Statins: a new approach to combat temozolomide chemoresistance in glioblastoma

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ABSTRACT

Patients with glioblastoma multiforme (GBM) have an average life expectancy of approximately 15 months. Recently, statins have emerged as a potential adjuvant cancer therapy due to their ability to inhibit cell proliferation and induce apoptosis in many types of cancer. The exact mechanisms that mediate the inhibitory actions of statins in cancer cells are largely unknown. The purpose of this proceeding paper is to discuss some of the known anticancer effects of statins, while focusing on GBM therapy that includes adjunct therapy of statins with chemotherapeutic agents.

INTRODUCTION

Glioblastoma multiforme (GBM) is the most common malignant brain tumor in adults, comprising 18.5% of all brain tumors and 54% of all gliomas in adults in the USA.[1] Standard treatment includes surgery, radiation and chemotherapy with the DNA alkylating drug temozolomide (TMZ)[2] but the average survival time stubbornly remains at approximately 15 months since TMZ was first introduced in the early 2000s.[3] Statins are exogenous inhibitors of the de novo cholesterol synthesis pathway and are among the most successful US Food and Drug Administration (FDA)-approved drugs for the prevention and treatment of cardiovascular diseases.[4] Recently, a large cohort study of approximately 200 000 individuals revealed a beneficial effect of long-term statin use on the survival rate of patients with different types of cancers.[5] A similar increase in survival time was reported for patients with GBM who had been taking statins for >1 year.[6] The beneficial effects of statins in patients with cancer are attributed, at least in part, to their effects on the post-translational prenylation of members of the small Rho-GTPase protein family.[7] However, the exact mechanism of action is not fully understood. Encouraged by the reports on the promising survival outcomes in patients with cancer receiving a combination therapy of chemotherapy drugs together with statins,[5 6 8] we have investigated this therapeutic strategy for the treatment of GBM.

STATINS ARE NEW CANDIDATES IN CANCER THERAPY

Statins act as competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), the key enzyme in the mevalonate pathway.[9] Mevalonate serves as the precursor of isoprenoids and cholesterol in this pathway.[10] Statins block HMGCR-mediated production of isoprenoid pyrophosphates (farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP)).[11] Besides lowering cholesterol, this inhibits the prenylation of small Rho GTPases and blocks their translocation to the plasma membrane which results in attenuated cell growth.[11] Statin-mediated inhibition of isoprenoid synthesis also induces apoptosis and inhibits cell cycle progression in different types of cancer cells.[10 12 13] Several reports have noted the benefits of statins in the treatment of cancer.[9] For better information, we have summarized some of the recent trials in cancer therapy that statins have been used as supplemental cancer therapy strategy or long-term use of statins show show lower risk of cancer in a population (table 1). In vitro studies have shown that statins can arrest cell cycle G1[14–17] or S phase[18] and induce apoptosis[19] in different cancer cells.[20–23] Our group has found recently that simvastatin can induce the intrinsic apoptotic pathway (activation of caspase-9 and caspase-3/-7) by depleting isoprenoids as precursors for prenylation of small Rho GTPases in different human cancer cell lines.[7] We confirmed that simvastatin caused the translocation of the small Rho GTPases RhoA, Cdc42 and Rac1/2/3 from the cell membrane to the cytosol in GBM, lung adenocarcinoma and breast cancer cell lines.[7] Statins inhibit both proliferation and invasiveness of tumor cells in a dose-dependent fashion, particularly in highly invasive tumor cell lines.[24 25] This effect was abolished after administration of GGPP, but not FPP, suggesting that farnesylated Ras protein plays only a minor role in the process. In vivo studies have also been conducted with intriguing results. Simvastatin treatment has been shown to decrease the tumor size of xenografts derived from human prostate cancer cells[26] and breast cancer cells in mice.[27] In the case of GBM, pitavastatin was found to induce

