on MP 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 cotreating with CoQ although this cotreatment method is controversial owing to conflicting clinical evidence.

The data discussed earlier suggest that statins have a high therapeutic index to target tumors in vivo despite the ubiquitous expression of the MP. This rationale has led to multiple clinical trials investigating the efficacy of various statins as a therapeutic option in a variety of tumor types. Two recent breast cancer window-of-opportunity clinical trials, using atorvastatin and fluvastatin, showed reductions in the Ki67 index in a subset of patients who were administered with cholesterol-management doses of statins between cancer 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 colorectal cancer and resulting in complete or partial response in 60% of patients with AML. 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.

Despite evidence of patient response to statins as anticancer agents, many patients remained nonresponsive to statin treatment in other cancer clinical trials. This is consistent with the current paradigm of interpatient tumor heterogeneity. This lack of response might also be expected considering the evidence that we discussed earlier showing that the MP 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 MP, 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, and deregulated MYC expression has been a proposed indicator of statin response in specific tumor types; 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 and epidemiological studies 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 MP end-product cholesterol is the precursor of hormones such as estrogen and androgens, which have a major role in the development of these types of cancers. Colorectal cancer also seems to be particularly responsive to statins, 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.

Targeting the SREBP in Combination With Statins

Crucial to the regulation of the MP is the tightly controlled, SREBP-mediated feedback mechanism, in which inhibition of the MP results in the activation of the SREBPs and an increase in the expression of MP 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. The SREBPs thus function to replenish MP metabolites, which can dampen the apoptotic response following statin treatment. This would be a classic resistance mechanism, similar to that seen with other anticancer therapeutics such as inhibitors of the BRAF protooncogene in BRAF-mutant melanoma. Cells treated with BRAF inhibitors, such as vemurafenib, can acquire an activating mutation in downstream kinases (e.g., the MEK1 mitogen-activated protein kinase kinase) or can have an increase in expression of receptor tyrosine kinases (e.g., epidermal growth factor receptor), bypassing the need for BRAF activity. These studies demonstrate that inhibiting both the cancer vulnerability and the resistance or feedback mechanism is crucial for maximum efficacy. 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.

Evidence that targeting the SREBPs in combination with statin therapy is a viable strategy has been provided by several studies. One study looking at breast and lung cancer cell lines used a short hairpin RNA screen to uncover genes that, when knocked down, potentiated the proapoptotic effects of statins. The MP genes *HMGCS1*, *GGPS1*, *SCAP*, and *SREBF2* all scored highly, adding credence to either inhibiting other enzymes in the MP or inhibiting the SREBP-mediated feedback response in combination with statin therapy. Another study showed that statin-induced SREBP processing can be blocked by another agent that has been approved for a noncancer indication, dipyridamole. 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, which is an effect that may be associated with their ability to degrade nuclear SREBP2 and inhibit its transcriptional activity. 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.

Therefore, clinical investigation into the utility of combined statins and SREBP inhibitors for the treatment of cancer is currently warranted.

Bisphosphonates

BPs are potent inhibitors of osteoclast mediated bone resorption and are currently the most important and effective class of drugs used to treat metabolic bone disease. BPs bind avidly and achieve therapeutic concentration at sites of active bone metabolism in the mineralized bone matrix. By inhibiting bone resorption and inducing osteoclast apoptosis, BPs reduce bone turnover, increase bone mass, and improve bone mineralization. Recent experimental studies have shown that nitrogen-containing BPs may have interesting antitumor properties. As shown in **Fig. 2**, they inhibit FPP synthase, a key enzyme in the MP, with consecutive decrease of the formation of isoprenoid lipids such as FPP and GGPP. It has been demonstrated that the capacity of inhibiting FPP synthase is correlated to the synthesis from the accumulated isopentenyl diphosphonate of an ATP analogue, named Apppi. This, in turn, prevents the prenylation of a number of small GTPases, such as Rho, Ras, Rac, and Rab, which play a role in malignant transformation of cells by regulating intracellular signaling, cell growth, motility, and invasion. Interestingly, the ability of nitrogen-containing BPs to inhibit FPP synthase appears to be clearly related to the presence of a nitrogen atom at critical positions in the side chain. Consistent with their recently identified biochemical effects on protein prenylation, there is extensive evidence from preclinical research that BPs also exhibit antitumor activity in a variety of human cancers: myeloma, breast cancer, prostate cancer, pancreatic cancer, mesothelioma, mesenchymal tumors, and osteosarcoma. Summarizing, inhibition of the MP is the underlying molecular mechanism of many of these antitumor properties of nitrogen-containing BPs: inhibition of integrin-mediated tumor cells adhesion to bone. BPs act by inhibition of prenylation of small GTPases required for integrin activation–inhibition of cancer cells migration and invasion by inhibition of metalloproteinases and by inhibition of Rho activation by preventing geranylgeranylation. Inhibition of proliferation and induction of apoptosis of cancer cells were observed at high BP concentrations. Although inhibition of the MP was found to be the main molecular mechanism of promoting apoptosis, other mechanisms have also been described, that is, reduction of bcl-2 expression and activation of caspase. In addition, immunomodulatory effects mediated through accumulation of mevalonate metabolites in tumor cells, which stimulate T lymphocytes expressing the T cell receptor, were described.

