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REVIEW

The effect of statins on cancer cells—review

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Abstract Statins [3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase, abbreviated HMGCR) inhibitors], are well-known cholesterol-depleting agents. Since the early 1990s, it has been known that statins could be successfully used in cancer therapy, but the exact mechanism(s) of statin activity remains unclear and is now an extensive focus of investigation. So far, it was proven that there are several mechanisms that are activated by statins in cancer cells; some of them are leading to cell death. Statins exert different effects depending on cell line, statin concentration, duration of exposure of cells to statins, and the type of statin being used. It was shown that statins may inhibit the cell cycle by influence on both expression and activity of proteins involved in cell-cycle progression such as cyclins, cyclin-dependent kinases (CDK), and/or inhibitors of CDK. Also, statins may induce apoptosis by both intrinsic and extrinsic pathways. Statin treatment may lead to changes in molecular pathways dependent on the EGF receptor, mainly via inhibition of isoprenoid synthesis. By inhibition of the synthesis of cholesterol, statins may destabilize the cell membrane. Moreover, statins may change the arrangement of transporter OATP1, the localization of HMGCR, and could induce conformational changes in GLUT proteins. In this review, we have tried to gather and compare most of the recent outcomes of the research in this field. We have also attempted to explain why hydrophilic statins are less effective than hydrophobic statins. Finally, we have gathered results from in vivo experiments, presenting the use of statins in combined therapies and discussed a number of molecular targets that could serve as biomarkers predisposing to statin therapy.

Keywords Statins · Cancer · Molecular pathways · Membrane rafts

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Introduction

Statins are competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase, abbreviated to HMGCR), the key enzyme in the cholesterol synthesis pathway. The ability of statins to inhibit HMG-CoA reductase stems from a structural similarity between the acidic form of statins and HMG-CoA, the natural substrate of HMGCR. HMGCR catalyzes the conversion of HMG-CoA into mevalonate, the precursor of cholesterol. Inhibition of HMG-CoA reductase leads to a decrease in mevalonate levels and thereby to an increase in the number of low-density lipoprotein (LDL) receptors on the surface of cells and, finally, to an increase in LDL catabolism [1]. Some statins can also inhibit the synthesis of LDL in the liver by preventing synthesis of their precursor, very low-density lipoprotein (VLDL) [2]. Moreover, the clinical data has indicated that some of them (lovastatin, pravastatin, and simvastatin) may increase the level of high-density lipoprotein (HDL). Therefore, statins are a commonly approved and established treatment of cardiovascular diseases.

There are many reports of promising attempts to use statins in the treatment of other diseases, not only cardiovascular diseases, such as rheumatoid arthritis [3], osteoporosis (reviewed in ref. [4]), and cancers. This property could be explained by the fact that the inhibition of the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate by statins is the main cause of not only the reduction of the

cholesterol level in the cell but also the inhibition of isoprenoid synthesis.

Although investigations on statins and their effects on cancer cells have continued from the early 1990s, the exact and full molecular mechanism(s) that could explain the anticancer activity of statins still remain unclear. There are many excellent reviews focused on the use of statins as anticancer drugs. They discuss antitumor potentials and effects of different statins against many cancer cell lines and the risk of cancer development connected with statin therapy. They also present clinical trials of statin use in cancer treatment both as a monotherapy and in combined therapy with other anticancer drugs [5, 6]. In this review, we attempt to gather and compare the most recent research outcomes concerning the effect of statins on cancer cells at the molecular level, and we also try to investigate step-by-step changes in molecular pathways in cancer cells. We also try to determine the influence of statins on the membrane domains of cancer cells and present results from the latest in vivo studies in support of this.

The origin of statins

Currently, there are around eight extensively investigated statins, which can be classified as either natural or synthetic, according to their origin. Natural statins (lovastatin and pravastatin) are secondary metabolites of fungi. They can be obtained from different types and species of filamentous fungi. Lovastatin is a product of fermentation carried out, among others, by *Aspergillus terreus* or *Monascus ruber*. Pravastatin is obtained as a result of the biotransformation of mevastatin (as a secondary metabolite of *Penicillium citrinum*), which is most efficiently carried out by actinomycetes, *Streptomyces carbophilus*, using an enzyme system containing cytochrome P-450 [7]. Among the producers of pravastatin, there are actinomycetes, *Actinomadura*, and, as mentioned above, fungi of the genus *Aspergillus* and *Monascus* [2]. Currently, pravastatin is produced by mutants of *Streptomyces*. Simvastatin is a semisynthetic derivative of lovastatin, generated as part of a process which requires chemical modification of the side chain of lovastatin at position C8. Lovastatin, simvastatin, and pravastatin together represent the first-generation of fungal-derived HMG-CoA reductase inhibitors.

Atorvastatin, cerivastatin, fluvastatin, rosuvastatin, and pitavastatin are fully synthetic statins. Atorvastatin and fluvastatin are obtained synthetically from mevalonate and pyridine, respectively. Cerivastatin, because of its many side effects, was withdrawn from the market in 2001.

Lovastatin and simvastatin are produced as prodrugs, forming a mixture of lactones and β-hydroxy acids. The lactone ring is then converted to the corresponding β-hydroxy acid in vivo, and only this form, through the similarity of its structure to the structure of HMG-CoA, inhibits HMGCR. All other statins are administered in the active form. The affinity of the statin to HMGCR is, however, several orders of magnitude higher than the affinity of the natural substrate. For example, the Michaelis–Menten constant, K_m, for lovastatin is about 6.4×10^{-10} M, while the K_m for the natural substrate is in the order of 4×10^{-6} M.

The structure of statins

All natural statins possess a common core polyketide structure, the hydroxy-hexahydro naphthalene ring system, to which different side chains are attached at positions C8 and C6 (Fig. 1). Lovastatin contains a methylbutyric side chain group at the C8 position and a 6-α methyl group at the C6 position. Pravastatin has β-hydroxylactone in the form of the 6-hydroxy sodium salt and is a C6-hydroxy analogue of mevastatin. Simvastatin comprises an additional methyl group at the 2′ position of the side chain.

Structures of the synthetic statins are not similar to each other. They also differ from the structures of natural statins. Only the HMG-CoA-like residue, which is responsible for inhibition of HMG-CoA reductase, is common to both groups. Synthetic statins are obtained in the form of the hydroxy acid and share a common fluorophenyl group. Fluvastatin was the first completely synthetic statin on the market, while atorvastatin and cerivastatin are pyridine derivatives. Pitavastatin has a unique cyclopropyl group that protects it from metabolism by cytochrome P450 enzymes, while rosuvastatin has a stable polar methanesulfonamide group that makes this statin hydrophilic [8].

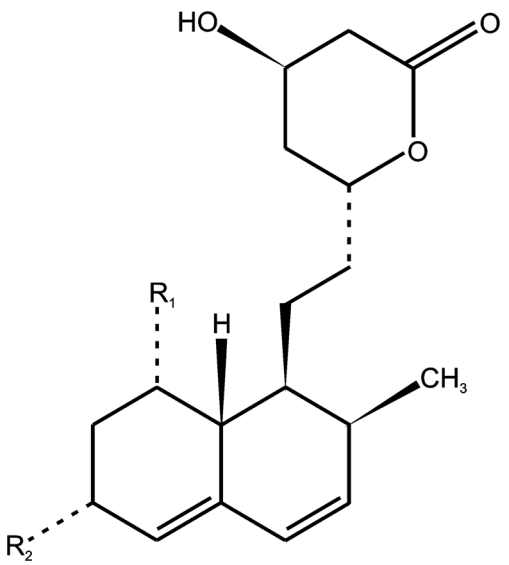

Fig. 1 The base structure of natural statins

The pharmacokinetics of statins

Statin pharmacokinetics vary depending on their hydrophilic or hydrophobic character and the presence, or absence, of suitable membrane transporters. It has been shown that hydrophilic statins, such as pravastatin and rosuvastatin, initially accumulate in the liver where they are a subject to uptake by hepatocyte-specific membrane transporters, especially organic anion-transporting polypeptide OATP1B1 [8–10]. OATP1B1 is also the most important transporter in the hepatic uptake of hydrophobic pitavastatin. Other hydrophobic statins, unlike the hydrophilic ones, are distributed to various tissues [10, 11]. Hydrophobic lovastatin, simvastatin, atorvastatin, and cerivastatin are metabolized mainly by the enzyme system containing cytochrome P-450 3A4, while fluvastatin uses a pathway involving cytochrome P-450 2C9 [11]. Hydrophilic pravastatin and rosuvastatin, as well as pitavastatin, undergo little metabolism via cytochromes and are excreted mainly unchanged [8].