Table 1 Effect of statins in different cancer therapy strategies

Type of cancer	Major findings
ER-negative breast cancer (stage I breast cancer)	Using fluvastatin (80 mg/day) for 3–6 weeks before surgery decreased tumor proliferation and increased tumor apoptosis in only high-grade tumor[78]
Glioblastoma multiform	Long-term prediagnostic statin (simvastatin) use may improve survival following glioblastoma multiform[6]
Extensive-disease small-cell lung cancer	The addition of simvastatin (40 mg daily) to irinotecan and cisplatin may improve the outcome of heavy smokers extensive-disease small-cell lung cancer[79]
Small-cell lung cancer	Pravastatin 40 mg combined with standard small-cell lung cancer therapy, although safe, does not benefit patients[80]
Prostate cancer	Using statins decreased advance prostate cancer risk[81]
Brain metastasis tumors	The addition of simvastatin 80 mg/day did not improve the clinical outcomes of patients with brain metastasis receiving whole-brain radiation therapy[82]
Different types of cancers	Patients aged 45 years or more and discharged from the hospital alive after admission for acute myocardial infarction after using high dose of statins show lower risk of cancers[83]

cell cycle arrest and inhibit cell proliferation in vitro.[28] Statins can also inhibit the formation of metastatic lesions by inhibiting the migration and invasion of cancer cells in vitro as well as tumor growth and bone metastasis in vivo.[29–32] Importantly, statins have been used in combination with commonly prescribed anticancer drugs, mainly to sensitize cancer cells to these drugs by inducing apoptosis and inhibiting cancer cells proliferation.[33] Synergistic treatment effects have been observed between statins and chemotherapeutic drugs[34 35] and radiotherapy. Statins are believed to sensitize cells to chemotherapy and radiation during late G1 phase.[36 37] The anticancer effects of statins are outlined in figure 1. Currently, there are 65 completed and 13 ongoing clinical trials investigating statins as therapeutic candidates in different cancer contexts (clinicaltrial.gov; search term "statin and cancer"). Most of these clinical trials have or are investigating statins in combination with other anticancer agents,[38 39] as monotherapy or combined therapy for different cancer treatments. Most of the clinical trials are phase I or II, and a few ongoing phase III clinical trials.[40]

STATINS AND GBM CANCER THERAPY

GBM has a poor prognosis with 5-year survival of only 3.3%.[41] It has been shown that statins can suppress invasion and promote apoptosis in GBM cells cultured in fibrin gel.[42 43] In this study, atorvastatin was effective in inhibiting growth and survival of GBM by suppressing Ras signaling in a prenylation-dependent manner. When used in combination with TMZ, atorvastatin significantly enhanced TMZ efficacy in vitro and in an in vivo mouse model.[44] In preclinical studies, statins have shown to synergize with antineoplastic drugs in different cancer cells, including GBM.[45–52] Statins also reduced invasiveness and migration in glioma cells.[53] In our recent investigation, inhibition of the mevalonate cascade by simvastatin boosted TMZ-induced apoptosis in GBM tumor cells, thereby highlighting the promising potential of statins in combination with TMZ on GBM (manuscript under revision). Combination of valproic acid and fluvastatin also has a synergistic effect in apoptosis induction in GBM8401 cells.[54] In line with our findings, Yanae *et al*[55] showed that statins inhibit cell proliferation and increase caspase-3 activity indicating apoptosis in C6 glioma cells. These effects were reversed by the addition of GGPP but not FPP.[7] A cohort study evaluated the influence of simvastatin use on survival of 339 patients with GBM and showed that long-term prediagnostic statin use may improve survival following GBM (a reduced HR of death (0.79; 95% CI 0.63 to 1.00)). The HRs decreased with increasing duration or intensity of prediagnostic statin use (long-term (5 years) statin use: HR 0.75 (95% CI 0.47 to 1.20); high-intensity statin use: HR 0.66 (95% CI 0.44 to 0.98)). Additional adjustment for oncotherapeutic modalities yielded similar results (overall HR 0.80, 95% CI 0.63 to 1.01).[6] Consistent with this, laboratory studies and a

