Statin-Dependent Suppression of the Akt/Mammalian Target of Rapamycin Signaling Cascade and Programmed Cell Death 4 Up-Regulation in Renal Cell Carcinoma

Jennifer Woodard,1 Antonella Sassano,1 Nissim Hay,2 and Leonidas C. Platanias1

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Purpose: Statins are pharmacologic inhibitors of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase with potent regulatory effects on cholesterol biosynthesis in vitro and in vivo. There is accumulating evidence that, beyond their cholesterol-lowering properties, statins inhibit cell proliferation and promote apoptosis of malignant cells in vitro, but the mechanisms by which they generate such responses remain to be defined.

Experimental Design: Combinations of experimental approaches were used, including immunoblotting and cell proliferation and apoptosis assays.

Results: We provide evidence that fluvastatin is a potent inducer of apoptosis and suppresses proliferation of renal cell carcinoma (RCC) cells in vitro. Such effects are mediated by direct targeting of the Akt/mammalian target of rapamycin (mTOR) pathway, as evidenced by the suppression of phosphorylation/activation of Akt, resulting in inhibition of its downstream effectors, mTOR and p70 S6 kinase. In addition, fluvastatin blocks the mTOR-dependent phosphorylation/deactivation of the translational repressor eukaryotic initiation factor 4E (eIF4E)-binding protein, leading to the formation of eIF4E-binding protein-eIF4E complexes that suppress initiation of cap-dependent mRNA translation. Importantly, inhibition of p70 S6 kinase activity by fluvastatin results in the up-regulation of expression of programmed cell death 4 (PDCD4), a tumor suppressor protein with inhibitory effects on the translation initiation factor eIF4A, suggesting a mechanism for the generation of antitumor responses.

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A two-tailed P value of 0.00002.

Apoptosis of 786-0 cells treated for 96 h versus control DMSO-treated cells showed a two-tailed P = 0.009. Paired t test analysis for the growth of 786-0 cells treated for 72 h versus control DMSO-treated cells showed a two-tailed P = 0.006. Paired t test analysis for the growth of 786-0 cells treated with 2 μmol/L fluvastatin versus control DMSO-treated cells showed a two-tailed P = 0.036. Paired t test analysis for the growth of 786-0 cells treated with 3 μmol/L fluvastatin versus control DMSO-treated cells showed a two-tailed P = 0.005.

Materials and Methods

Cell lines and reagents. The 786-0 cell line was grown in RPMI 1640 supplemented with 10% fetal bovine serum, antibiotics, 10 mmol/L HEPES buffer, and 1 mmol/L sodium pyruvate. The CAKI-2 cell line was grown in McCoy's medium supplemented with 10% fetal bovine serum and antibiotics. Rat1a (MycER) cells were retrovirally transfected with an activated form of Akt with the Src myristoylation signal fused in-frame to the c-Akt coding sequence (Myr-Akt; ref. 29) were grown in DMEM supplemented with 10% fetal bovine serum and antibiotics. Fluvastatin was provided by Novartis. Atorvastatin was purchased from 21CEC Pharmaceuticals. Rapamycin was purchased from Calbiochem. Geranylgeranyl pyrophosphate (GGPP) ammonium salt was purchased from Sigma-Aldrich. Antibodies against the phosphorylated forms of Akt (Ser 473; Thr 308), mTOR (Ser 2448), S6K (Thr 389), S6 ribosomal protein (rpS6; Ser 240/244), and eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP1; Ser 65) were obtained from Cell Signaling Technology. Antibodies to detect Akt, mTOR, S6K, rpS6, 4E-BP1, and eIF4E were also purchased from Cell Signaling Technology. An anti-phospho-PRAS antibody was obtained from Abcam and Santa Cruz Biotechnology. Agarose-conjugated eIF4E antibody and control nonimmune mouse immunoglobulin were obtained from Santa Cruz Biotechnology. HA-Myr-Akt vector was generated previously as described in ref. 30.

Cell lysis and immunoblotting. Cells were treated with fluvastatin or DMSO (control) for the indicated times and were subsequently lysed in phosphorylation lysis buffer as described previously (31, 32). Immunoprecipitations and immunoblotting using an enhanced chemiluminescence method were done as in previous studies (27, 31, 32). For
immunoblotting, an anti-GAPDH antibody (Chemicon) was diluted 1:5,000, an anti-tubulin antibody (Abcam) was diluted 1 μg/mL, and an anti-tubulin antibody (Santa Cruz Biotechnology) was diluted 1:200. Unless otherwise specified, all other antibody dilutions for Western blotting were 1:1,000.