Studies using pairs of cancer cells of different epithelial origin, including breast (MCF7 and SKBr-3), prostate (LNCaP and PC-3), colon (Caco-2 and HCT-116), skin (SCC-M7 and SCC-P9), and lung cancer (Calu-3 and Calu-6) cell lines, indicate that the inhibition of cell growth caused by simvastatin is better in the less differentiated cells, compared to the more differentiated cancer cells. Poorly differentiated cancer cells do not resemble normal cells and may lack normal tissue structures. Such tumors grow and spread faster than well-differentiated tumors. Furthermore, prolonged exposure of cancer cells to simvastatin causes more extensive cell death in poorly differentiated cancer cell lines [10]. Other studies confirm that the cytostatic and cytotoxic effects of statins are much more pronounced in malignant, highly metastatic tumor cells compared to benign, low metastatic tumors of the same origin. This feature is explained by the fact that cells of malignant tumors that are spreading faster show a greater demand for isoprenoids arising from mevalonate, probably to improve pro-survival signaling in cells [12].

Studies have shown that the most pronounced inhibitory effect on cell proliferation is observed with cerivastatin, followed by lovastatin, fluvastatin, simvastatin, and atorvastatin, and, finally, pravastatin [13, 14]. This is confirmed by a comparative study on the effect of hydrophilic pravastatin and hydrophobic simvastatin which showed that simvastatin has a higher cytotoxic potential than pravastatin [10]. On the other hand, comparing the effectiveness of different inducers of apoptosis led to the conclusion that lovastatin is much more effective than other known inducers of apoptosis, such as cycloheximide, etoposide, or serum starvation [15].

In vitro studies on cell lines have shown that the anticancer effect of statins is based mainly on the inhibition of proliferation and induction of apoptosis in cancer cells such as various leukemia cells [14], lymphoma cells [16, 17], and solid tumor cells of different origins [13, 18–20].

The effect of statins on the cell cycle

In vitro studies have shown that statins arrest cells in G1 [13, 17–19, 21] or S phase [22] by affecting cell-cycle regulatory proteins. Their effect is time and dose dependent. However, the effect of statins on the cell cycle depends on many factors, and there are many different mechanisms used by statins to arrest cells in a particular phase (Fig. 2).

Experiments performed on a multiple myeloma cell line (nonsolid tumor) show that simvastatin has the ability to retain such cells in the S phase. The arrest of cells in the S phase is caused by the increased phosphorylation and, therefore, activation of Chk1 kinase, but no changes in the level of the protein kinase was observed. Also, a decrease in the level and activity of Cdc25 phosphatase and a reduction in the level of cyclin A and CDK2 expression were observed. These effects are dependent on the concentration of simvastatin. Although silencing of Chk1 after simvastatin treatment unlocked cell arrest in the S phase, it enhanced simvastatin-induced downregulation of Bcl-2, caspase 9 cleavage, and subsequent apoptosis [22]. However, since the activity of Chk1 and Cdc25 may be associated with the activity of caspases, further studies are required in this direction.

Another mechanism was demonstrated after performing experiments with another fungal-derived statin, lovastatin. It was shown that lovastatin at a concentration of 5 μM [21] induces breast cancer cells to arrest in the G1 phase by inhibiting the proteasome which, in turn, leads to the accumulation of inhibitors of cyclin-dependent kinases (CKI), such as $p21^{WAF1/CIP1}$ and p27. Lovastatin may exist in two forms, which have two independent effects, either as a prodrug, comprising a β-lactone ring, which is responsible for the inhibition of the proteasome and thus inhibition of $p21^{WAF1/CIP1}$ and p27 degradation, while it does not inhibit the activity of HMG-CoA reductase, or in the form of an open ring, which inhibits HMG-CoA reductase. It has been proven that mevalonate restored and increased the activity of proteasome, which caused degradation of CKI and facilitate entry into the next phase of the cell cycle.

More recent in vitro and in vivo studies have confirmed that lovastatin reduces the proliferative capacity of breast cancer cells (MCF-7 cell line). Additionally, in MCF-7 cells which were transfected with the *BRCA1* gene, even more significant decrease in the proliferative ability was observed after lovastatin treatment in comparison to nontransfected MCF-7 cancer cells. It was demonstrated that the reduction in proliferation occurred via downregulation of expression of cyclin D1, cyclin-dependent kinase 4 (CDK4), retinoblastoma protein (pRb), and upregulation of $p21^{WAF1/CIP1}$ [23].

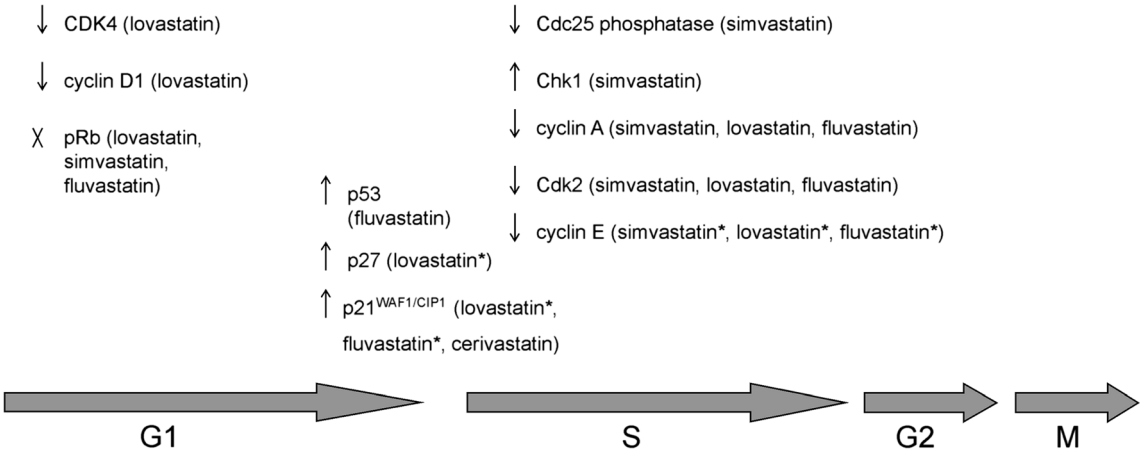

Fig. 2 Cell cycle genes/proteins which expression/activation is disrupted by statins. *Asterisks* (*) indicate not in prostate cancer cells, *arrows pointing down* (↓) indicate decrease, and *arrows pointing up* (↑) indicate increase. *X* inhibition

A similar mechanism was observed in the case of kidney cancer and breast cancer cells, where the synthetic statins, fluvastatin and cerivastatin, arrested cells in the G1 phase by upregulation of $p21^{WAF1/CIP1}$ and p53 [18, 19]. The authors of these studies point to the very important fact that $p21^{WAF1/CIP1}$ is inhibited by prenylated, membrane-bound RhoA. In cerivastatin-treated cells, there is a significantly reduced level of membrane-bound RhoA, and this protein is translocated to the cytosol, which decreases the inhibition of $p21^{WAF1/CIP1}$.

On the other hand, in the case of prostate cancer cells (LNCaP and PC cell lines), cell-cycle arrest in the G1 phase, after treatment with simvastatin, lovastatin, and synthetic statin, fluvastatin seems to occur via inhibition of cyclin E/Cdk2 kinase complex [13] but is independent of $p21^{WAF1/CIP1}$ and p27. There is evidence that cell proliferation in prostate cancer cells is arrested by inhibition of cyclin E/Cdk2 activity at a step after the assembly of the kinase, but before activation, through phosphorylation on T160 of Cdk2. The exact mechanism still remains to be established.

