

Marketed drugs used for the management of hypercholesterolemia as anticancer armament

Panagiota Papanagnou¹
Theodora Stivarou²
Ioannis Papageorgiou¹
Georgios E Papadopoulos³
Anastasios Pappas¹

¹Department of Urology, Agios Savvas Cancer Hospital, Athens, Greece; ²Immunology Laboratory, Immunology Department, Hellenic Pasteur Institute, Athens, Greece; ³Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece

Abstract: The design of novel pharmacologic agents as well as their approval for sale in markets all over the world is a tedious and pricey process. Inevitably, oncologic patients commonly experience unwanted effects of new anticancer drugs, while the acquisition of clinical experience for these drugs is largely based on doctor–patient partnership which is not always effective. The repositioning of marketed non-antineoplastic drugs that hopefully exhibit anticancer properties into the field of oncology is a challenging option that gains ground and attracts preclinical and clinical research in an effort to override all these hindrances and minimize the risk for reduced efficacy and/or personalized toxicity. This review aims to present the anticancer properties of drugs used for the management of hypercholesterolemia. A global view of the antitumorigenicity of all marketed antihypercholesterolemic drugs is of major importance, given that atherosclerosis, which is etiologically linked to hypercholesterolemia, is a leading worldwide cause of morbidity and mortality, while hypercholesterolemia and tumorigenesis are known to be interrelated. In vitro, in vivo and clinical literature data accumulated so far outline the mechanistic basis of the antitumor function of these agents and how they could find application at the clinical setting.

Keywords: antihypercholesterolemic agents, cancer, synergism, repurposing

Introduction

Many pharmacologic agents that are commercially available find application in multiple fields in everyday clinical practice. Characteristically, the chemotherapeutic agent mitomycin C exhibits multilevel therapeutic exploitability functioning, both for killing cancer cells and regulating wound healing.^{1,2} Importantly, a growing body of evidence suggests that several pharmacologic agents could be “repositioned” or “repurposed” in the field of oncology, owing to their interference with signaling pathways which are crucial for tumorigenesis. This notion is experimentally (at the preclinical level, clinical level or even at both levels) corroborated for marketed agents currently used for the management of different pathologic entities ranging from pain,³ gastrointestinal disorders,⁴ depression,⁵ hypercholesterolemia⁶ and diabetes mellitus type II^{7,8} to human immunodeficiency virus^{9,10} and parasitic infection.^{11,12}

Herein, for the first time, we concurrently review the antitumor activity of the widely prescribed marketed antihypercholesterolemic drugs, statins and ezetimibe. Notably, although a concernable amount of theoretical evidence regarding the anticancer activity of statins in different types of malignancies has already been published,^{13,14} to the best of our knowledge, so far, there has been no published work summarizing the anticancer function of both statins and ezetimibe. The fact that hypercholesterolemia is etiologically linked to a leading cause of morbidity and mortality worldwide, that is, atherosclerosis¹⁵

Correspondence: Panagiota Papanagnou
Department of Urology, Agios Savvas
Cancer Hospital, 171 Alexandra's
Avenue, GR-11522, Athens, Greece
Tel +30 210 771 8060
Email panagiota1983rr@yahoo.com

and that high blood cholesterol levels are interrelated with tumorigenesis¹⁶ underlines the impetus for presenting the antitumorigenicity of this category of drugs. Bile acid sequestrants (cholestyramine, colestipol and colesevelam) are also used for the management of hypercholesterolemia. Yet, to the best of our knowledge, no evidence has been provided so far suggesting any anticancer activity for these drugs. The objective of this article is to prompt further basic and/or clinical research regarding the repositioning of the aforementioned or other marketed drugs in cancer therapeutics by presenting the antitumorigenicity of non-vitamin antilipidemic agents that are either synthetic (atorvastatin, fluvastatin, rosuvastatin and ezetimibe) or fermentation derived (lovastatin, simvastatin and pravastatin). Of note, vitamin B3 (niacin), which is known to reduce low-density lipoprotein (LDL)-cholesterol levels,¹⁷ participates to antitumor pathways upon its *in vivo* conversion into nicotinamide.^{18,19} However, only the antitumor functions of non-vitamin antilipidemic are presented here.

Current clinical use of statins and ezetimibe

Statins are LDL-cholesterol-lowering agents. They serve as 5-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, thereby blocking the rate-limiting step in mevalonate metabolic pathway, which is responsible for the biosynthesis of cholesterol and isoprenoids, exemplified by geranylgeranyl pyrophosphate (GGPP).²⁰ Statins are currently commonly prescribed both to prevent the onset of cardiovascular disease (CVD) as well as to patients who already suffer from CVD, since in the latter case, they reduce the mortality rates.²¹

Like statins, ezetimibe is an LDL-cholesterol-lowering drug. Yet, it works through a different mechanism to prevent the exogenous uptake of cholesterol as well as its biliary absorption. Hence, until now, ezetimibe finds clinical application as an antihypercholesterolemic agent.^{22,23} It is used as a second-line option in hypercholesterolemic individuals who do not satisfactorily respond to statin therapy, either alone or in combination with a statin.

Possible therapeutic repurposing

Overview

Hypercholesterolemia has been associated with accelerated tumor growth,¹⁶ while statins interfere with the pathways modulating molecules that are crucial for tumorigenesis; the fact that prenylation of the cancer-associated G-protein superfamily members (eg, RAS isoforms and RhoA) is required for their activation²⁰ has instigated researchers to explore

the antitumor activity of statins. Although there are many reports indicating that the anticancer function(s) of statins can be reversed by mevalonate pathway intermediates,^{24,25} it becomes increasingly clear that statins may actually employ far more sophisticated mechanism of action to affect tumor biology.^{26–30}

In striking contrast to statins which have long been known to target oncoproteins (eg, RAS), ezetimibe targets a molecule that is not an oncoprotein. In fact, ezetimibe blocks the intestinal sterol influx transporter Niemann–Pick C1-Like 1 (NPC1L1).²² To the best of our knowledge, however, no evidence has been provided that ezetimibe's anticancer mechanism of action is NPC1L1-dependent. As it is presented immediately below, ezetimibe's function goes beyond NPC1L1 regulation to interfere with tumor microvascularization,²² as well. These data underline the fact that the pharmacologic agents that either target the oncoproteins or interfere with different pathways that involve cancer-associated proteins merit research interest as possible anticancer drugs.

Preclinical data

Simvastatin, pravastatin and rosuvastatin

Simvastatin exerts antiproliferative effects on non-small cell lung cancer (NSCLC) A549 cells by impairing the activation of ERK.^{25,31} These effects are counteracted by fueling the mevalonate pathway with the terpenoid precursor, farnesyl pyrophosphate.²⁵ Downregulation of transforming growth factor- β receptor II (TGF- β RII) has also been documented as a molecular event that is associated with simvastatin-dependent inhibition of ERK.²⁵ This is of major importance, since deregulated TGF- β signaling is a well-known culprit in lung carcinogenesis.³² Other mechanism linked to the simvastatin-mediated deterioration of NSCLC cell viability is the pro-oxidant activity of this drug³³ as well as its ability to decrease the expression of antiapoptotic proteins (XIAP, Bcl-2) and to stimulate the expression of proapoptotic ones, including Bax and p53.^{31,34} Moreover, simvastatin acts in a cytostatic fashion through negating the expression of cell cycle-regulatory molecules (cyclin D1, cyclin-dependent kinase 4 [CDK4]) governing the G1/S phase transition in NSCLC A549 and NCI-H460 cells.^{34,35} Importantly, experiments indicate that treatment of different cancer cell lines with simvastatin regulates Bcl-2/Bax expression and triggers apoptosis, but leaves the human normal small airway epithelial cells unaffected.³⁶