Other Therapeutic Agents That Target MP

Extensive preclinical evidence suggested that manipulation of the MP through inhibition of FTase and GGTase, and, in consequence, inhibition of protein prenylation, can result in alteration of malignant cells' capacity of proliferation, growth, and migration. FTIs represent a new generation of signal transduction inhibitors, designed to target the critical posttranslational modification of Ras, a protooncogene that is believed to play a critical role in tumor growth or progression and may have prognostic significance.

Some studies suggest that FTIs inhibit not exclusively Ras proteins but also the farnesylation of additional cellular polypeptides that have not been identified, thereby exerting antitumor effects independent of the presence of activating Ras gene mutations. Lonafarnib SCH-66336 has been the first of these compounds to undergo clinical development. Several other FTIs have entered clinical trials for various cancer indications: examples are tipifarnib (R115777), BMS-214662, and L-778. Although phase I and II studies have shown that FTIs have a significant antitumor activity and an acceptable toxicity profile, the results of phase III trials have been disappointing, showing no overall significant amelioration of survival in solid cancers. The most promising activity to date has been demonstrated in patients with hematological malignancies, in particular AML and myelodysplastic syndrome. GGTIs are a novel class of drugs that are still in the experimental stage. It has been recently demonstrated that when FTase is blocked by FTIs, additional inhibition of geranylation by inhibitors of GGTase can augment antiproliferative properties of FTIs. Treatment of tumor cell lines in culture with varying doses of a GGTI in conjunction with a FTI can inhibit Ki-Ras prenylation and determine an apoptotic response that is greater than the apoptotic response elicited by either agent alone in vitro. However, the tolerability of this protocol in vivo was poor and suggested that the use of this approach in humans should be cautious because of a possible narrow therapeutic index. Recent preclinical research has identified a new inhibitor of protein prenylation, a selective, highly potent, and cell-active GGTase-I inhibitor, GGTI-DU40, and suggested that investigation of GGTIs, as potential new anticancer drugs should continue, in order to define their exact role and toxicity profile. In conclusion, in the last few years, there has been intense interest in understanding the clinical and therapeutical implications of MP, a metabolic pathway which plays a key role in regulation of cellular cholesterol synthesis and in controlling cell proliferation by generating prenyl intermediates. Since the discovery of MK deficiency as the direct biochemical and molecular cause of MVA and hyperimmunoglobulinemia D syndrome, considerable progress has been made in understanding the pathophysiology of these antiinflammatory disorders. However, it is still not known what is the causal relationship between the enzymatic defect and the inflammation and no effective treatment has been established so far. Also, MP is an important, attractive target for many areas of therapeutical research and application, through inhibition of the isoprenylation process of small intracellular proteins. Although extensively studied, many important issues remain unanswered, and this fascinating area of interference between biochemistry, clinical implications, and pharmacology remains a major focus for potential future investigation.

Outlook

Understanding tumor metabolism in the context of oncogenic signals has the potential to drive the development of targeted personalized therapies. The various signaling pathways that we describe in this book chapter are important drivers in many cancers, and they all have the ability to deregulate the MP, making these cancers potentially vulnerable to MP 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 MP in patients. Rapidly developing technologies for the comprehensive flux-based analysis of MP metabolites will provide further advances in understanding how the MP 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 MP genes, which may identify patients who will respond to MP 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 MP 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 and many other diseases.

Acknowledgments

Related works in our laboratory are supported in part by the Fonds zur Förderung der wissenschaftlichen Forschung (FWF; The Austrian Science Fund) Project P24370-B19, the WGKK (Social Health Insurance Vienna), and the AUVA (Austrian Social Insurance for Occupational Risks).

Further Reading

Buhaescu, I., Izzedine, H., 2007. Mevalonate pathway: a review of clinical and therapeutical implications. Clinical Biochemistry 40 (9–10), 575–584.

Miziorko, H.M., 2011. Enzymes of the mevalonate pathway of isoprenoid biosynthesis. Archives of Biochemistry and Biophysics 505 (2), 131–143.

Karlic, H., Thaler, R., Gerner, C., Grunt, T., Proestling, K., Haider, F., Varga, F., 2015. Inhibition of the mevalonate pathway affects epigenetic regulation in cancer cells. Cancer Genetics 208 (5), 241–252.

Moutinho, M., Nunes, M.J., Rodrigues, E., 2017. The mevalonate pathway in neurons: it's not just about cholesterol. Experimental Cell Research (Epub ahead of print).

Mullen, P.J., Yu, R., Longo, J., Archer, M.C., Penn, L.Z., 2016. The interplay between cell signalling and the mevalonate pathway in cancer. Nature Reviews. Cancer 16 (11), 718–731.

Räikkönen, J., Mönkkönen, H., Auriola, S., Mönkkönen, J., 2010. Mevalonate pathway intermediates downregulate zoledronic acid-induced isopentenylpyrophosphate and ATP analog formation in human breast cancer cells. Biochemical Pharmacology 79 (5), 777–783.