The results described above indicate that statins can inhibit proliferation of cells by modulation of both expression and activity of many proteins involved in cell-cycle progression. Among those proteins are cyclins, cyclin-dependent kinases, and inhibitors of CDK.

Statins and apoptosis

Numerous in vitro experimental data confirmed that incubation of cells with statins leads not only to aforementioned effects on cell-cycle arrest but also to cell death, via apoptosis, especially when exposure of cells to statins is prolonged. It was shown that after statins treatment, almost all known molecular mechanisms of apoptosis become activated (Fig. 3).

Spampanato et al. [20] demonstrated that simvastatin administered at concentrations as low as 20 μM, after exposure between 24 and 72 h, induced malignant cells to undergo apoptosis. In their work, the authors studied different cancer cell lines, breast, liver, stomach, and lungs, and demonstrated that administration of simvastatin results in all of the significant features of apoptotic cells: fragmentation of DNA, decrease in the expression level of *Bcl-2*, and increase in the expression of *Bax*, both at the level of transcripts and proteins. What is worth emphasizing is that normal fibroblasts, which were used as a control in the cited study, were not sensitive to simvastatin and, even after 72 h of simvastatin treatment, did not exhibit the characteristics of apoptotic cells. Also, studies using lovastatin, performed on breast cancer cells, have shown that after statin treatment, clear translocation of the Bax protein to mitochondria (cells lacking Bax were resistant to apoptosis induced by simvastatin), and release of cytochrome c into the cytosol was noted [24]. Induction of both cytochrome c release and PARP cleavage after lovastatin treatment was shown also in LNCaP and HL60 cells (promyelocytic leukemia cells) [25].

Detailed complementary mechanisms of apoptosis in cells exposed to statins were proposed after a series of experiments were conducted using nonsolid tumor cells such as T lymphocyte (Jurkat T), lymphocyte B, myeloma [14], and lymphoma cell lines [16]. Firstly, after statin treatment in Jurkat T and myeloma cells, a reduction of mitochondrial membrane potential and release of the second mitochondria-derived activator of caspases, Smac/DIABLO along with cytochrome c into the cytosol in the intrinsic apoptosis pathway was observed. Smac/DIABLO directly interacts with IAP proteins by blocking their inhibitory effects on activation of both caspases 9 and 3. Indeed, after administration of statins, an increase in both caspase 3 [22, 26, 27] and caspase 9 activity was observed (see Fig. 3). There was also a clear increase in the activity of caspase-8, which amplifies the death signals. The effect of simvastatin on caspase 3 activity was reversed by incubation of cells with either mevalonate or geranylgeranyl

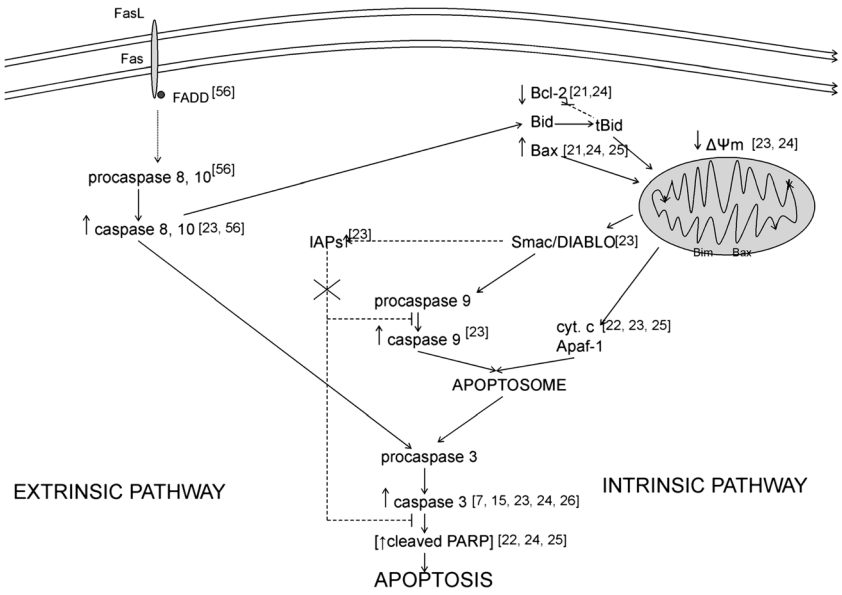

Fig. 3 Apoptosis pathways affected by statins. The *arrows pointing down* (↓) indicate decreased activity, while the *arrows pointing up* (↑) indicate increased activity. *Numbers in parentheses* indicate references from the reference list. *X,* inhibition of activity

pyrophosphate (GGPP) [26]. No effect was observed by addition of squalene to the medium of statin-treated cells, indicating that the restoration of cholesterol level did not inhibit apoptosis [14].

Secondly, recent experiments with lymphoma cells have shown that the synthetic statin, fluvastatin [16], at concentrations of 5 and 10 μM for 24 h caused chromatin condensation, DNA fragmentation, and formation of apoptotic bodies. The same studies indicated that fluvastatin treatment could lead to a dose-dependent decrease in the mitochondrial membrane potential and increase in the activation of caspase 3. As a significant increase in the level of cleaved PARP (the substrate of caspase 3) was observed after fluvastatin treatment, it is very likely that the decrease in membrane potential is accompanied by a release of cytochrome c into the cytosol, which could lead to apoptosis. In addition, it was shown that in fluvastatin-treated cells, the level of Bax proteins was increased while the level of Bcl-2 proteins was decreased (Fig. 3) [16].

In addition, studies performed on LNCaP, HL60 [25], and cholangiocarcinoma cell lines [27] have shown that after statin treatment, there is proteolytic activation of another caspase that is involved in the execution of apoptosis, namely, caspase 7. Activation of caspase 7 occurs probably by caspase 8 or 10 [25]. Furthermore, it was shown that in cholangiocarcinoma cells [27] apoptosis is closely associated with a reduction in the level of cholesterol, disruption of Rac-1 localization in cell membrane (in particular, membrane rafts), and inhibition of the Rac1 activity. Interestingly, simvastatin had no effect on caspase activity in normal human cholangiocytes.

Another study has shown that statins, by interruption of the synthesis of cholesterol and then by changing the organization of cholesterol-rich membrane rafts, may trigger the activation of the signaling pathway induced by Fas (CD95) [28]. The study was performed on HaCaT cells (keratinocytes) and showed that disruption of lipid rafts by administration of mevastatin led to a spontaneous, ligand-independent clustering of Fas in the nonraft compartment of the plasma membrane, along with the formation of Fas–FADD complexes, activation of caspase-8, and apoptosis.

In addition to the abovementioned biochemical changes, numerous studies have also focused on changes in phenotype of cells treated with statins. Simvastatin reduces the ability of cancer cells to form colonies in vitro [26], and in highly sensitive cell lines (such as PC-3 and Panc 28), simvastatin at a concentration as low as 10 μM causes significant changes in cell shape, which is associated with redistribution of mitochondria—they aggregate into perinuclear deposits [10].

All the data presented above indicate that statins may cause cell death via apoptosis not only by activation of intercellular mechanisms but probably also by inhibition of cholesterol synthesis and prenylation of G proteins.

Molecular pathways affected by statins

Studies that have been carried out since the 1990s on the effects of statins on cancer cells have prompted researchers to pay attention to the molecular mechanisms leading to changes in cell functions and, consequently, their apoptosis (Fig. 4).