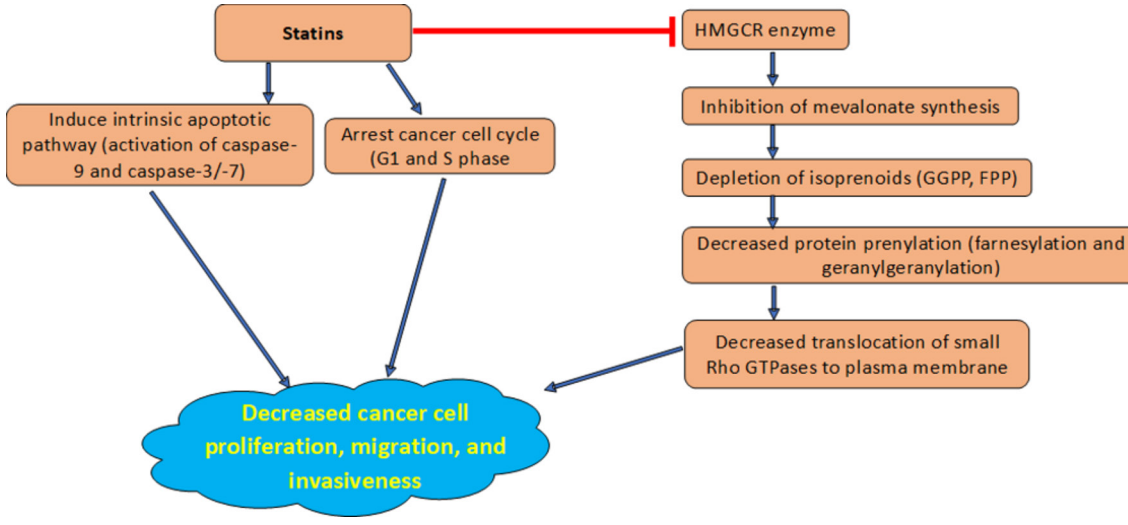

Figure 1 The effects of statins on cancer cells. Statins decreases cancer cells proliferation, migration and invasions via targeting cancer cell cycle, apoptosis and regulation of small Rho GTPase protein activity (through changes in small Rho GTPase prenylation).

single case–control study have suggested a protective effect of statins on the risk of glioma which also suggests the potentially beneficial long-term use of statin to increase life expectancy in glioma patients.[1 42] A phase II clinical trial has evaluated the synergistic effects of statins (atorvastatin) in combination with radiotherapy and TMZ in patients with GBM with promising results.[8] However, the benefit of statins in combination treatments warrants further studies to explore and validate the use of statins in GBM.[40]

CAN STATINS/TMZ COMBINATION THERAPY BENEFIT PATIENTS WITH GBM?

TMZ is the standard chemotherapeutic choice for the treatment of GBM.[56] TMZ acts as a DNA alkylating agent that induces single-strand DNA damage and depletes DNA repair enzyme O6-methylguanine-DNA methyltransferase from targeted GBM cells.[56] These molecular changes in TMZ-treated GBM cells activate several signaling cascades which induce apoptosis.[57–59] While TMZ has slightly improved the survival rate of patients with GBM, most will die in <2 years, mostly due to resistance of glioma cells to TMZ resulting in recurrences.[60] Combination therapy with statins is a novel approach aimed at overcoming chemoresistance and enhancing drug cytotoxicity in GBM cells.[61–64] FDA-approved cholesterol pathway inhibitors, statins, are well known for their cholesterol-lowering effects and have been commonly prescribed to prevent and/or treat cardiovascular diseases.[7 65 66] Statins have been identified by many researchers to have pleiotropic effects independent of the classical cholesterol biosynthesis pathway (mevalonate cascade).[4 65 67–69] The intermediate products of the mevalonate cascade, including FPP and GGPP, are important factors in the prenylation of small GTPases.[4 7 70 71] Ras and Ras-related proteins, the cardinal small GTPase proteins, play pivotal roles in cell adhesion, proliferation, trafficking, cytoskeletal dynamics and malignant transformations.[70 72 73] Exhaustion of GTPase proteins is associated with programmed cell death in a variety of cancer cells.[7 74–76] It is conceivable that statins sensitize GBM cells to TMZ-induced apoptosis via inhibition of small Rho-GTPases. Our recent investigations showed that (1) autophagy might be involved in sensitizing GBM cells to TMZ-induced apoptosis via statin co-treatment[77] and (2) simvastatin possibly increases TMZ-induced apoptosis via modulation of the lysosomes/autophagosomes interaction, as such affecting end-stage autophagy.[77] Our team is currently working on identifying the exact mechanisms involved in sensitizing GBM cells to TMZ-induced apoptosis.