In highly metastatic triple-negative MDA-MB-231 breast cancer cells, simvastatin functions in an antigrowth manner

to inhibit NF κ B/PTEN- and NF κ B/Bcl_{XL}-dependent routes.³⁷ Also, the metastatic potency of these cells is antagonized by simvastatin or lovastatin that upregulate the expression of p53 which, in turn, represses the expression of the adhesion molecule, CD44.³⁸ Another mechanism by which simvastatin decreases the migratory and invasive potential of these cells is by interfering with a Rho GTPase/Hippo-YAP pathway, which modulates the expression of the oncogenic protein CD168 (hyaluronan-mediated motility receptor) whose gene promoter is responsive to the transcription coactivator YAP and TEAD family transcription factors.³⁹ In breast cancer cells which are positive for HER2, simvastatin seems to be a promising anticancer agent that shuts off the expression of HER2 through upregulating its negative transcriptional modulator, namely, PEA3.²⁴ In MCF-7 breast cancer cells (these cells are ER-positive and HER2-negative), simvastatin and fluvastatin stimulate apoptosis through positively modulating the expression of inducible nitric oxide synthase (iNOS) at the transcriptional level.⁴⁰ In ER-negative breast cancer cells, simvastatin displays synergistic antigrowth function with the cardiac glycoside, digitoxin.⁴¹

Experimental evidence pinpoints to an interrelation between the retinoblastoma protein (RB) status and the tamoxifen resistance phenotype. In fact, analyses have shown that in a molecular background where the tumor-suppressive retinoblastoma protein (pRb) axis is dysfunctional (eg, in SaOS2 osteosarcoma cells), the interference with the assembly of the minichromosome maintenance (MCM) complex and DNA replication causes more extensive DNA damages than in the presence of functional pRb (eg, in U2OS osteosarcoma cells).⁴² Besides, premenopausal ER-positive breast cancer patients carrying a functional pRb are benefited from adjuvant tamoxifen treatment, in striking contrast to premenopausal ER-positive breast cancer patients with nonfunctional pRb, who do not experience any benefit.⁴³ Simvastatin negatively affects MCM7 expression, resulting in the elevation of the DNA damage marker, γ H2AX. Remarkably, the combined treatment of tamoxifen-resistant human breast cancer cells (these cells express pRb at lower levels in comparison to their wild-type counterparts) with simvastatin and tamoxifen evokes a genotoxic signal, which is efficient to trigger apoptosis.⁴²

In prostate cancer cells characterized by androgen-independent growth, simvastatin displays synergistic toxicity with another mevalonate pathway inhibitor, the bisphosphonate alendronate. Unlike statins that deplete isoprenoid biosynthetic route products by inhibiting HMG-CoA reductase, alendronate and other bisphosphonates inhibit the mevalonate pathway at the level of farnesyl diphosphate synthase by

virtue of molecular mimicry. Hence, at low doses, simvastatin along with alendronate decreases the viability of these cells. This event is accompanied by the stimulation of the c-JUN and the induction of apoptosis. However, this is not the case in androgen-dependent prostate cancer cells, where no such anticancer synergy is observed.⁴⁴ When prostate cancer cells are incubated with conditioned medium obtained from mouse calvaria, insulin-like growth factor-1 receptor (IGF1R) is upregulated. Simvastatin abolishes this induction of IGF1R. Given that this receptor mediates the prosurvival effects of IGF-1 which is released by the bone matrix, this molecular event is of pivotal importance. Moreover, simvastatin acts in a synergistic manner with the IGF1R antagonist NVP-AEW541 to trigger apoptosis in prostate cancer cells. These findings indicate the value of simvastatin in the modulation of the dynamic interactions between prostate cancer cells and bone microenvironment in prostate cancer skeletal metastases.⁴⁵ Another antimetastatic property of simvastatin is its ability to disrupt the interaction among the adhesion molecules expressed on prostate cancer cells (av β 3 integrin) and vascular endothelial cells (ICAM-1) as well as to preserve the endothelial barrier integrity.⁴⁶

Simvastatin exhibits cytostatic effects (G0/G1 arrest) in a peroxisome proliferator-activated receptor- γ (PPAR- γ)-dependent manner, as well as antimigratory and anti-invasive properties in bladder cancer cells. Further, in these cells, simvastatin controls the expression of epithelial-to-mesenchymal transition (EMT)-associated molecules, exemplified by E-cadherin, which is elevated, and β -catenin, which is decreased upon exposure to simvastatin.⁴⁷

In leiomyoma cells, the ability of simvastatin to trigger apoptosis has been attributed to the targeting of calcium-permeable voltage-gated channels,⁴⁸ while in renal cancer cells, the proapoptotic and antimetastatic functions of this drug have been linked to perturbed mammalian target of rapamycin (mTOR), JAK2/STAT3 and ERK signaling. Colon cancer cell viability is reduced by simvastatin through the down-regulation of the prosurvival factors Bcl-xL and Bcl-2 and by the suppression of the expression of the apoptosis inhibitors, cIAP1 and cFLIP.⁴⁹ In a seemingly paradoxical manner, the sustained activation of ERK by simvastatin is required for drug-induced apoptosis in HT-29 human colon cancer cells, whereas, in contrast, IGF-1-triggered ERK/Akt activation is counteracted by simvastatin that stimulates apoptosis.⁵⁰

Further, simvastatin increases the cytotoxic effects of irradiation combined with the anti-epidermal growth factor receptor (anti-EGFR) antibody cetuximab in head and neck squamous cell carcinoma (HNSCC) cells. Experimental

data indicate that this antitumor activity is mechanistically associated with the suppression of ERK1/2-, STAT3- and AKT-dependent pathways and the induction of apoptotic cell death.⁵¹ In human lymphoma and melanoma cells, the antitumor function of simvastatin is attributed both to its ability to sensitize to bortezomib-mediated apoptosis or to induce apoptosis, respectively, as well as to its ability to impinge on the expression of CDK inhibitors (p21 Waf1/Cip1, p27 Kip1), thereby halting cell cycle progression.^{52,53} In melanoma cells, simvastatin as well as lovastatin and a statin that is not used at the clinical setting (mevastatin) are known to exhibit antiproliferative and anti-invasive properties.⁵⁴ On the other hand, in multiple myeloma cells, simvastatin can induce both apoptosis and necrosis.⁵⁵ Also, simvastatin enhances the chemosensitivity of multiple myeloma cells by disrupting an HMG-CoA/GGPP/Rho/ROCK pathway, which confers cell adhesion-mediated drug resistance (CAM-DR) to them.⁵⁶

A mechanism by which simvastatin triggers apoptosis in SH-SY5Y neuroblastoma cells is through causing depletion of dolichol phosphate, which is produced throughout the mevalonate pathway. This isoprenoid compound serves as a sugar donor in the multistep process of protein N-glycosylation in the endoplasmic reticulum (ER). Impaired protein N-glycosylation results in the activation of the unfolded protein response (UPR)-related molecules BiP and CHOP, as well as in cellular stress and apoptotic death. Of note, this proapoptotic effect of simvastatin is dolichol-reversible. In addition, in neuroblastoma and rhabdomyosarcoma cells, simvastatin-mediated dolichol depletion precludes the full glycosylation of ABCB1 transporter, a protein that has been linked to the phenomenon of multidrug resistance in cancer cells. Also, simvastatin causes a drop in the transportation rates of ABCB1 in neuroblastoma cells.⁵⁷

Interference of simvastatin with the Rho GTPase/Hippo-YAP/CD168 pathway is not only documented in vitro as mentioned above,³⁹ but also in human breast cancer xenografts. The importance of this finding is underlined by the fact that CD168 along with YAP is found to be overexpressed in clinical specimens from patients with invasive breast cancer.³⁹ Also, in MDA-MB-231 xenografts, simvastatin prevents the occurrence of bone metastasis, and this has been attributed to an inverse relationship between the expression of CD44 and p53, which is associated with drug administration.³⁸ In immunodeficient mouse strains xenografted with tamoxifen-resistant breast cancer cells, simvastatin acts as a negative modulator of the replication licensing factor (RLF), MCM7. Simvastatin restores the sensitivity of tamoxifen-resistant tumors to tamoxifen and suppresses tumor growth.⁴² In animal

models of triple-negative breast cancer (TNBC), simvastatin has been found to engage a FOXO3a-mediated pathway to decrease the metastatic potency of malignant cells.⁵⁸ Another notable in vivo antitumor activity of simvastatin is that it inhibits lung cancer metastasis to the bones in murine xenografts.³¹ Moreover, simvastatin exhibits synergistic anticancer effects in mouse HNSCC xenografts, in conjunction with radiotherapy and cetuximab.⁵¹