It is well known that statins that inhibit conversion of HMG-CoA into mevalonate inhibit not only cholesterol but also isoprenoid synthesis. The isoprenoids, such as farnesyl pyrophosphate (FPP) and GGPP, are a group of essential components involved in the posttranslational modification (prenylation) of many intracellular signaling proteins, such as nuclear lamins, transducin γ, rhodopsin kinase, and, in

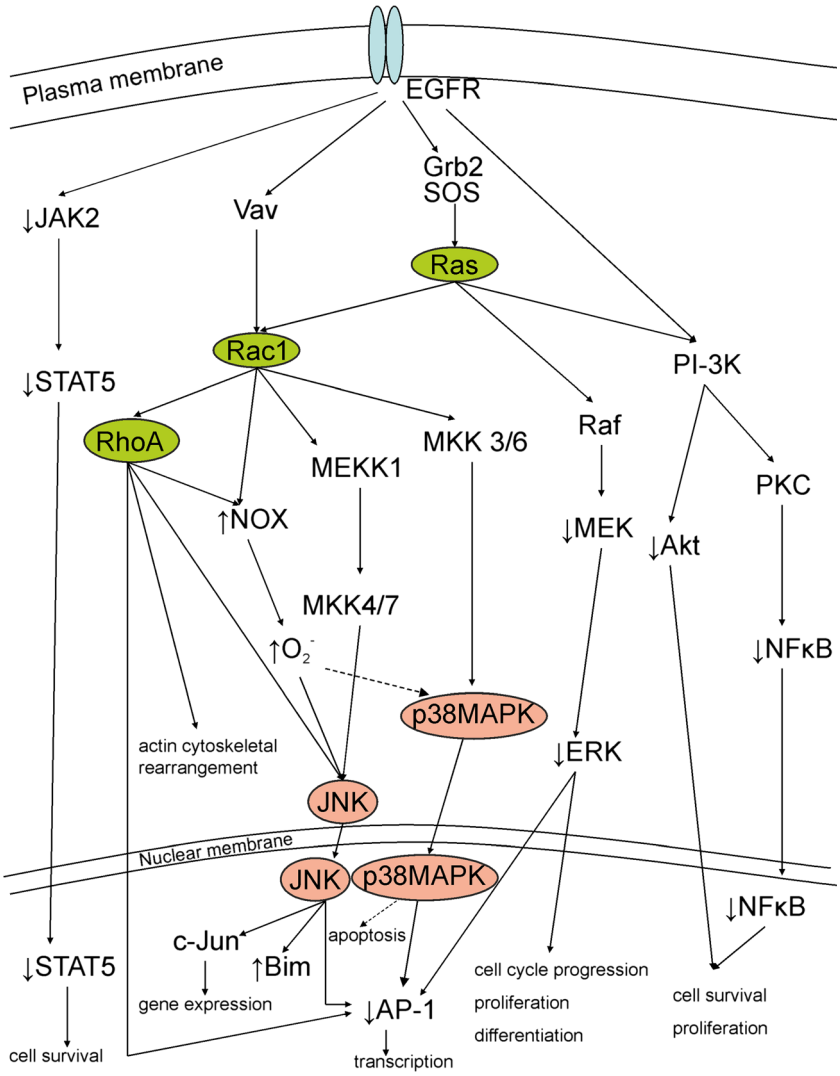

Fig. 4 Molecular pathways affected by statins in cancer cells. In *red* are kinases which activity can be either increased or decreased. In *light green* are cytosolic forms of proteins. *Arrows pointing up* (↑) indicate activation, while the *arrows pointing down* (↓) indicate inhibition

particular, the G proteins, among other Ras proteins (which could be farnesylated), and the Rho family of proteins (which could be geranylgeranylated).

FPP and GGPP are necessary for both attachments of small GTP-binding proteins of the GTPase family to the cell membrane and for their biological activity. In cell signal transduction, these proteins, such as Ras and RhoA, translocate from the cytosol to the membrane after covalent attachment of FPP (Ras) or GGPP (RhoA) [14]. After statin treatment [14, 26], an increase of inactive, nonisoprenylated, cytosolic forms of Ras, Rho, and Rac proteins could be observed. Among molecular pathways affected by statins are those involving phosphoinositide 3-kinase (PI-3K), serine–threonine kinases, nuclear factor kappa-B (NF−κB), and mitogen-activated kinases (MAPKs).

So far, the most extensively studied proteins of the GTPase family are the changes associated with Ras protein activity in cancer cells. Mutations in *Ras* genes that lead to loss of the intrinsic GTPase activity have been observed in approximately 20–30 % of human cancers. These mutations result in the constant activation of Ras that, consequently, leads to uncontrolled proliferation of cells. It was shown that inhibition of farnesylation of mutated (constantly active) Ras prevents its activity in cancer cells. In this case, inhibition of farnesylation seems to offer a promising way to impede the progression of cancer [29]. The participation of statins in the inhibition of prenylation has been confirmed by many investigations [14, 19, 26, 27]. The inhibition can be abolished by introducing external isoprenoids (see Fig. 4).

It was shown that the synthetic statin, cerivastatin, inhibited both proliferation and invasiveness of tumor cells in a dose-dependent way particularly of highly invasive tumor cell lines. Studies on the highly invasive breast cancer cell line, MDA MB 231 (a cell line with overexpression of RhoA and constitutive activation of Ras) [19], have demonstrated that inhibition of proliferation in those cells is caused mainly by inhibition of the signaling pathways activated by RhoA. The effect

was abolished after administration of GGPP, but not FPP, indicating that usually, farnesylated Ras protein plays only a minor role in the process. Two mechanisms that may be responsible for the inhibition of invasiveness caused by cerivastatin have been proposed. The first is based on the fact that in cells that are not exposed to statins, membrane-bound, prenylated RhoA is connected to caveolae-enriched membrane domains [19]. Since it is known that RhoA is involved in the regulation of actin filaments and in the formation of the focal adhesion complex, the impact of cerivastatin on these functions of RhoA was investigated. Cerivastatin treatment caused changes in cell shape with only a few organized actin filaments, the loss of focal adhesion points, and a general decrease in motility of statin-treated cells. These changes were accompanied by significant reduction of the membrane-bound RhoA. Secondly [19], it is suggested that cerivastatin was responsible for inactivation of NFκB factor in MDA MB 231 cell line, exhibiting its overexpression. In cells treated with cerivastatin, a decrease in NFκB DNA-binding activity was shown. Moreover, after statin treatment, large amounts of the RelA subunit were found in the cytoplasm. The inhibitory effect of cerivastatin on NFκB was prevented by coincubation with GGPP. Therefore, it is suggested that inhibition of NFκB is related to RhoA inhibition. NFκB is involved in the regulation of expression of tissue factor (TF), urokinase-type plasminogen (u-PA), and metalloproteinase 9 (MMP9). After cerivastatin treatment, u-PA and MMP9 expression levels were reduced, and inhibition of TF protease was observed. TF activity is regulated by AP-1 factor, and AP-1 is regulated by RhoA. Prolonged exposure of the MDA MB 231 cells to high concentrations of cerivastatin leads to the loss of the ability of cells to adhere to the surface, although it does not lead to induction of cell death by apoptosis. It was observed that the efficiency of cerivastatin was significantly lower in the weak invasive MCF7 cell line, which is not characterized by overexpression of RhoA. The relationship between RhoA activity and statin effect was also confirmed for other statins and cancer cell lines.

Experiments performed on renal cancer cells showed that after exposition of cells to another synthetic statin, fluvastatin, there was a significant decrease in the phosphorylation of another member of the Rho-like GTPase family, Rac1, although total Rac1 expression levels did not change [18]. It has been reported that Rac1 mediates the distinct actin cytoskeleton changes required for cell invasion, and sustained activation of Rac1 is correlated with invasion and metastasis [30]. Indeed, fluvastatin significantly inhibited the in vitro invasive activity of Renca cells. Studies on cholangiocarcinoma cells revealed that simvastatin, apart from a reduction in cell viability and induction of apoptosis, decreases total cellular cholesterol, disrupts membrane rafts, and significantly inhibits Rac1 activity in cancer cell lines. Rac1 activity is dependent upon its localization in membrane rafts. It has been shown that cholangiocarcinoma cells treated with simvastatin exhibited a lack of membrane raft localization of Rac1. This effect was reversed by cholesterol treatment. What is interesting is that in normal human cholangiocytes, simvastatin also inhibited cell proliferation and reduced the level of cholesterol, but it did not lead to apoptosis and had no effect on Rac1 activity [31]. It is suggested that normal cells do not proliferate as fast as cancer cells and that this is the reason they are not as sensitive to the effects of statins as cancer cells.