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REFERENCES

1 Altwairgi AK. Statins are potential anticancerous agents (review). *Oncol Rep* 2015;33:1019–39.
2 Ostrom QT, Gittleman H, Fulop J, *et al*. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008-2012. *Neuro Oncol* 2015;17 Suppl 4:iv1–iv62.
3 Hombach-Klonisch S, Mehrpour M, Shojaei S, *et al*. Glioblastoma and chemoresistance to alkylating agents: Involvement of apoptosis, autophagy, and unfolded protein response. *Pharmacol Ther* 2018;184:13–41.
4 Yeganeh B, Wiechec E, Ande SR, *et al*. Targeting the mevalonate cascade as a new therapeutic approach in heart disease, cancer and pulmonary disease. *Pharmacol Ther* 2014;143:87–110.
5 Nielsen SF, Nordestgaard BG, Bojesen SE. Statin use and reduced cancer-related mortality. *N Engl J Med* 2012;367:1792–802.
6 Gaist D, Hallas J, Friis S, *et al*. Statin use and survival following glioblastoma multiforme. *Cancer Epidemiol* 2014;38:722–7.
7 Alizadeh J, Zeki AA, Mirzaei N, *et al*. Mevalonate cascade inhibition by simvastatin induces the intrinsic apoptosis pathway via depletion of isoprenoids in tumor cells. *Sci Rep* 2017;7:44841.
8 Altwairgi AK, Alghareeb W, Alnajjar F, *et al*. *Phase II study of atorvastatin in combination with radiotherapy and temozolomide In patients with glioblastoma (ART): interim analysis report Annals of Oncology*. 2016;27.
9 Matusewicz L, Meissner J, Toporkiewicz M, *et al*. The effect of statins on cancer cells-review. *Tumour Biol* 2015;36:4889–904.
10 Garcia-Ruiz C, Morales A, Fernandez-Checa JC. Statins and protein prenylation in cancer cell biology and therapy. *Anticancer Agents Med Chem* 2012;12:303–15.
11 Cordle A, Koenigsknecht-Talboo J, Wilkinson B, *et al*. Mechanisms of statin-mediated inhibition of small G-protein function. *J Biol Chem* 2005;280:34202–9.
12 Oh B, Kim TY, Min HJ, *et al*. Synergistic killing effect of imatinib and simvastatin on imatinib-resistant chronic myelogenous leukemia cells. *Anticancer Drugs* 2013;24:20–31.
13 Tapia-Pérez JH, Kirches E, Mawrin C, *et al*. Cytotoxic effect of different statins and thiazolidinediones on malignant glioma cells. *Cancer Chemother Pharmacol* 2011;67:1193–201.
14 Young-Xu Y, Jabbour S, Goldberg R, *et al*. Usefulness of statin drugs in protecting against atrial fibrillation in patients with coronary artery disease. *Am J Cardiol* 2003;92:1379–83.
15 Durazzo AE, Machado FS, Ikeoka DT, *et al*. Reduction in cardiovascular events after vascular surgery with atorvastatin: a randomized trial. *J Vasc Surg* 2004;39–967–75.
16 Kertai MD, Boersma E, Westerhout CM, *et al*. Association between long-term statin use and mortality after successful abdominal aortic aneurysm surgery. *Am J Med* 2004;116:96–103.
17 Lindenauer PK, Pekow P, Wang K, *et al*. Lipid-lowering therapy and in-hospital mortality following major noncardiac surgery. *JAMA* 2004;291:2092–9.
18 Ridker PM, Cannon CP, Morrow D, *et al*. C-reactive protein levels and outcomes after statin therapy. *N Engl J Med Overseas Ed* 2005;352:20–8.
19 Spampanato C, De Maria S, Sarnataro M, *et al*. Simvastatin inhibits cancer cell growth by inducing apoptosis correlated to activation of Bax and down-regulation of BCL-2 gene expression. *Int J Oncol* 2012;40:935–41.
20 Lewis KA, Holstein SA, Hohl RJ. Lovastatin alters the isoprenoid biosynthetic pathway in acute myelogenous leukemia cells in vivo. *Leuk Res* 2005;29:527–33.