The antitumor properties of simvastatin are also evident in human renal carcinoma xenografts.⁵⁹ Liposomal simvastatin administration yielded encouraging results in mice with melanoma. Mechanistically, the observed antitumor effect was demonstrated to be related to the ability of simvastatin to oppose the pro-oxidant function of tumor-associated macrophages (TAMs).⁶⁰ This is also the case of pravastatin, which, upon administration in a liposomal formulation to melanoma-bearing mice, exhibits anti-inflammatory properties. Moreover, liposomal pravastatin was reported to upregulate major histocompatibility complex (MHC) class I expression in tumors, thereby possibly positively influencing tumor immunosurveillance.⁶¹

Simvastatin opposes the development of tumors in the mouse model of colitis-associated colon cancer and exhibits proapoptotic and antiangiogenic effects in colon cancer xenografts.⁴⁹ In addition, simvastatin acts in synergy with bevacizumab to inhibit the growth of human colorectal cancer (CRC) xenografts and their ability to give rise to metastases.⁶² More recently, simvastatin has been reported to serve as an antiangiogenic factor in CRC in vivo by regulating a HER2/VEGF signaling pathway.⁶³

Moreover, simvastatin prevents virus-driven lymphomagenesis in immunodeficient mice⁶⁴ and functions to impede mammary tumorigenesis in rodents induced by irradiation at the earliest stages.⁶⁵ In the bone marrow of immunodeficient mice, simvastatin reduces the clonogenic capacity of human myeloblastic leukemia cells.⁶⁶ In rhabdomyosarcoma xenografts, clinically relevant doses of simvastatin inhibit tumor growth, while in xenograft models of neuroblastoma and rhabdomyosarcoma, simvastatin displays synergistic proapoptotic effects with cyclophosphamide.⁵⁷

Aside from simvastatin, rosuvastatin and pravastatin also exhibit antitumor function. Rosuvastatin, has been reported to suppress the activity of arginase (an urea cycle enzyme) and to reduce the levels of polyamines, both in the serum and tumor tissue in mouse models of breast cancer.⁶⁷ These data add another level of complexity to statins' antitumor mechanism of action, given that arginase, its substrate arginine and polyamines participate in perplexed signaling pathways and

are involved in tumor biology.^{68–71} On the other hand, pravastatin displays remarkable chemopreventive efficacy in rodent models of chemical-induced colon carcinogenesis.^{72,73}

Lovastatin

Lovastatin stimulates caspase-dependent apoptotic death of melanoma cells in a GGPP-reversible manner.⁷⁴ Aside from melanoma cells, lovastatin induces apoptosis in a wide range of human cancer cells, including medulloblastoma and malignant mesothelioma cells.^{75–80}

Lovastatin triggers apoptosis in different lung cancer cell lines by affecting Bax levels and stimulating p53.⁸¹ Further, lovastatin overrides resistance to gefitinib-induced apoptosis in NSCLC cells carrying mutant K-RAS by positively and negatively modulating the expression of Bax and Bcl-2, respectively,⁸² while it inhibits NSCLC cell adhesion and migration through depleting lipid rafts from cholesterol.⁸³ Of note, the RLF MCM2 has been identified as a target of putative therapeutic activity of lovastatin in human NSCLC. Actually, exposure of NSCLC cells to lovastatin results in the decrease of MCM2 levels in a JNK-dependent fashion.⁸⁴

In colon cancer cell lines, lovastatin potentiates the cytotoxic effects of commonly used chemotherapeutics such as cisplatin and 5-fluorouracil (5-FU) by upregulating the expression of Bax and suppressing Bcl-2.⁸⁵ Celecoxib-induced apoptosis in colon cancer cells is increased by lovastatin,⁸⁶ while in colon cancer cells, NSCLC cells as well as in SCC9 cells, this statin potentiates the cytotoxicity of gefitinib.⁸⁷

Lovastatin along with simvastatin induces cell cycle arrest and stimulates apoptosis in prostate cancer cells by affecting the expression of a series of cell cycle regulators (D-type cyclins, pRb and the CDK inhibitors, p21 Waf1/Cip1 and p27 Kip1) and through activating a RhoA/caspase-dependent pathway.⁸⁸ In these cells, lovastatin has also been reported to decrease E2F1 levels to trigger apoptosis.⁸⁹ Antitumor properties of lovastatin have also been reported in glioblastoma cells, where lovastatin exhibits antiproliferative and anti-invasive properties by inhibiting small GTPase (Rac1 and H-Ras) isoprenylation.⁹⁰

Another mechanism by which lovastatin indirectly displays anticancer function is through prevention of the adenosine triphosphate (ATP)-dependent extrusion of the antileukemic drug imatinib from the cytosol of CD34 antigen-expressing chronic myeloid leukemia (CML) cells by multidrug resistant transporters, namely, ABCG2 and ABCB1. These effects of lovastatin are cholesterol-dependent.⁹¹ In human B lymphoma cells, the antiproliferative effects of lovastatin have been

attributed to its antioxidant potency as well as to its ability to suppress the expression of the calcium-permeable channel transient receptor potential canonical 6 (TRPC6).⁹²

An antibody microarray analysis showed that the expression of a set of 37 proteins is altered in response to lovastatin in MDA-MB-231 triple-negative aggressive breast cancer cells. Remarkably, the apoptosis-related proteins, death receptor 3 (DR3) and caspase-7, as well as the tumor-suppressor, p57Kip2, were found to be upregulated among other proteins, whereas the cell-cycle regulatory protein CDK4 and apoptosis-inducing factor (AIF) were downregulated. Transglutaminase II (a mediator of N-cadherin switching) and hypoxia-inducible factor 1 α (HIF-1 α) that are associated with the EMT phenomenon and matrix metalloproteinase (MMP)-7, which is known to be related with metastasis, were also reported to be negatively modulated by lovastatin in these cells.⁹³ In leukemia cells of human origin, lovastatin displays synergistic effects with the clinically used antimetabolite, cytarabine.⁹⁴

Intriguingly, lovastatin dampens the dimerization of the cancer-associated molecule EGFR and downstream Akt-dependent signaling in a GGPP-reversible fashion. This leads to the suppression of global protein synthesis in squamous cell carcinoma cells.⁹⁵ In addition, lovastatin may prevent tumorigenesis, which takes place on the ground of chronic inflammation, given that in vitro experimentation showed that this statin markedly prevents the oxidative damage of DNA evoked by stimulated phagocytes.⁹⁶ Also, Ames testing suggests that lovastatin as well as atorvastatin may exhibit chemopreventive activity, since both these drugs inhibit the action of mutagens.⁹⁷

In vivo, lovastatin is known to enhance the antimelanoma effects of cisplatin at the preclinical level⁹⁸ and increase the anticancer function of tumor necrosis factor- α ⁹⁹ and doxorubicin in different mouse tumor models.⁹⁹ In a mouse model of ascites tumor, lovastatin triggers apoptosis in Dalton's lymphoma ascitic cells,¹⁰⁰ while in rats, this drug halts metastatic dissemination of fibrosarcoma and lymphoma cells.^{101,102} Besides, lovastatin reduces the metastatic potential of mouse mammary tumors and melanomas.^{103,104} Further, in a manner that is unrelated to K-RAS mutational status or expression, lovastatin inhibits nitrosamine-induced tumorigenesis in murine lungs.¹⁰⁵ Of note, in different murine tumor models, lovastatin is known to protect the myocardium from doxorubicin-induced cell damage.⁹⁹ In tumors of HER2/neu transgenes, lovastatin decreases the population of macrophages exhibiting an M2-like phenotype; a cell population which is known to control various aspects of carcinogenesis. In contrast, the population of effector T

lymphocytes increases upon lovastatin treatment. At the same time, tumors get more oxygen and they become more accessible to anticancer drugs by virtue of lovastatin's property to trigger vascular remodeling in tumors.²⁷