Another study, using simvastatin, performed on colorectal cancer cells [26] confirmed that simvastatin could induce apoptosis as a result of inhibition of geranylgeranylation and resulted in an increase in the amount of cytosolic forms both RhoA and Rac1. Surprisingly, it was shown that simvastatin significantly increases both the level of GTP binding by RhoA, Rac1, and Cdc42, and the total cellular level of RhoA and Cdc42. There is an increased expression of the aforementioned proteins, and the level of bound GTP is effectively blocked by preincubation of cells with GGPP (to a lower extent by FPP). After simvastatin treatment, cytosolic, unprenylated RhoA-GTP, and Rac1-GTP retain at least part of their functional activities and are likely to stimulate NADPH oxidase complex (NOX) and increase the production of intracellular superoxide signaling molecule involved in the execution of apoptosis. Increased concentration of superoxide in the cell as well as signaling by Rho GTPases leads to the activation of the JNK pathway and increased levels of proapoptotic Bim protein, isoform of Bcl-2. Activation of the JNK pathway is extremely important, as some reports have shown that activation of JNK may increase the sensitivity of tumor cells to chemotherapy and radiotherapy [32, 33].

The data obtained by another group of researchers [34] showed that lovastatin induces apoptosis in acute myeloid leukemia (AML) cells by inhibiting Raf-1 [35] proto-oncogene (Raf)-Mitogen/Extracellular signal-regulated Kinase (MEK)-Extracellular-signal-Regulated Kinase (ERK) signaling pathway. Similarly, in osteosarcoma cells, statin-induced apoptosis was associated with inhibition of ERK and reduced expression level of *Bcl-2*, which was induced by inhibition of RhoA geranylgeranylation [36]. These results are of great importance as it is known that ERK activation usually promotes survival and is essential for carcinogenesis.

Very interesting outcomes have been revealed by experiments performed using three breast cancer cell lines of different phenotypes: MCF7 (activation of esterogen receptor), SKBr3 (activation of ErbB2/HER2), and MDA MB 231 (mutated Ras) [37]. All of these three cell lines display different activation of the NF-κB transcription factor complex, which is thought to be continuously active in many types of cancer cells and protects them from apoptosis; MCF7 has the lowest, and MDA MB 231 the highest level of activation of NF-κB. First of all, it was demonstrated that cell lines with activated Ras or ErbB2 pathways are more sensitive to statins, and their overall pattern of statin responsiveness correlate with the

endogenous level of activated NF-κB (the higher the activation of NF-κB, the higher the sensitivity to the statins). After statin treatment, all of the three cell lines showed a decline in the phosphorylated form of MEK1/2, an intermediate effector of the Ras/Raf/MEK/ERK pathway. Moreover, after 12-h statin exposure, all cells showed declined activation of NF-κB, which correlated with a significant increase in the level of the phosphorylated form of IκBα, the NF-κB inhibitor, and a slight decline in cyclin D1 protein levels. After 48 h, a decrease in AP-1 levels, as well as the phosphorylated forms of various MAP kinase pathway proteins (ERK1/2, JNK, p38), was observed. There is also evidence of increased levels of p21. Surprisingly, in the SKBr3 cell line, levels of phosphorylated form of Akt (protein kinase B) did not show a change following statin exposure, in contrast to the response observed with many cancer cell lines (see below). Akt kinase is one of the most frequently activated signaling molecules in cancers; its activation is very common in prostate cancers, among others, and is related to enhanced cell survival.

Others [38] have demonstrated that the administration of simvastatin inhibits phosphorylation and activity of Akt in prostate cancer cells and also inhibits proliferation, migration, and invasion in these cells and induces apoptosis. Inhibition of invasion of PC3 cells by simvastatin was greater in cells stimulated with epidermal growth factor (EGF) compared with nonstimulated cells. These effects are time- and dose-dependent. On the other hand, the same study has shown that prostate cancer cells with expression of constitutively active Akt (myrAkt) are resistant to simvastatin-mediated inhibition of prostate cancer cell functions. Studies on mice that had engrafted prostate cancer cells (PC-3 cell line) indicate that the effectiveness of simvastatin strongly depends not only on the dose but also on the frequency of administration and so is effectively dependent upon its actual concentration. Administration of simvastatin at a concentration of 2 mg/kg body weight/day for 14 days did not lead to any substantial effect, while increasing the dose to two administrations daily resulted in a significant reduction in tumor size and also a significant decrease in the level of p-Akt and expression of PSA in mice. These experiments led to the conclusion that in prostate cancer cells, simvastatin treatment could significantly inhibit cell migration and invasiveness caused by EGF stimulation, the ability of colony formation (possibly by inhibition of Akt), and proliferation in vitro as well as tumor growth in vivo.

On the other hand, studies carried out on lymphoma cells showed that fluvastatin suppressed the activation of both the Akt and Erk pathways in a time-dependent manner [16]. Besides, fluvastatin markedly increased phosphorylation of p38 MAPK, a MAP kinase that in response to oxidative stress, for example, leads to apoptosis. These effects were reversible by the addition of mevalonate, FPP, and GGPP. Moreover, the treatment of lymphoma cells with fluvastatin significantly increased intracellular ROS generation before apoptosis. In contrast, the administration of fluvastatin in the presence of the thiol antioxidant, *N*-acetylcysteine (NAC), inhibited the cytotoxic effect. These results suggest that there is a potential involvement of oxidative stress in the cytotoxic action of fluvastatin. To be more precise, it is known that cancer cells produce higher levels of ROS than normal cells, as they possess a specific antioxidant defense system, which eliminates ROS. There is a hypothesis that statins cause a breakdown of the antioxidant defense system, thereby increasing intracellular oxidative stress and, in this way, exerting at least a part of their cytotoxic effects [16]. The cytotoxic effect may be connected with the fact that statins inhibit biosynthesis of not only cholesterol, GGPP, and FPP but also the electron transport chain intermediates CoQ10, dolichol, heme A, and ubiquinone that are related to antioxidant status. A deficit of these compounds might cause oxidative stress and lead to cell death [39, 40].

Another mechanism that could be complementary to those presented above has been presented recently. Song et al. [17] demonstrated that after exposing Daudi cells (human Burkitt's lymphoma cell line) to lovastatin, there was a substantial inhibition of proliferation and decrease in intracellular ROS level. The authors showed that lovastatin reduces superoxide levels by decreasing the expression of two subunits of NADPH oxidase, p47-phox and gp91-phox, in Daudi cells. Apart from that, it is known that the assembly and activity of NADPH oxidases depend on well-functioning membrane rafts, which can be disrupted by statins. These effects are accompanied by inhibition of TRPC6 (transient receptor potential canonical channel 6) expression/activity in cancer cells by lovastatin. TRPC6 is overexpressed in cervical and breast cancers and plays an important role in malignant cell proliferation in a variety of cancers. In Daudi cells, TRPC6 was shown to mediate the effects of cholesterol on cell proliferation. Reduced expression of this receptor leads to a decrease in intracellular Ca^{2+} level.

Apart from the roles of statins in interfering with signaling pathways that have already been discussed, the effects of statins on other signaling pathways are currently under investigations. One of these pathways is the JAK/STAT pathway. This can be stimulated via growth hormone (GH), which induces growth, mitogenesis, and proliferation in various tissues and cell types by activation mainly of JAK2 along with both isoforms of STAT5, A and B. Recently, simvastatin treatment of UMR-106 (rat osteosarcoma) cells stimulated by GH led to a reduction in STAT5 serine phosphorylation and a decrease in transcriptional activity of STAT5 as well as induction of *SOCS-3* and *CIS* expression. All these effects depend on the dose and time of exposure of UMR-106 cells to simvastatin [41]. It is suggested that decreased JAK2 phosphorylation following simvastatin treatment might be associated with changes in membrane lipid raft composition.

The data presented above indicate that statins may interfere with many signaling pathways, not only those in which prenylated proteins are involved. Statins may also generate oxidative stress in cells and, in this way, lead to apoptosis.