21 Kim WS, Kim MM, Choi HJ, *et al*. Phase II study of high-dose lovastatin in patients with advanced gastric adenocarcinoma. *Invest New Drugs* 2001;19:81–3.
22 Sivaprasad U, Abbas T, Dutta A. Differential efficacy of 3-hydroxy-3-methylglutaryl CoA reductase inhibitors on the cell cycle of prostate cancer cells. *Mol Cancer Ther* 2006;5:2310–6.
23 Yu X, Luo Y, Zhou Y, *et al*. BRCA1 overexpression sensitizes cancer cells to lovastatin via regulation of cyclin D1-CDK4-p21WAF1/CIP1 pathway: analyses using a breast cancer cell line and tumoral xenograft model. *Int J Oncol* 2008;33:555–63.
24 Herrero-Martin G, López-Rivas A. Statins activate a mitochondria-operated pathway of apoptosis in breast tumor cells by a mechanism regulated by ErbB2 and dependent on the prenylation of proteins. *FEBS Lett* 2008;582:2589–94.
25 Zhu Y, Casey PJ, Kumar AP, *et al*. Deciphering the signaling networks underlying simvastatin-induced apoptosis in human cancer cells: evidence for non-canonical activation of RhoA and Rac1 GTPases. *Cell Death Dis* 2013;4:e568.
26 Kochuparambil ST, Al-Husein B, Goc A, *et al*. Anticancer efficacy of simvastatin on prostate cancer cells and tumor xenografts is associated with inhibition of Akt and reduced prostate-specific antigen expression. *J Pharmacol Exp Ther* 2011;336:496–505.
27 Ghosh-Choudhury N, Mandal CC, Ghosh-Choudhury N, *et al*. Simvastatin induces derepression of PTEN expression via NFkappaB to inhibit breast cancer cell growth. *Cell Signal* 2010;22:749–58.
28 Jiang P, Mukthavaram R, Chao Y, *et al*. In vitro and in vivo anticancer effects of mevalonate pathway modulation on human cancer cells. *Br J Cancer* 2014;111:1562–71.
29 Liu H, Wang Z, Li Y, *et al*. Simvastatin prevents proliferation and bone metastases of lung adenocarcinoma in vitro and in vivo. *Neoplasma* 2013;60:240–6.
30 Collisson EA, Kleer C, Wu M, *et al*. Atorvastatin prevents RhoC isoprenylation, invasion, and metastasis in human melanoma cells. *Mol Cancer Ther* 2003;2:941–8.
31 Farina HG, Bublik DR, Alonso DF, *et al*. Lovastatin alters cytoskeleton organization and inhibits experimental metastasis of mammary carcinoma cells. *Clin Exp Metastasis* 2002;19:551–60.
32 Horiguchi A, Sumitomo M, Asakuma J, *et al*. 3-hydroxy-3-methylglutaryl-coenzyme a reductase inhibitor, fluvastatin, as a novel agent for prophylaxis of renal cancer metastasis. *Clin Cancer Res* 2004;10:8648–55.
33 Manu KA, Shanmugam MK, Li F, *et al*. Simvastatin sensitizes human gastric cancer xenograft in nude mice to capecitabine by suppressing nuclear factor-kappa B-regulated gene products. *J Mol Med* 2014;92:267–76.
34 Holstein SA, Hohl RJ. Synergistic interaction of lovastatin and paclitaxel in human cancer cells. *Mol Cancer Ther* 2001;1:141–9.
35 Feleszko W, Młynarczuk I, Olszewska D, *et al*. Lovastatin potentiates antitumor activity of doxorubicin in murine melanoma via an apoptosis-dependent mechanism. *Int J Cancer* 2002;100:111–8.
36 Rozados VR, Hinrichsen LI, McDonnell J, *et al*. Lovastatin enhances in vitro radiation-induced apoptosis of rat B-cell lymphoma cells. *J Exp Clin Cancer Res* 2005;24:55–61.
37 Mace AG, Gantt GA, Skacel M, *et al*. Statin therapy is associated with improved pathologic response to neoadjuvant chemoradiation in rectal cancer. *Dis Colon Rectum* 2013;56:1217–27.
38 Knox JJ, Siu LL, Chen E, *et al*. A Phase I trial of prolonged administration of lovastatin in patients with recurrent or metastatic squamous cell carcinoma of the head and neck or of the cervix. *Eur J Cancer* 2005;41:523–30.
39 Hindler K, Cleeland CS, Rivera E, *et al*. The role of statins in cancer therapy. *Oncologist* 2006;11:306–15.
40 Chae YK, Yousaf M, Malecek MK, *et al*. Statins as anti-cancer therapy; Can we translate preclinical and epidemiologic data into clinical benefit? *Discov Med* 2015;20:413–27.
41 Bondy ML, Scheurer ME, Malmer B, *et al*. Brain tumor epidemiology: consensus from the Brain Tumor Epidemiology Consortium. *Cancer* 2008;113(7 Suppl):1953–68.
42 Gaist D, Andersen L, Hallas J, *et al*. Use of statins and risk of glioma: a nationwide case-control study in Denmark. *Br J Cancer* 2013;108:715–20.
43 Gjerstorff ML. The Danish Cancer Registry. *Scand J Public Health* 2011;39(7 Suppl):42–5.
44 Peng P, Wei W, Long C, *et al*. Atorvastatin augments temozolomide's efficacy in glioblastoma via prenylation-dependent inhibition of Ras signaling. *Biochem Biophys Res Commun* 2017;489:293–8.
45 Bil J, Zapala L, Nowis D, *et al*. Statins potentiate cytostatic/cytotoxic activity of sorafenib but not sunitinib against tumor cell lines in vitro. *Cancer Lett* 2010;288:57–67.