Atorvastatin

In vitro experimentation using human pancreatic cancer cells demonstrated that atorvastatin displays antitumor synergy with the FTI tipifarnib and the cyclooxygenase-2 (COX-2) inhibitor celecoxib.¹⁰⁶ In HNSCC cells, atorvastatin displays inhibitory activity on RhoC, a small GTPase and metastasis marker in HNSCC that has to be prenylated in order to be functional. Atorvastatin impairs the proliferation of HNSCC cells and reduces their motility and invasiveness.¹⁰⁷ In A549 cells, a TGF- β 1/sphingosine kinase 1 (SphK1) axis has been recently characterized. Inhibition of this axis by atorvastatin is associated with the partial suppression of the EMT phenomenon and, hence, mitigation of the metastatic potency of these NSCLC cells. In addition, atorvastatin prevents the TGF- β 1-mediated induction of the EMT-promoting transcription factor, ZEB1. This molecular event is also associated with the suppression of the migratory activity of A549 cells by atorvastatin.¹⁰⁸

EH domain-binding protein 1 (EHBP1) has been found to serve as a mediator of atorvastatin's purinergic (P2X7), signaling-dependent, anti-invasive activity in prostate cancer cells, which are positive for the tumor suppressor protein PTEN. In these cells, EHBP1 physically associates with the invasiveness-related guanine nucleotide exchange factor (GEF), P-Rex1. In other cancer cell lines, statins also suppress invasive growth through interfering with a P2X7-dependent pathway that modulates the nuclear phospho-Akt levels. The importance of these findings is substantiated by population genetic data suggesting that a loss of function allele coding for a P2X7 receptor variant is correlated with nonaggressive cancer, in contrast to the common allele which is associated with aggressiveness.¹⁰⁹ Mechanistically, the antimigratory/anti-invasive function of atorvastatin in breast cancer principally involves suppression of the expression of urokinase-type plasminogen activator (uPA), MMP-9 and the basic helix-loop-helix (bHLH) transcription factor, Twist.²⁹ The different signaling routes used by atorvastatin to suppress the migratory and invasive potential of malignant cells in vitro are collectively depicted in Figure 1. Importantly, atorvastatin dampens the expression of the RLF MCM7 and induces genomic damage and apoptosis in tumor cells in a pRb-deficient background to a greater extent than in the presence of pRb in a similar way as simvastatin does, both in an in vitro and an in vivo setting.⁶

In *Pan^{kras/p53}* mice which are engineered to carry Kras^{G12D} and p53^{R172} mutations, thereby genetically and phenotypically resembling the human pancreatic carcinogenesis, nutritional supplementation with atorvastatin resulted in a decrease of the proliferation index of the tumors and their shrinkage. In fact, treatment with atorvastatin or the FTI tipifarnib (R115777) prevented protein prenylation and halted the growth of malignant cells. Importantly, atorvastatin decreased neutrophil infiltration in chronic pancreatitis near the tumor in transgenes.²⁸ The latter finding is important given, the interrelation between inflammation and tumorigenesis.^{110,111}

In addition, analysis in pancreatic tumor xenografts in mice demonstrated that atorvastatin displays antitumor synergy with the COX-2 inhibitor celecoxib and tipifarnib.¹⁰⁶ In rats, combined administration of atorvastatin with either naproxen or sulindac, two marketed nonsteroidal anti-inflammatory drugs (NSAIDs), could markedly inhibit inflammatory signaling and lower the incidence of colon adenocarcinomas.¹¹² In line with the latter finding, atorvastatin acts in synergism with the COX-2 inhibitor celecoxib to suppress tumorigenesis in a prostate cancer xenograft mouse model¹¹³ or the progression of the androgen-dependent pattern of prostate tumor growth to androgen independence¹¹⁴ as well as the formation of intestinal polyps in the mouse familial adenomatous polyposis (FAP) model APCmin.¹¹⁵

Further, there are reports indicating the in vivo antitumor function of atorvastatin in head and neck cancer, MYC-induced lymphoma and breast cancer. In mice with head and neck cancer, atorvastatin suppresses tumor angiogenesis and the occurrence of distant metastases.¹⁰⁷ The antitumor activity of atorvastatin in murine conditional transgenes for modeling MYC-driven lymphomagenesis is mechanistically associated with the suppression of the Ras/ERK/MYC axis upon the pharmacologic inhibition of HMG-CoA reductase.¹¹⁶ In an orthotopic model of breast cancer metastasis, a micelle-based nanosystem for the delivery of atorvastatin inhibited distant metastases of breast cancer cells.²⁹

Fluvastatin

The fluorinated compound fluvastatin displays selective toxicity toward malignant rat glial cells, but it does not affect normal neurons. Its multilevel antitumor activity is molecularly associated with the stimulation of the JNK1/2 axis, the suppression of ERK1/2-dependent signaling, as well as the impaired secretion of VEGF and MMP-9.¹¹⁷ Paradoxically, statins are not only involved in the inhibition of GTPase isoprenylation, but also function as glycosylation inhibitors to suppress tumor growth. In fact, fluvastatin inhibits glycosylation of the class III receptor tyrosine kinase FMS-like tyrosine

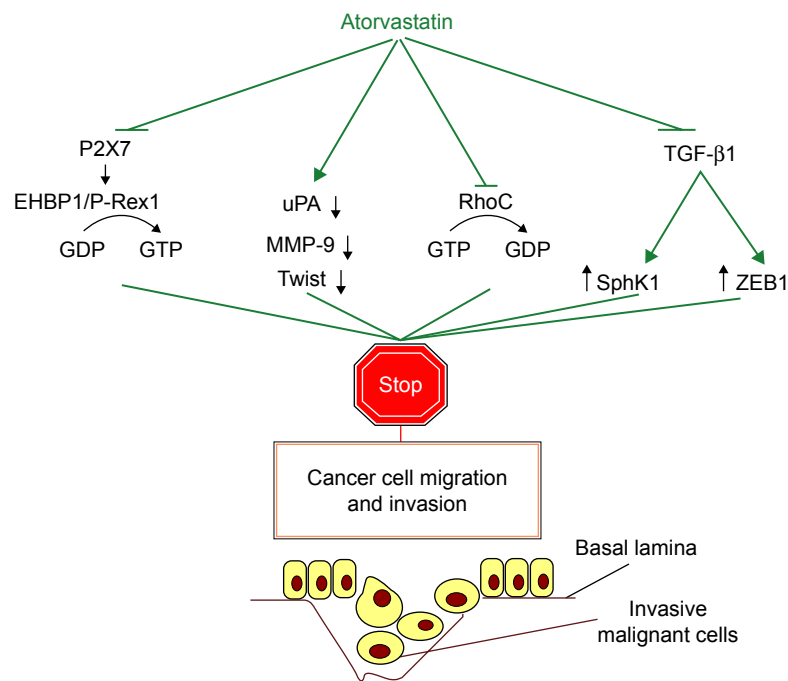


Figure 1 Signaling pathways mechanistically involved in atorvastatin's ability to inhibit the migratory and invasive potential of different types of cancer cells in vitro.

Notes: One of the mechanisms that have been reported in PTEN-positive prostate cancer cells involves the blockage of a purinergic P2X7/EHBP1/P-Rex1 route. On the other hand, the antimigratory/anti-invasive properties of atorvastatin in breast cancer cells have been linked to the downregulation of the expression of a series of different proteins, that is, uPA, the bHLH transcription factor Twist as well as MMP-9. In HNSCC cells, the ability of atorvastatin to inhibit cancer cell migration/invasion relies on the drug-induced impaired function of the small GTPase, RhoC. In NSCLC cells, atorvastatin hinders the metastatic potency through inhibiting TGF-β1-dependent pathways which are associated with the upregulation of SphK1 and the EMT-promoting transcription factor, ZEB1. Downward pointing arrows refer to negative regulation of the expression by atorvastatin. P-Rex1 exhibits GEF activity (ie, enables the release of GDP and the subsequent binding of a GTP molecule by a GTPase), whereas RhoC exhibits GTPase activity (ie, it catalyzes the hydrolysis of GTP to GDP).