Statins and membrane domains

It is worth noting that changes in almost all of the abovementioned molecular pathways after statin treatment are connected with the disruption of membrane rafts. Many receptors for growth factors, such as the EGF receptor, may be localized within membrane rafts. Inhibition of signal transduction involving epidermal growth factor receptor (EGFR) may be a very important function of statins as anticancer drugs.

There are some discrepancies in the case of membrane domain terminology within the literature. In this review, plasma membrane microdomains are considered as caveolae-enriched membrane domains and lipid rafts enriched in cholesterol. For the purpose of this text, we have accepted the terminology used by the authors of the particular publication.

Influence of statins on caveolin-1 and cofilin

Menter et al. [10], studying the time dependence of the simvastatin effect, observed a biphasic response of cancer cells. The first phase, 6–24 h after drug administration, is characterized by changes in cell morphology and arrest of cell growth (among others, via inhibition of isoprenylation of G proteins), and the second phase, after 24–72 h, is associated with a significant reduction in the level of cholesterol, which leads to a reduction in the content of membrane rafts in the cell membrane, inhibition of the phosphorylation of caveolin-1 (Cav-1), disruption of caveolae, and loss of membrane integrity. Cav-1 is an integral membrane protein. It binds and transports cholesterol, and hence, it increases the ordering of lipids in membrane domains. It is believed that the phosphorylated form of Cav-1 might promote cell survival. In tumors, Cav-1 expression correlates with increased cell survival and drug resistance via different mechanisms. Although direct Cav-1 regulation of EGFR function is signaling pathway dependent, in the majority of cases, Cav-1 prevents activation of EGFR [42]. Cav-1 binds to and regulates many structural and signaling proteins, among others; it prevents activation of ERK1/2 and Ras GTPase [42]) and maintains Akt kinase in the activated state in PC cells [42, 43]). However, further work is required in this field.

The role of Cav-1 in maintaining stability of the cell membrane is associated with the ability of the phosphorylated form of the protein to form dimers that interact with actin filaments. Administration of statins results in the inhibition of phosphorylation of caveolin-1 and in the displacement of RhoA from the plasma membrane to the cytosol, which leads to all of the above consequences and which collectively leads to disorganization of actin fibers and failure of the formation of focal adhesions.

Cav-1 plays another very important role in the cell. It is an upstream regulator of fatty acid synthase (FASN), the main producer of intracellular palmitate, which is significantly upregulated in cancer [44]. This feature of Cav-1 is in agreement with the hypothesis that Cav-1, cooperating with FASN, can alter the lipid content of biological membranes [45]. Experiments [46] have shown that these two proteins directly interact, and as a result, FASN is transiently redistributed into membrane domains in response to genetic and pharmacological manipulation of oncogenic signals in PC cells. The level of Cav-1 in PC cells correlates with the level of FASN, and both proteins are quantitatively increased in metastatic tumors in comparison to locally confined tumors. This suggests that Cav-1 upregulation promotes prostate cancer progression and points to the relationship between the expression level of *Cav-1* and the degree of aggressiveness of the tumor [43, 47]. In the light of the abovementioned information, inhibition of phosphorylation of caveolin by statins appears to be a feature of substantial importance.

Statins, by inhibition of prenylation of G proteins, contribute to the formation of clusters of phosphorylated cofilin and thus to the reduction of cofilin–actin interactions. Cofilin is a protein involved in depolymerization of actin filaments. Because the cycle of cofilin activation and inactivation is essential for maintaining cell shape, prolonged exposure of cells to statins not only causes a reduction in the amount of cholesterol in the cell membrane but also induces morphological changes in the cells. A cofilin-mediated mechanism is likely to underlie these changes [10].

Consequences of cholesterol depletion

The most interesting and still not fully understood observation is that of the role of cholesterol in the development of cancer. Evidence exists that several cancer cell types including those most commonly used in statin studies, such as breast cancer and prostate cancer cell lines, have higher membrane cholesterol levels and contain elevated levels of membrane rafts (probably as a result of cholesterol accumulation) than their normal counterparts. These cancer cells are more sensitive to apoptosis induced by cholesterol-depleting agents than normal cells, which have lower membrane cholesterol levels [20, 27, 48, 49]. Furthermore, cholesterol is a major lipid component of rafts and its level in the cell membrane is critical for the formation and maintenance of rafts.

It is known that the integrity of membrane rafts is critical for the correct functioning of cells including regulation of cell proliferation and apoptosis, because many signaling molecules are associated with rafts. It is accepted that rafts serve as molecular platforms which spatially organize appropriate

molecules to facilitate signaling cascades [50]. Studies show that cholesterol-rich membrane rafts have been implicated also in tumor progression and metastasis. One of the most extensively studied was the relationship between cholesterol depletion and EGFR activation. There is no doubt that cholesterol controls EGFR activation, and its depletion causes changes in EGFR localization and/or activation. However, the types of molecular pathways being stimulated and the extent of their activation by growth factors, such as EGF, seem to differ depending on cell type and, more precisely, on the level of cholesterol in the cell membrane. Studies performed using the human epidermal carcinoma cell line A431 that overexpress EGFR demonstrated that cholesterol-depleting drugs cause an increased accumulation of EGFR in the plasma membrane without changing the total level of EGFR and an increase in EGFR dimerization and phosphorylation [51, 52]. These changes were explained by the fact that localization of EGFR to lipid rafts partially suppress the binding of EGF and the kinase functions of the receptor. Cholesterol depletion increases the fluidity of the membrane and hence enhances the possibility of lateral movement of the EGFR and thereby enables phosphorylation of specific sites of the receptor that triggers the activation of downstream signaling protein. On the other hand, it is proposed that in prostate cancer cells, elevated cholesterol levels lead to raft expansion and coalescence, which might potentiate oncogenic pathways of cell signaling [53]. In these cells, cholesterol depletion may cause EGFR detachment from membrane rafts and subsequent disruption of cell signaling.

Indeed, it was proven that in A431 cancer cells, in contrast to prostate cancer cells, cholesterol depletion activates phosphorylation of EGFR and ERK1/2. However, in A431 cells treated with MβCD, there is a clear induction of apoptosis despite EGFR and ERK1/2 activation. The effect is dose dependent. After MβCD treatment, a number of cholesterol depletion effects that include a decrease in the level of membrane rafts, fragmentation, condensation, and segmentation of nuclei, loss of mitochondrial membrane potential, Bcl-xL downregulation (at both protein and mRNA levels), caspase-3 activation, and decrease in Akt phosphorylation without changes in the protein levels were demonstrated [48]. Even EGF stimulation could not reverse cholesterol depletion effects. Cell viability, Akt activation, and replenished rafts on the cell surface could be restored by the addition of cholesterol in the absence of EGF. The role of Akt is underlined in a study which points to the relationship between cholesterol-rich membrane rafts, activation of EGFR, and phosphorylation of Akt [49]. In the study, membrane rafts were isolated from prostate cancer cells after simvastatin treatment and localization of EGFR and p-Akt was determined. It was shown that EGFR was localized outside the raft compartment, but p-Akt, although its level was decreased, was still in the raft compartment. These studies suggest that cholesterol-rich membrane rafts are strongly implicated in both basal Akt activity and EGF-induced Akt activation and that Akt is crucial for cancer cell viability. In contrast to prostate tumor cells, normal prostate epithelial cells also show inhibition in Akt phosphorylation but do not undergo apoptosis caused by a decrease in the level of cholesterol [49].

Similar results were obtained when cancer cells were treated with simvastatin which was able to induce cell death not only by inhibition of lipid modifications of signaling molecules but also through reduction of cholesterol levels as well as disruption or inhibition of raft formation, consequently downregulating Akt activity. This was confirmed by performing experiments on prostate cancer (LNCaP), human epidermoid carcinoma (A431), and breast cancer (MDA MB 231 and MCF7) cell lines [48]. In the case of prostate and breast cancer cell lines, it was confirmed that increased induction of apoptosis caused by cholesterol depletion is related to decreased phosphorylation of Akt kinase [54] as well as decreased Bcl-xL expression via inhibition of NF-κB, derepression of phosphatase and tensin (PTEN) homolog, and subsequent inhibition of PI3 kinase. Cholesterol addition rescued cells from simvastatin-induced cell death along with raft reformation and Akt reactivation.