46 Calabro A, Tai J, Allen SL, *et al*. In-vitro synergism of m-TOR inhibitors, statins, and classical chemotherapy: potential implications in acute leukemia. *Anticancer Drugs* 2008;19:705–12.
47 Cemeus C, Zhao TT, Barrett GM, *et al*. Lovastatin enhances gefitinib activity in glioblastoma cells irrespective of EGFRvIII and PTEN status. *J Neurooncol* 2008;90:9–17.
48 Khanzada UK, Pardo OE, Meier C, *et al*. Potent inhibition of small-cell lung cancer cell growth by simvastatin reveals selective functions of Ras isoforms in growth factor signalling. *Oncogene* 2006;25:877–87.
49 Kozar K, Kaminski R, Legat M, *et al*. Cerivastatin demonstrates enhanced antitumor activity against human breast cancer cell lines when used in combination with doxorubicin or cisplatin. *Int J Oncol* 2004;24:1149–57.
50 Martirosyan A, Clendening JW, Goard CA, *et al*. Lovastatin induces apoptosis of ovarian cancer cells and synergizes with doxorubicin: potential therapeutic relevance. *BMC Cancer* 2010;10:103.
51 Roudier E, Mistafa O, Stenius U. Statins induce mammalian target of rapamycin (mTOR)-mediated inhibition of Akt signaling and sensitize p53-deficient cells to cytostatic drugs. *Mol Cancer Ther* 2006;5:2706–15.
52 Stirewalt DL, Appelbaum FR, Willman CL, *et al*. Mevastatin can increase toxicity in primary AMLs exposed to standard therapeutic agents, but statin efficacy is not simply associated with ras hotspot mutations or overexpression. *Leuk Res* 2003;27:133–45.
53 Yongjun Y, Shuyun H, Lei C, *et al*. Atorvastatin suppresses glioma invasion and migration by reducing microglial MT1-MMP expression. *J Neuroimmunol* 2013;260(1-2):1–8.
54 Chang YL, Huang LC, Chen YC, *et al*. The synergistic effects of valproic acid and fluvastatin on apoptosis induction in glioblastoma multiforme cell lines. *Int J Biochem Cell Biol* 2017;92:155–63.
55 Yanae M, Tsubaki M, Satou T, *et al*. Statin-induced apoptosis via the suppression of ERK1/2 and Akt activation by inhibition of the geranylgeranyl-pyrophosphate biosynthesis in glioblastoma. *J Exp Clin Cancer Res* 2011;30:74–81.
56 Stupp R, Mason WP, van den Bent MJ, *et al*. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;352:987–96.
57 Zhang WB, Wang Z, Shu F, *et al*. Activation of AMP-activated protein kinase by temozolomide contributes to apoptosis in glioblastoma cells via p53 activation and mTORC1 inhibition. *J Biol Chem* 2010;285:40461–71.
58 Thanasupawat T, Natarajan S, Rommel A, *et al*. Dovitinib enhances temozolomide efficacy in glioblastoma cells. *Mol Oncol* 2017;11:1078–98.
59 Roos WP, Batista LF, Naumann SC, *et al*. Apoptosis in malignant glioma cells triggered by the temozolomide-induced DNA lesion O6-methylguanine. *Oncogene* 2007;26:186–97.
60 Weatherbee JL, Kraus JL, Ross AH. ER stress in temozolomide-treated glioblastomas interferes with DNA repair and induces apoptosis. *Oncotarget* 2016;7:43820–34.
61 Palmer AC, Sorger PK. Combination cancer therapy can confer benefit via patient-to-patient variability without drug additivity or synergy. *Cell* 2017;171–1678–91.
62 Doroshow JH, Simon RM. On the design of combination cancer therapy. *Cell* 2017;171:1476–8.
63 Minn AJ, Wherry EJ. Combination cancer therapies with immune checkpoint blockade: Convergence on interferon signaling. *Cell* 2016;165:272–5.
64 Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* 2015;161:205–14.
65 Wang CY, Liu PY, Liao JK. Pleiotropic effects of statin therapy: molecular mechanisms and clinical results. *Trends Mol Med* 2008;14:37–44.
66 Likus W, Siemianowicz K, Bieńk K, *et al*. Could drugs inhibiting the mevalonate pathway also target cancer stem cells? *Drug Resist Updat* 2016;25:13–25.
67 Olivieri F, Mazzanti I, Abbatecola AM, *et al*. Telomere/Telomerase system: a new target of statins pleiotropic effect? *Curr Vasc Pharmacol* 2012;10:216–24.
68 Ghavami S, Mutawe MM, Hauff K, *et al*. Statin-triggered cell death in primary human lung mesenchymal cells involves p53-PUMA and release of Smac and Omi but not cytochrome c. *Biochim Biophys Acta* 1803;2010:452–67.
69 Jang HJ, Hong EM, Park SW, *et al*. Statin induces apoptosis of human colon cancer cells and downregulation of insulin-like growth factor 1 receptor via proapoptotic ERK activation. *Oncol Lett* 2016;12:250–6.
70 Swanson KM, Hohl RJ. Anti-cancer therapy: targeting the mevalonate pathway. *Curr Cancer Drug Targets* 2006;6:15–37.
71 Ghavami S, Yeganeh B, Stelmack GL, *et al*. Apoptosis, autophagy and ER stress in mevalonate cascade inhibition-induced cell death of human atrial fibroblasts. *Cell Death Dis* 2012;3:e330.
72 Lawson CD, Ridley AJ. Rho GTPase signaling complexes in cell migration and invasion. *J Cell Biol* 2018;217:447–57.
73 Kazanietz MG, Caloca MJ. THe rac GTPase in cancer: from old concepts to new paradigms. *Cancer Res* 2017;77:5445–51.