Abbreviations: ATP, adenosine triphosphate; bHLH, basic helix-loop-helix; EMT, epithelial-to-mesenchymal transition; GDP, guanosine diphosphate; GEF, guanine-nucleotide exchange factor; GTP, guanosine triphosphate; HNSCC, head and neck squamous cell carcinoma; MMP, matrix metalloproteinase; NSCLC, non-small cell lung cancer; PTEN, phosphate and tensin homolog deleted on chromosome 10; SphK1, sphingosine kinase 1; uPA, urokinase-type plasminogen activator.

kinase-3 (FLT3), which commonly harbors activating mutations in acute myeloid leukemia (AML) and cannot be successfully targeted. This molecular event is associated with hampered MAPK and AKT activation and killing of cells expressing mutant FLT3 (FLT3/ITD).²⁶ This is a good example of how a class of pharmacologic agents somehow interferes with more than one type of protein's posttranslational modification to achieve their pleiotropic biologic effects. Regarding the antitumor activity of statins in hematologic malignancies, evidence has been provided for the combined antileukemic effects of fluvastatin with all-trans retinoic acid (ATRA).¹¹⁸

In renal cancer cells, fluvastatin's antitumor activity has been suggested to be mediated by the derepression of the function of a negative modulator of translation initiation, namely, programmed cell death 4 (PDCD4), through the inhibition of the mTOR.¹¹⁹ Interestingly, fluvastatin as well as lovastatin reduce EGF-stimulated human pancreatic cancer cell invasion by the inhibition of RhoA isoprenylation.¹²⁰ Of note, the stimulation of the production of the antitumor cytokine interleukin-18 by human monocytes is

thought to account for an antitumor mechanism of action of all the aforementioned statins (fluvastatin, simvastatin and pravastatin).¹²¹

Fluvastatin is known to confer a survival benefit to leukemic mice with FLT3/ITD.²⁶ In addition, fluvastatin negatively influences the ability of mouse renal cancer cells to give rise to metastases in the lungs of mice.¹²²

Preclinical data

Ezetimibe

A study carried out by Solomon et al indicates that not only statins, but also ezetimibe exhibit antitumor activity. Actually, ezetimibe was reported to act independently, but additively with the application of a low fat/no cholesterol (LFNC) diet in retarding tumor growth in severe combined immunodeficiency (SCID) mice implanted with human prostate tumors. Moreover, it was demonstrated that ezetimibe and LFNC exhibit additive, independent inhibitory effects on LNCaP cell proliferation and viability, as well as on tumor microvascularization through upregulating the expression of thrombospondin 1 (TSP-1), a well-established antiangiogenic

factor.²² More experimentation is required to shed light on whether ezetimibe can halt the growth of other cancer cell types as well.

Clinical data

Data from Phase I and II clinical trials support that monthly administration of lovastatin at relatively high doses (25 mg/kg/day) for a week followed by a 3-week pause in patients with prostate cancer or central nervous system (CNS) tumors causes, in most cases, ubiquinone-reversible myopathy, but yields only minor and partial responses.^{123,124} Almost no objective responses were reported in patients with advanced gastric adenocarcinoma who received 7-day courses of lovastatin at a dose of 35 mg/kg/day every 28 days.¹²⁵ However, a clinically relevant dose of pravastatin (40 mg/day) was found to extend life expectancy by two times in patients with advanced hepatocellular carcinoma (HCC) who had previously undergone transcatheter arterial embolization (TAE) followed by 5-FU administration, without causing myalgia, indicating its usefulness in adjuvant treatment.¹²⁶ In line with this, chemoembolization is more efficacious in improving survival in HCC patients when it is combined with pravastatin at a dose of 20–40 mg/day.¹²⁷

A Phase II clinical study yielded promising results with clinically relevant doses of simvastatin (40 mg/day) combined with the conventional chemotherapeutic scheme FOLFIRI (folinic acid, 5-FU and irinotecan) in patients with metastatic CRC.¹²⁸ Of note, certain monoclonal antibodies targeting the EGFR, exemplified by cetuximab, have been shown to be of clinical benefit, provided that KRAS does not harbor mutations. Hence, it has been hypothesized that simvastatin, which interferes with RAS posttranslational modification (prenylation), might restore the sensitivity to cetuximab. Unfortunately, this hypothesis is not valid, as reported by a Phase II clinical study in previously treated, metastatic CRC patients. Specifically, only 4 out of 18 patients who were enrolled into the study experienced disease stabilization at 12.5 weeks after cetuximab was first administered. This does not suggest any clinical applicability of simvastatin as a KRAS modulator and, hence, an agent that would restore sensitivity to cetuximab in metastatic CRC patients carrying KRAS mutations.¹²⁹ This also holds true for another anti-EGFR monoclonal antibody, namely, panitumumab, as indicated by another Phase II clinical study.¹³⁰ An analysis retrospectively assessing the data stemming from the CAIRO2 Phase III clinical trial reported that the usage of statins does not beneficially influence either progression-free survival (PFS) or overall survival of metastatic CRC patients

with the KRAS mutant gene who received chemotherapeutic agents in conjunction with the monoclonal antibodies, cetuximab and bevacizumab.¹³¹ On the other hand, in Danish individuals suffering from different types of cancer, the use of statin reduces the rates of malignancy-associated death, as shown by a retrospective analysis.¹³² Considering the latter analysis, however, some phenomena including the preferential prescription of statins to nonsmokers by some general practitioners or other comorbidities in statin users (eg, CVD) may have led to data misinterpretation and/or incorrect inferences.^{133,134}

Hopefully, certain statins, in a manner that depends upon their physicochemical properties (ie, their hydrophobicity), increase the PFS rates of patients suffering from the most life-threatening type of primary breast cancer; inflammatory breast cancer, as demonstrated in an observational study.¹³⁵ This is in line with the results from a Phase II study where it was found that the preoperative administration of high doses of atorvastatin manages to decrease the proliferation of tumor cells that express HMG-CoA reductase in women with primary invasive breast cancer.¹³⁶ In window trials enrolling breast cancer patients, comparison of the transcriptome before and after treatment with high dose (80 mg/day) of atorvastatin for 14 days prior to surgery has shown that the mRNA levels of molecules associated with immune response, apoptosis (GADD45B) and MAPK signaling (eg, the DUSP1 phosphatase) are statistically significantly affected,¹³⁷ while this atorvastatin dosage regimen also modulates cyclin D1 and the oncosuppressor p27Kip1 proteins in breast cancer, negatively and positively, respectively.¹³⁸ Remarkably, a pilot perioperative study demonstrated that fluvastatin at clinically attainable dosages (20 or 80 mg/day) exhibits potent chemopreventive properties in high-grade early-stage breast cancer patients.¹³⁹

In a cohort of white postmenopausal women, the self-reported consumption of simvastatin, lovastatin as well as other lipophilic statins over a follow-up period of 10.5 years was associated with an elevated risk for non-melanoma skin cancer (NMSC), irrespective of other factors including sunlight exposure, vitamin D intake, smoking and body mass index (BMI). Therefore, researchers did not exclude the scenario of a protumorigenic – possibly immunomodulatory – activity of statins and recommended that subjects with a family history of NMSC or those who are carcinogenesis prone due to their skin type should avoid the consumption of the aforementioned statins.¹⁴⁰

Intriguingly, the results of other previous studies regarding the putative link between NMSC and statin usage are

discrepant, both incriminating this category of antihypercholesterolemic agents for NMSC^{141–144} and pinpointing to a protective role of statins against the development of cancer^{145–147} or even not suggesting any statistically significant relationship between them.^{148–151} In accordance with the latter scenario, patients suffering from metastatic CRC who received chemotherapy and then were enrolled into a Phase III study in South Korea did not experience any clinical benefit (PFS) when 40 mg of simvastatin were added to a XELIRI/FOLFIRI chemotherapy regimen.¹⁵² Based on the statistics on statin prescription (simvastatin ranked first in statin prescription in the UK¹⁵³) and the fact that atorvastatin is one of the most extensively studied statins as an antitumor

agent at the clinical basis, Table 1 summarizes selected completed or active clinical trials evaluating the usage of these two antihypercholesterolemic agents in different types of malignancy or precancerous conditions.