Hydrophobic vs. hydrophilic statins

Epidemiological studies and meta-analyses on the use of statins and their potential impact on the risk of developing cancer provide inconsistent data, ranging from risk reduction, through no effect, to an increase in the risk of tumor development. The effect that statins exert on cells depends on many factors, primarily on the structure of the statin and its ability to penetrate cell membranes, time of exposure, and statin concentration.

When the level of cholesterol in plasma decreases, there is a compensation system in extra-hepatic cells that results in an increase in the synthesis of mevalonate. Since lipophilic statins, such as simvastatin, passively penetrate through the plasma membrane, including extra-hepatic cell membranes, they inhibit the compensating processes. The situation is different for hydrophilic statins and their effects on compensating processes as extra-hepatic cells do not have OATP1B1 transporter, the key for hydrophilic statins. Therefore, hydrophilic pravastatin does not penetrate through cell membrane and does not cause HMGCR inhibition in those cells. There is a hypothesis that an increase in mevalonate synthesis in extra-hepatic cells may be related to an increased risk of cancer [55]. A number of epidemiological studies seem to be in agreement with this suggestion, pointing to an increased risk of cancer associated with the use of the poorly penetrating cell membrane pravastatin [11], in comparison to patients who are taking other statins.

In a great majority of proliferation and apoptosis experiments, pravastatin has little or no effect on the viability of cell lines, even at concentrations higher than those for other statins [13, 14]. It has been shown that pravastatin at a concentration of about 20 μM, even after 72 h of incubation, has no effect on the viability of cancer cells [10]. The cancer cell lines examined in this study included liver, prostate, lung, breast, colon, bladder, skin, and pancreatic cancer cells, i.e., cells that do not express the OATP1B1 transporter, which explains the weak effect of pravastatin. In healthy hepatocytes, expression of OATP1B1 is high, and they respond to pravastatin treatment almost equally as to simvastatin. These data indicate the important role and interdependence between the activity of pravastatin and the presence of its cellular transporter, OATP1, within the target cell.

It has been shown [10] that both pravastatin and simvastatin administration changes the arrangement of OATP1 (after statin treatment, the OATP1 transporter is distributed mainly in the perinuclear area of the cell) and localization of HMGCR (after statin administration, the reductase is located in areas that resemble endoplasmic reticulum). Although the activity of the HMGCR is regulated by statins, the expression level of this enzyme does not always correlate with the response to statins. The major features of hydrophobic and hydrophylic statins are compared in Table 1.

In addition, it has been shown that cholesterol metabolism is disregulated in many malignancies, as cancer cells usually exhibit constitutively elevated levels and activity of HMG-CoA reductase and low-density lipoprotein receptor, presumably to satisfy their increased need for isoprenoids and cholesterol for new membrane synthesis, potentially making them more sensitive than normal cells to the isoprenoid-depleting effects of statins [48]. But in some cancer types, such as cholangiocarcinoma, expression levels of HMGCR are not significantly different between normal and cancer cell lines [31], which does not reconcile with increased cholesterol levels in cancer cells. However, it has been reported that there is a deficient feedback control of HMG-CoA reductase in some types of tumor [56]. It should be also mentioned that two alternatively spliced isoforms of HMGCR have been identified, namely, the full-length HMGCR and a version that lacks exon 13 [57]. In colorectal cancer, the overall risk of developing this type of cancer is associated with the expression of HMG-CoA reductase lacking exon 13, which suggests that patients expressing this isoform may be unresponsive to statin therapy [58].

Recent studies have shown that apart from all of the effects that statins exert on intracellular mechanisms leading to induction of apoptosis and inhibition of the cell cycle as well as the inhibition of molecular pathways promoting cell survival, statins could also impair glucose uptake in cancer cells. In tumor cells, the expression of the glucose transporter, GLUT-1, is frequently upregulated, as tumor cells require a high glucose supply for their increased metabolic demands. Studies using statins performed on human Burkitt lymphoma, human follicular lymphoma, and human colon adenocarcinoma have shown that statins are not able to inhibit expression of GLUT proteins, but by inhibition of cholesterol synthesis, they could induce conformational changes in GLUT proteins that possess multiple membrane-spanning domains [59]. It was previously shown that cholesterol can associate with a number of membrane proteins via covalent or noncovalent interactions and also induces condensation of membrane lipids and formation of membrane rafts. Depriving cells of cholesterol, for example, using statins, could lead to changes in the activity of these membrane proteins.

Although statins seem to be perfect anticancer drugs, it should not be forgotten that they can cause serious side effects. Predominant are myopathies, rhabdomyolysis, and hepatotoxicity (for reviews, see refs. [60, 61]). There is another problem connected with statin therapy: because of high doses that are required to trigger antitumor effect in humans, statin use as a monotherapy is rather doubtful. Combined therapies of statins with other drugs should be carefully considered, since statins, because of their metabolism, mainly via the cytochrome P450 isoenzyme systems, may interact with many common drugs.

Table 1 Comparison of hydrophilic and hydrophobic statins

	Hydrophilic statins	Hydrophobic statins
Type of statin	Pravastatin and rosuvastatin	Cerivastatin, simvastatin, lovastatin, fluvastatin, atorvastatin, and pitavastatin
Origin	Pravastatin—natural and rosuvastatin—synthetic	Lovastatin—natural; simvastatin—semisynthetic; and cerivastatin, fluvastatin, atorvastatin, pitavastatin—synthetic
Distribution in the organism	Accumulate mainly in the liver (uptake by OATP1B1)	Distributed to various tissues
Metabolism	Pravastatin—sulfation and rosuvastatin—only little metabolism via cytochromes	Metabolized by cytochromes
Cytotoxic potential	Low, in comparison to hydrophobic statins	High
Plasma membrane penetration	Poor; the OATP1B1 transporter is needed	Passively penetrate through the plasma membrane

Studies in vivo

Every year, there are more and more published studies which indicate that the effects of statins shown by in vitro models are reflected in vivo. Apart from the studies mentioned above [38] that revealed that simvastatin treatment can decrease the tumor size in mice that had engrafted prostate cancer cells, many more such examples exist. Favorable effect of statins in vitro that were subsequently confirmed by studies in mice xenografts have also been demonstrated in the case of gastric [62], renal [63], and breast cancer [37, 54], as well as for glioblastoma [64]. For example, it was shown that simvastatin inhibited the growth of the tumors derived from MDA MB 231 human breast cancer cell xenografts in mice [54]. The phosphorylation level of Akt and the level of Bcl_{XL} protein were significantly reduced in tumor samples, but the expression of tumor suppressor protein PTEN was increased. In the case of glioblastoma [64], pitavastatin was shown to inhibit cell proliferation in vitro and to induce cell-cycle arrest and cellular autophagy. In accordance with in vitro studies, pitavastatin administered to mice delayed subcutaneous U87 tumor growth, was more effective when injected intraperitoneally in comparison to oral administration, and was more potent than fluvastatin.

It was demonstrated that statins may also inhibit formation of metastatic lesions. For instance, simvastatin inhibited migration and invasion of lung adenocarcinoma cells in vitro as well as tumor growth and bone metastasis in vivo [65]. In the case of melanoma, although atorvastatin did not inhibit cell growth in vivo, it retained formation of metastatic lesions [66]. Atorvastatin treatment was also shown to reduce cell motility, invasion, proliferation, and colony formation of human head-and-neck squamous cell carcinoma cell lines in vitro. In vivo studies confirmed this result: the reduction in neovascularization and distant lung metastasis was observed in SCID mice to which cancer cells were administrated either via intravenous or subcutaneous injections [67]. It was demonstrated that inhibition of metastasis is most probably caused by inhibition of RhoC geranylgeranylation [66, 67] (overexpression of RhoC is known to occur in many types of invasive carcinomas). Worth emphasising is the fact that in SCID mice treated by atorvastatin, neither weight loss nor toxic effect of the drug was not observed [67].