74 Matzno S, Yasuda S, Juman S, *et al*. Statin-induced apoptosis linked with membrane farnesylated Ras small G protein depletion, rather than geranylated Rho protein. *J Pharm Pharmacol* 2005;57:1475–84.
75 Tsubaki M, Fujiwara D, Takeda T, *et al*. The sensitivity of head and neck carcinoma cells to statins is related to the expression of their Ras expression status, and statin-induced apoptosis is mediated via suppression of the Ras/ERK and Ras/mTOR pathways. *Clin Exp Pharmacol Physiol* 2017;44:222–34.
76 Zandvakili I, Lin Y, Morris JC, *et al*. Rho GTPases: Anti- or pro-neoplastic targets? *Oncogene* 2017;36:3213–22.
77 Shojaei S, Alizadeh J, Thliveris J, *et al*. Inhibition of autophagy by mevalonate pathway inhibitors, a new therapeutic approach to sensitize glioblastoma cells to temozolomide induced apoptosis. *FASEB Jounral* 2018;32.533.41.
78 Garwood ER, Kumar AS, Baehner FL, *et al*. Fluvastatin reduces proliferation and increases apoptosis in women with high grade breast cancer. *Breast Cancer Res Treat* 2010;119:137–44.
79 Han JY, Lim KY, Yu SY, Sy Y, *et al*. A phase 2 study of irinotecan, cisplatin, and simvastatin for untreated extensive-disease small cell lung cancer. *Cancer* 2011;117:2178–85.
80 Seckl MJ, Ottensmeier CH, Cullen M, *et al*. Multicenter, Phase III, Randomized, Double-Blind, Placebo-Controlled Trial of pravastatin added to first-line standard chemotherapy in small-cell lung cancer (LUNGSTAR). *J Clin Oncol* 2017;35:1506–14.
81 Allott EH, Howard LE, Vidal AC, *et al*. Statin Use, Serum Lipids, and Prostate inflammation in men with a negative prostate biopsy: results from the REDUCE trial. *Cancer Prev Res* 2017;10:319–26.
82 El-Hamamsy M, Elwakil H, Saad AS, *et al*. A randomized controlled open-label pilot study of simvastatin addition to whole-brain radiation therapy in patients with brain metastases. *Oncol Res* 2016;24:521–8.
83 Karp I, Behlouli H, Lelorier J, *et al*. Statins and cancer risk. *Am J Med* 2008;121:302–9.