More clinical trials are necessary to evaluate the antitumor function of statins. The results from the clinical trials that have already been completed or are still ongoing (NCT02026583, NCT01992042) are, therefore, much awaited. Regarding ezetimibe, yet, no clinical evidence has been provided supporting its antitumor function. The relationship between ezetimibe and tumorigenesis is actually obscure.¹⁵⁴ A multinational study, namely, SEAS, investigating the occurrence of cancer in patients with aortic stenosis who received

Table 1 Selected active or completed clinical trials on simvastatin and atorvastatin for cancer therapeutics and/or cancer prevention

Setting	Pharmacologic intervention(s)	Phase	Status	Clinical trials Gov identifier
Gastric cancer	Simvastatin; placebo	Phase III	Completed	NCT01099085
Breast cancer	Simvastatin	Phase II	Completed	NCT00334542
Pancreatic cancer	Gemcitabine + simvastatin; Gemcitabine + placebo	Phase II	Completed	NCT00944463
Bladder cancer	Metformin; simvastatin	Phase II	Recruiting	NCT02360618
Metastatic colorectal cancer	Cetuximab/irinotecan/simvastatin	Phase II	Completed	NCT01281761
Colorectal cancer	Simvastatin	Phase II	Recruiting	NCT02026583
Lung cancer	Simvastatin; gefitinib only	Phase II	Completed	NCT00452244
Small cell lung cancer	Irinotecan; cisplatin; simvastatin	Phase II	Completed	NCT00452634
Breast cancer	Metformin/simvastatin/fulvestrant	Phase II	Recruiting	NCT03192293
Breast cancer	Anastrozole; simvastatin; other: pharmacologic study; procedure: adjuvant therapy	Phase II	Completed	NCT00354640
Breast cancer	Nimesulide; simvastatin; placebo	Phase II	Completed	NCT01500577
Metastatic colorectal cancer	Simvastatin	Phase II	Completed	NCT00313859
Adenocarcinoma of rectum	Simvastatin	Phase II	Recruiting	NCT02161822
Relapsed and/or refractory pediatric solid and CNS tumors	Simvastatin; cyclophosphamide; topotecan; myeloid growth factor	Phase I	Recruiting	NCT02390843
Prostate cancer	Ezetimibe 10 mg–simvastatin 40 mg	Early Phase I	Recruiting	NCT02534376
Cirrhosis (liver cancer prevention)	Simvastatin; placebo	Phase II	Recruiting	NCT02968810
Brain metastases	Simvastatin (in addition to radiation therapy)	Phase II	Completed	NCT02104193
Multiple myeloma	Simvastatin	Phase II	Completed	NCT00399867
Chronic lymphocytic leukemia	Simvastatin	Phase I	Completed	NCT00828282
Breast cancer	Atorvastatin; metformin	Early Phase I	Recruiting	NCT01980823
Breast cancer	Letrozole and atorvastatin; fulvestrant; letrozole	Phase II	Recruiting	NCT02958852
Prostate cancer	Atorvastatin calcium; celecoxib	Phase II	Completed	NCT01220973
Prostatic neoplasms	Atorvastatin; placebo	Phase II	Active, not recruiting	NCT01821404
Endometrial cancer	Atorvastatin	Early Phase I	Recruiting	NCT02767362
Breast cancer; postmenopausal	Atorvastatin	Phase II	Completed	NCT00816244
Colorectal cancer; precancerous condition	Atorvastatin calcium; oligofructose-enriched inulin; sulindac; placebo	Phase II	Completed	NCT00335504
Breast cancer; precancerous condition	Atorvastatin calcium	Phase I	Completed	NCT00637481
Prostate cancer	Atorvastatin; metformin; placebo (corresponding to metformin)	Phase II	Recruiting	NCT02497638
Glioblastoma multiforme	Atorvastatin; temozolomide	Phase II	Recruiting	NCT02029573
Hepatocellular carcinoma	Atorvastatin; placebo oral tablet	Phase IV	Recruiting	NCT03024684

Abbreviation: CNS, central nervous system.

placebo compared to those who received simvastatin (40 mg daily) and ezetimibe (10 mg daily) reported that the latter are at higher risk for carcinogenesis.¹⁵⁵ However, no such association was reported in a subsequent study enrolling a SEAS trial cohort from the UK, Denmark and Scandinavia.¹⁵⁶ Clinical trials evaluating the possible anticancer therapeutic potency of ezetimibe in oncologic patients are necessary to enable its future repositioning in the field of oncology.

Considerations for using and repurposing antihypercholesterolemic drugs

In many studies, the anticancer function of statins on various cancer cell lines has been demonstrated, using them at clinically irrelevant doses.^{157–159} In other cases, however, statins display their anticancer properties at dosages that are clinically attainable. For instance, the IC₅₀ (ie, 50% of growth suppression) of lovastatin in various cancer human cells is within the concentration range 0.3–2 µM.¹⁶⁰ According to data stemming from experiments in animals, 2–4 µM of lovastatin in serum is safe and toxicity becomes evident only at a concentration of 20–25 µM.¹⁶¹ Clinical data indicate that in cancer patients, even high plasma concentrations of lovastatin (up to 12 mM) administered for a short period (≤4 days) are well tolerated.¹⁵⁹

Statins display additivity and synergism with conventional chemo drugs and monoclonal antibodies (eg, anti-metabolites, cisplatin, doxorubicin and cetuximab).^{85,94,98,162–164} In addition, there is accumulating evidence that statins act at low doses in synergism with NSAIDs as chemopreventive agents.¹⁶⁵ Moreover, owing to the phenomenon of biotransformation by cytochrome P450 (CYP) 3A4 (expressed in gut and liver), the concentrations of statins required to achieve their antitumor effects may be actually much lower than the concentrations used for in vitro experiments.¹⁵⁸ Hence, all these data open the possibility for the future use of statins as adjuvant agents for the treatment of cancer at dose schemes close to the current therapeutic anticholesterolemic dose.

Some people receiving statins experience myotoxicity and/or hepatotoxicity. In addition, coadministration of statins with certain drugs (including clarithromycin, erythromycin, azole antifungals and cyclosporine A) which inhibit CYP3A4—the major statin-metabolizing CYP isoform—is prohibitive, since these agents increase the serum statin concentration, thereby exacerbating myopathy.^{166,167} Therefore, frequent monitoring of serum transaminases is highly recommendable, while statin-induced reduction of serum Q10 (a mevalonate pathway product) levels that has been associated with myopathy could be restored by ubiquinone supplementation.¹⁶⁸

Great caution should also be exercised with regard to those patients who receive medication that is incompatible with statins exemplified by cyclosporine and macrolide antibiotics that inhibit CYP3A4.^{169,170} Regarding statin myotoxicity, particular caution should be paid in case that these drugs will eventually be repositioned in the field of oncology since malignancy and myopathy have been interrelated, while myopathy may also be ascribed to steroid consumption by cancer patients.^{171,172} Conceivably, in cases of myopathic patients, the use of statins as antitumor drugs would not be recommended. A major concern is that subjects suffering from type II diabetes mellitus who consume atorvastatin are likely to develop atorvastatin-induced autoimmune myositis (AtorAIM). Hallmarks of AtorAIM are: 1) detection of anti-HMG-CoA reductase autoantibodies in the serum of patients, 2) abnormal creatine kinase (CK) levels even when the administration of atorvastatin is being discontinued and 3) responsiveness to the intravenous immunoglobulin (IVIG) induction therapy, although AtorAIM patients do not respond to corticosteroids.¹⁷³

Since ezetimibe is devoid of the severe adverse effects of statins (eg, hepatotoxicity, myotoxicity) and has been proved to be safe and well tolerated, either in monotherapy or in combinational hypolipidemic therapy,^{174–176} it would be of great interest if future clinical trials would evaluate it as an antitumor agent. However, it should be noted that, ezetimibe in combination with cholesterol diet stimulates NFκB and local intestinal inflammation in guinea pigs.¹⁷⁷ Given the interrelation between inflammation and intestinal cancer,¹⁷⁸ more studies are needed to address whether ezetimibe could fuel intestinal carcinogenesis in humans under certain circumstances.