However, before statins could be considered as therapy for any type of cancer in humans, it is necessary to precisely determine the association of statin use with cancer prevention and/or progression. Thus, of great importance are data analyzed in cohort studies. In such a study, it is crucial to note not only the type of cancer and type of statin being used but also, among other factors, the dose of drug and the duration of treatment.

A cohort study on prostate cancer [68] concluded that the use of statins is not associated with the risk of prostate cancer overall, but it is associated with a reduced risk of advanced (metastatic or fatal) prostate cancer.

In the case of breast cancer, most of the studies emphasize that statin use is associated with a reduced risk of breast cancer recurrence [69, 70], but not reduced breast cancer incidence. In accordance with this observation is the study from Finland [69], which has shown that statins may exert a greater effect on cancer progression vs. initiation. In the study, women were separated into a number of groups referred to as "statin nonusers," "postdiagnostic statin users," and "prediagnostic statin users." It was shown that postdiagnostic statin users and prediagnostic statin users had lower risk of breast cancer death compared to statin nonusers, and all-cause mortality was the lowest in the prediagnostic statin users who did not stop statin therapy after cancer diagnosis. The risk decrease was observed in both localized and metastatic disease at diagnosis. More importantly, it was suggested that the risk decrease remained even in the case of long-term therapy. A study performed in Denmark [71] among women diagnosed with stages I–III breast carcinoma has shown that hydrophilic statin users had approximately the same rate of breast cancer recurrence as nonusers, whereas lipophilic statin users had a reduced rate of recurrence compared with nonusers. Currently, it is proposed to move from conclusions based on observational epidemiology studies to evidences that could be delivered by randomized clinical trials [70].

Statins in combination therapies

There are also many studies in which statins are used in combination with commonly prescribed anticancer drugs, mainly to sensitize cancer cells to the effects of established, anticancer drugs. Of great importance is the fact that studies on the use of statins in combination with other anticancer drugs are much more advanced than studies on statins alone.

A study was carried out to investigate the use of simvastatin in a combination therapy with capecitabine in gastric cancer treatment [62]. It transcribed that simvastatin inhibited proliferation and enhanced apoptotic effect on cancer cells in vitro. Moreover, in a xenograft mouse model, simvastatin alone caused a decrease in tumor growth which was then potentiated by capecitabine treatment. Both in vitro and in vivo analyses demonstrated that molecular mechanisms that may underlie these effects are related to inhibition of expression of genes that are regulated by NF-κB such as cyclin D1, COX-2, Bcl, survivin, and MMP9.

Another study was performed to find novel therapeutic agents that could be used in glioblastoma treatment [72]. A number of 446 FDA-approved drugs were tested, and among them, statins seemed to be promising candidates for therapeutic agents, as they inhibited cell proliferation and induced autophagy in glioblastoma cells in vitro. Further studies have

shown that pitavastatin used in combination with irinotecan, a topoisomerase 1 inhibitor commonly prescribed in cancer treatment, caused a 40- to 70-fold decrease of the IC_{50} of glioblastoma cells compared to irinotecan alone. The most probable explanation for this effect is that statins are able to prevent glycosylation of the multidrug resistance protein (MDR-1), a protein that is overexpressed in glioblastoma, that impairs MDR-1 functionality and allow irinotecan to accumulate intracellulary. In vivo studies confirmed that a combination of irinotecan with pitavastatin inhibits tumor growth. Because pitavastatin use allowed for a reduced dosage of irinotecan, this combined therapy was less toxic and safer in comparison to irinotecan monotherapy.

There is also a randomized phase II study of gefitinib used alone or in combination with simvastatin in patients with advanced nonsmall cell lung cancer (NSCLC) [73]. Gefitinib is an EGFR tyrosine kinase inhibitor that is effective particularly in cancers with mutated EGFR. The study demonstrated that in a nonselected group of patients, there was not a significant difference in response rate and progression-free survival between patients who took gefitinib alone or gefitinib with simvastatin. However, in the exploratory group of patients with wild-type EGFR nonadenocarcinomas, higher response rate, progression-free survival, and overall survival were shown for combined therapy in comparison to gefitinib monotherapy. Worth emphasizing is the fact that the therapy did not lead to any serious adverse effects. All of the above examples suggest that statins should be considered as promising anticancer drugs, if not as single agents then, for sure, as an adjuvant treatment.

Possible biomarkers predisposing to statin therapy

Statin therapy seems a promising future cancer treatment. However, to determine which patients could benefit from statin treatment, researchers are still looking for reliable biomarkers that may be predisposed to statin therapy. A number of such candidates have been proposed. The first one, the most frequently discussed, is HMGCR [70, 72, 74, 75]. Considering the role of HMGCR as a biomarker, a study including 50 women suffering from primary invasive breast cancer should be mentioned [75]. It demonstrated that even short-term administration of atorvastatin at a high dose caused a decrease in proliferation in HMGCR-positive breast cancer.

Higher expression of HMGCR is usually correlated with higher expression of ERα. The status of this receptor in cancer cells could facilitate selection of the group of patients susceptible to statin therapy. Related to HMGCR expression is also the status of mutant p53 in cancer cells. However, it is not clear if this protein could have a predictive value [70]. In 2006, there was a report prepared by the AACR Cancer Prevention Task Force [74] in which a list of molecular targets, possible risk/progression markers, and agents for chemoprevention was published. As possible biomarkers of cancer risk that could predispose to statin therapy, apart from HMGCR, were listed IGF/IGFR and MAPK. All these possible biomarkers should be taken into account before considering statin therapy.

Conclusions

Statins are a promising group of drugs in cancer treatment because of their ability to reduce both cholesterol and isoprenoid levels. Many years of investigations have shown that there is not just one mechanism explaining the anticancer activity of statins. Mechanisms that lead to cell-cycle arrest, induction of apoptosis, or changes in molecular pathways depend on the type of statin being used, the type of cancer cells, dose of statins being used, and time of exposure of cells to statins. Changes in cells that follow statin treatment usually occur according to a common scheme. At first, arrest of cells in the G1- or S-phase is observed. Inhibition of cell-cycle progression involves cyclins, cyclin-dependent kinases, and inhibitors of CDK. At the same time, inhibition of prenylation of G proteins is observed. These proteins are inactivated as signal transducers and cause changes in molecular pathways, leading to the arrest of proliferation and/or induction of apoptosis in cancer cells. In the meantime, a significant decrease in the amount of cholesterol leads to a reduction in the content of membrane rafts in the cell membrane and to further changes in cell signaling, as many signaling molecules are associated with membrane rafts. Finally, depletion of cholesterol leads to loss of membrane integrity.

There are no significant differences between mechanisms of activity of fungal derived and synthetic statins. However, it should be noted that hydrophilic statins are significantly less effective than hydrophobic ones.

Apart from changes in activity of proteins associated with cell survival, statins can also lead to changes in distribution of HMGCR and to inhibition of phosphorylation of caveolin-1. By inhibition of cholesterol synthesis, they could possibly induce conformational changes in GLUT proteins.

Many in vivo experiments have confirmed results from in vitro studies. Additionally, in vivo studies showed that statins may inhibit formation of metastatic lesions. Because of the pleiotropy of intracellular processes they affect, statins are promising anticancer drugs. There are a number of molecular targets that could serve as biomarkers predisposing to statin therapy. Confirming the adequacy of biomarkers may make future statin therapy even more effective. Statins are less toxic than cytostatics, and consequently, precise study of their effects on cancer cells of different types may lead to progress in cancer treatment and may result in reduced side effects that usually occur in anticancer therapies. Cohort studies have led

to the conclusion that statin use is associated with a reduced risk of cancer recurrence though not with an overall risk of cancer, suggesting that statins could be used as a salvage therapy alongside conventional therapeutic treatments. However, before any final conclusions are drawn, randomized clinical trials should be performed. If statins would not be efficient as a monotherapy, there is sufficient evidence to suggest that this group of drugs could be effectively used in combined therapies.

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Conflicts of interest None

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