Conclusion and future perspectives

The putative usage of the pharmacologic agents discussed here, especially statins, in the field of oncology is not based on the same rationale as those for the mechanism of action of either chemotherapeutics^{179–181} or targeted anticancer drugs.¹⁸² Statins were first isolated and characterized as HMG-CoA reductase inhibitors in 1970s, while lovastatin was the first statin that gained Food and Drug Administration approval as an antihypercholesterolemic drug in 1987. Hence, researchers started to make efforts to target cholesterol synthesis as a blood cholesterol-lowering strategy, even though two decades ago, published data¹⁸³ indicated an interrelation between the biosynthetic pathway of cholesterol and cellular transformation. In early 1990s, it was hypothesized that the blockage of mevalonate pathway could suppress cancer cell growth.¹⁸⁴

However, accruing experimental data presented above suggest that statins may be exploited as antitumor agents owing not solely to their isoprenoid biosynthesis-blocking property. Actually, statins are somehow molecularly “endowed” with pleiotropism, and therefore, they exhibit multilevel antitumor function(s) associated with the modulation of divergent signaling pathways, including cell life and death decisions,³¹ cell-to-cell adhesion,⁴⁶ EMT,³² protein N-glycosylation, the UPR,⁵⁷ DNA replication licensing,⁴² TAMs⁶⁰ or even angiogenesis.⁶³ Thus, it becomes increasingly clear that statins act as versatile anticancer bulletins targeting pathways far beyond those that are known to link cholesterol and lipoprotein metabolism to carcinogenesis.¹⁸⁵ Versatility at the signaling and physiologic level also holds true for the blood cholesterol-lowering drug ezetimibe, which displays an antiangiogenic mode of antitumor action.²²

Additional clinical data are needed for statins to assess their antitumor potency. The results of ongoing clinical studies (eg, NCT01992042 and NCT02026583) are much awaited. It would also be very interesting to evaluate the antitumor properties of ezetimibe in oncologic patients, given that no such evidence has been provided for this antihypercholesterolemic agent so far, even though it is devoid of the major adverse effects associated with the use of statins.

A darling enterprise of the future is to delineate the mechanism(s) of the anticancer activity of non-antineoplastic marketed agents, which, in a similar manner to statins, are currently used for managing CVD – either prophylactically or therapeutically – given that CVD and cancer rank as the two leading causes of mortality globally.¹⁸⁶ Aspirin, similar to statins, is a widely prescribed NSAID with well-established antitumor properties.^{9,187} Caffeine is commonly combined with aspirin in commercially available formulations and is also well known to exhibit antitumor function.^{188–190} Serum proteomics data (Figure S1) indicate that at the clinical level, administration of aspirin combined with caffeine compared to aspirin alone may affect (either positively or negatively) the levels of proteins in human serum that have been linked to cancer biology, cancer prognosis/diagnosis and prediction of the efficacy of anticancer medication. These proteins are TTR,¹⁹¹ RBP4,¹⁹² Hp¹⁹³ and ApoA1.¹⁹⁴ These hints would be very interesting to be experimentally validated in the future. Paradoxically enough, although several experimental data have been provided so far regarding the multilevel pharmacologic activity of aspirin or caffeine alone,^{9,195–198} only less amount of data has been published regarding the effects of aspirin combined with caffeine.^{199,200} Most importantly, research so far has focused on the analgesic properties of this

combinational scheme. To the best of our knowledge, there is no evidence addressing whether such a pharmacologic combination is associated with superior antitumor function in comparison to that of either agent alone. If the scenario for anticancer synergism holds true, it would be interesting to delineate the signaling pathway(s) that is/are involved in the synergistic effect. Intriguingly, caffeine is an inhibitor of the activity of the master DNA damage response (DDR) kinases ATM and ATR,²⁰¹ while an interplay between COX and ATR has been described. Actually, COX positively regulates ATR levels through a COX/PGE₂/ERK/ATR axis in cancer cells and this pathway has been functionally associated with the acquisition of resistance to genotoxic agents.²⁰² Thus, a major future challenge would be to assess whether aspirin combined with caffeine displays antitumor synergism and which molecular circuits are affected in the presence of both these agents with regard to tumorigenesis. Besides, another scenario which is worthy of investigation is a putative synergism between an NSAID such as aspirin and atorvastatin or another statin against tumorigenesis, or even the possible antitumorigenic synergism of a triple combination of an NSAID, a statin and caffeine, given that atorvastatin and celecoxib are known to display anticancer synergism.¹¹³ A synergism of such a double or triple combination in the prevention of androgen-independent pattern of growth of prostate cancer is also possible and merits investigation, as indicated by the former findings.¹¹⁴

Finally, another future challenge is to identify new cancer-associated molecular targets of statins by exploiting the predictive power of bioinformatics tools in order to develop novel antitumor therapeutic schemes. Consistent with this, an *in silico* analysis indicates that simvastatin not only serves as a negative modulator of MCM7 expression as it was previously mentioned,^{6,42} but also may physically interact with another RLF, the human protein CDT1. Specifically, bioinformatics indicate that simvastatin possibly binds at one up to three different binding sites on human CDT1 and predict that this putative binding of the drug interferes with the intermolecular interaction between CDT1 and its physical inhibitor, geminin (Figure 2). The importance of this prediction is substantiated by previous findings showing that silencing the expression of geminin is an emerging cancer cell-eliminating strategy irrespective of the p53 status which leaves normal or immortalized cells unaffected. This methodology is based on the geminin silencing-induced DNA re-replication and the consequent induction of genomic damage and programmed cell death. However, as it is reported, not all malignant human cells

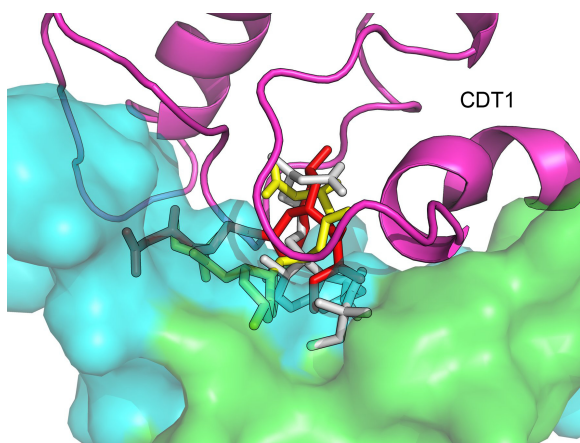


Figure 2 Docking of simvastatin on the human DNA replication licensing factor CDT1.

Notes: Autodock vina has been used to predict binding sites of simvastatin as a ligand (structure taken from Protein Data Bank, PDB:2WVVR) on CDT1 (chain C, PDB:4XW2) allowing flexible docking for the ligand. Three of the nine predicted binding sites of similar affinity (~ 5.5 kcal/mol) are positioned in the interaction interface between CDT1 and geminin (chains A and B, PDB:4XW2), indicating that simvastatin interferes with the CDT1:geminin complex and possibly destabilizes it. The figure has been produced using PyMol using the following coloring code: magenta for CDT1 in cartoon representation, green and cyan for the geminin dimer in surface representation, red, yellow and gray for the three poses of simvastatin in sticks representation.

are equally affected by geminin silencing. In fact, HeLa and skin melanoma cells are not sensitive to geminin silencing.²⁰³ Conceivably, an attractive goal is to carry out in vitro assays to address whether simvastatin and/or another statin physically interferes with the binding of geminin at CDT1 as in silico analysis predicts. If this assumption holds true, it would be very interesting to assess the physiologic relevance of this interference. In case simvastatin or another statin hinders the CDT1:geminin interaction, it is plausible that the inhibition of geminin activity and statin treatment could exhibit synergistic cancer cell-killing effects in cancer cells that are sensitive to geminin silencing. In addition, geminin silencing-resistant cells could be sensitized in the presence of simvastatin. This testable prediction merits experimental validation that could open new avenues in cancer therapeutics.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

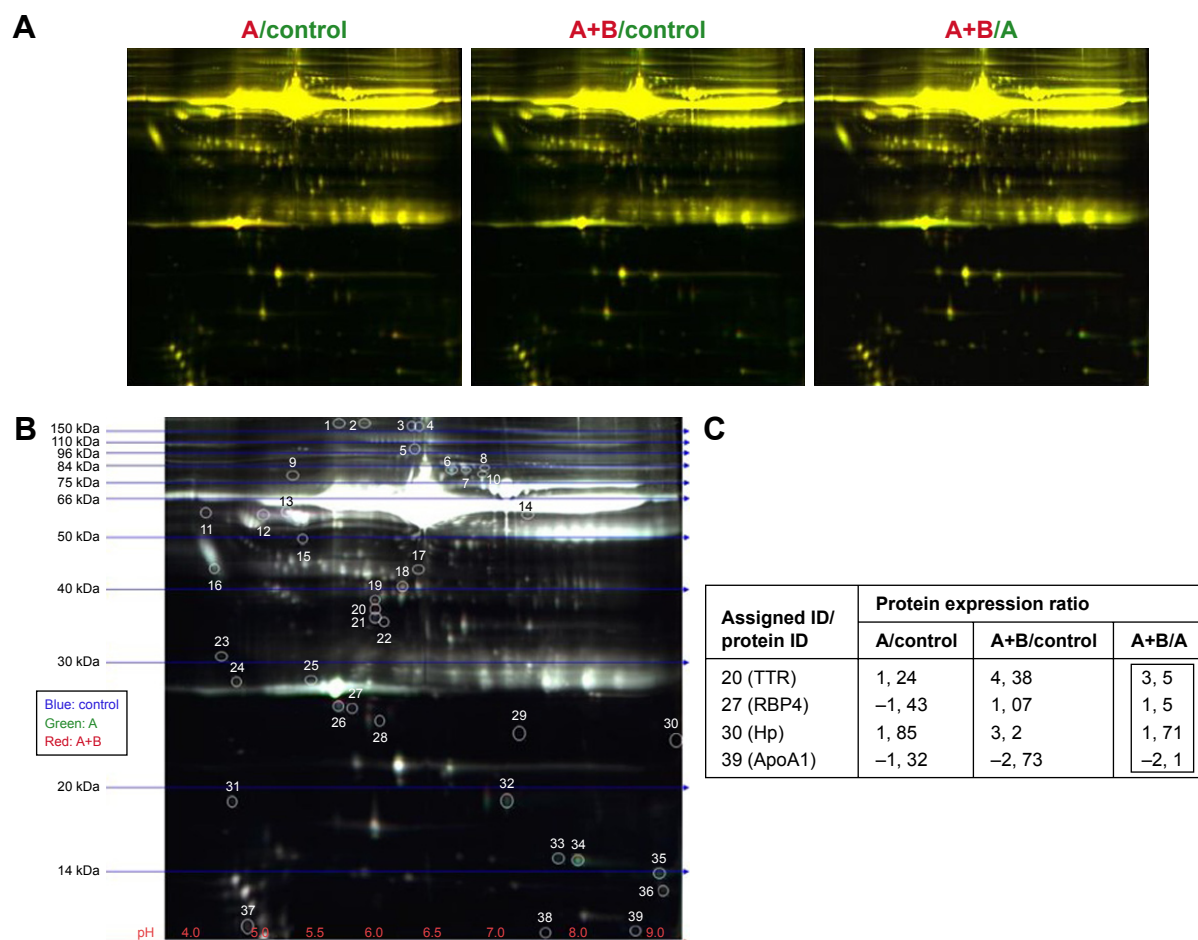


Figure S1 Proteome profiling indicating alterations in the expression levels of human proteins evoked by the consumption of aspirin alone or combined with caffeine at clinically attainable doses.

Notes: Proteomic analysis in the serum of a 33-year-old healthy female individual 16 hours after the consumption of 500 mg of a marketed (ASPIRIN 500MG/TAB, Bayer Hellas A.B.E.E., Marousi 151 25, Attiki, Greece) tablet of aspirin ("A") or a marketed (KALMALINE AC [500+30]MG/TAB, Coup A.B.E.E., Agias Varvaras 53, P.C. 17235 Athens, Greece) tablet containing 500 mg of aspirin and 30 mg of caffeine ("A+B"). None of these pharmacologic agents or even a placebo pill was consumed by the volunteer in the case of "control" serum analysis, while the individual voluntarily did not consume any food or beverage containing caffeine (chocolate, tea and so on) at least 24 hours before any of the three distinct blood sample collections, which took place throughout a 3-day period (ie, 1st, 2nd and 3rd days when control, sample A and sample A+B were collected). Approximately 8 mL of blood was collected in serum separating tubes (BD Vacutainer SST II 8.5 mL Advance Tubes-367958; Scientific Diagnostics Ltd, Attiki, Greece), and 2 mL of serum obtained after sample centrifugation for 15 min at 3,000 rpm was transferred into cryovials (Greiner Bio-One GmbH 5 mL U-Bottom cryovials CE-IVD; Bioline Scientific, Athens, Greece) and stored at -20°C , until their transfer in dry ice shortly after (1 day) the completion of the 3-day sample collecting period. All the experimental procedures associated with 2D-DIGE from protein extraction and sample labeling with CyDye DIGE fluors (blue, green and red for "Control", "A" and "A+B" samples, respectively) to spot picking and protein identification by mass spectrometry were performed at Applied Biomics (Hayward, CA, USA) according to the established protocol as it has been described elsewhere.¹ Shown here (**A**) are 2D-DIGE gel images from overlay of each paired sample, A/control, A+B/control and A+B/A. The protein spots shown in yellow color means that protein expression is similar in the paired samples (two-color image overlay). White color (**B**) means that the protein expression is the same in all these samples (three-color image overlay). For the aforementioned three paired comparisons, a fold ≥ 1.3 was used as a cut-off value in the DeCyder analysis and most significant changed spots were selected. All the 39 spots were circled and numbered in the triple color overlay gel image with the estimated MW and isoelectric point (pI) grid (**C**). Each spot number was assigned (Assigned ID/protein ID) if the ratio of changes in at least one paired sample was ≥ 1.3 fold. Some of the statistically significant spot ratios corresponding to the expression of the 15 proteins which were mostly affected (either positively or negatively) in the A+B/A paired sample were chosen for identification by mass spectrometry. The rationale for focusing on this category of proteins is based on the fact that these proteins indicate the differential biologic outcome of aspirin when it is received in combination with caffeine ("A+B" or the numerator of the fraction) or not ("A" or the denominator of the fraction), respectively. Mass spectrometry revealed that the ID of four randomly chosen spots (**C**, shown as boxed values of the "A+B" paired sample) corresponding to the 15 most influenced proteins in the A+B/A paired sample is TTR (protein expression ratio 3, 5, spot number 20), RBP4 (protein expression ratio 1, 5, spot number 27), Hp (protein expression ratio 1, 71, spot number 30) and ApoA1 (protein expression ratio -2, 1, spot number 39). The role of these proteins in tumorigenesis and/or cancer diagnosis/prognosis is discussed in the text.

Abbreviations: 2D-DIGE, two-dimensional difference in gel electrophoresis; ApoA1, apolipoprotein A1; Hp, haptoglobin; MW, molecular weight; TTR, transthyretin; RBP4, retinol-binding protein 4.

Reference

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