Cell proliferation assays. Cells were seeded in 96-well plates and incubated in the presence of DMSO (control) or the indicated concentrations of fluvastatin at 37°C for 4 days. Cell proliferation was assessed using a methyl-thiazolyl-tetrazolium assay system as described previously (33).

Flow cytometric analysis. Flow cytometric studies to detect apoptosis by Annexin V/propidium iodide staining were done as in our previous studies (27, 34).

Quantitative reverse transcription-PCR. Cells were treated with either solvent control (DMSO) or fluvastatin (5 μmol/L) for the indicated times, and quantitative reverse transcription-PCR was carried out as described (35). Real-time reverse transcription-PCR to determine expression of PDCD4 mRNA was carried out using commercially available FAM-labeled probes and primers (Applied Biosystems). GAPDH was used for normalization.

Results

We initially examined whether fluvastatin promotes growth suppression and induces proapoptotic effects on human RCC cells. For these experiments, two human RCC cell lines, 786-0 and CAKI-2, were used. Fluvastatin significantly inhibited the growth of both cell lines as shown in cell proliferation assays (Fig. 1A). Such growth-inhibitory effects were dose dependent, with significant inhibition seen at final concentrations of 2 to 3 μmol/L (Fig. 1A), concentrations that are achievable when statins are administered to humans (36). In a similar manner, treatment of either 786-0 or CAKI-2 cells with fluvastatin resulted in induction of apoptosis, with a maximum effect seen after 72 to 96 h of treatment of the cells (Fig. 1B).

The Akt kinase plays a critical role in the induction of anti-apoptotic, prosurvival signals and acts as an upstream effector of the mTOR pathway in response to cytokines and growth factors (37–39). There is emerging evidence that constitutive activation of mTOR plays a critical role in the survival and growth of RCC cells, and targeted therapies using mTOR inhibitors have been established as the most efficacious approach to date in the treatment of RCC (40, 41). We examined the effects of fluvastatin on the phosphorylation/activation of Akt and mTOR. When RCC cells were treated with fluvastatin for 48 h, there was suppression of the phosphorylation of Akt at Ser473 (Fig. 2A), a site important for its kinase activity, and Thr308 (Fig. 2A), the PDK1 phosphorylation site (37, 38). Thus, fluvastatin treatment of RCC cells blocks phosphorylation/activation of Akt, suggesting a mechanism by which statins may be blocking activation of mTOR to generate proapoptotic and growth-inhibitory effects on RCC cells.

To directly determine whether downstream engagement of mTOR and p70 S6K is suppressed by statin treatment, cells were treated with fluvastatin and the phosphorylation/activation of mTOR and p70 S6K was assessed by anti-phospho-mTOR and anti-phospho-p70 S6K immunoblotting. Treatment of cells with fluvastatin resulted in suppression of phosphorylation of mTOR on Ser2448 (Fig. 2B) and p70 S6K on Thr389 (Fig. 2C), consistent with deactivation of the pathway, apparently due to inhibition of Akt activity. In addition, fluvastatin treatment of the cells resulted in dephosphorylation of PRAS40 (Fig. 2D), a mTORC1 phosphorylation substrate that was recently shown to act upstream of p70 S6K and 4E-BP1 (42). Thus, by suppressing

![Fig. 2. Statin-dependent inhibition of phosphorylation/activation of Akt signaling in RCC cells. A, top left, top, 786-0 cells were treated with solvent control (DMSO) or fluvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of Akt at Ser473. Middle, the same blot was stripped and reprobed with an antibody to detect anti-GAPDH to control for protein loading. Top right, top, 786-0 cells were treated with solvent control (DMSO) or fluvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/ activated form of Akt at Ser473 and GAPDH. Bottom, the same blot was subsequently reprobed with an antibody to detect anti-GAPDH to control for protein loading. B, top left, top, 786-0 cells were treated with solvent control (DMSO) or atorvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of Akt at Ser473. Middle, the same blot was reprobed with an antibody to detect anti-GAPDH to control for protein loading. Bottom right, top, 786-0 cells were treated with solvent control (DMSO) or atorvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of Akt at Ser473 and GAPDH. Bottom, the same blot was reprobed with anti-GAPDH antibody to control for protein loading.](www.aacrjournals.org)
Akt, fluvastatin appears to negatively regulate sequential activation of the mTOR-p70S6K signaling cascade. Similarly, suppression of phosphorylation/activation of Akt was observed when another new generation statin, atorvastatin, was used (Fig. 2A).

To examine whether the downstream signals generated by the mTOR pathway are blocked in response to statin treatment of RCC cells, experiments were done to examine the effects of statin treatment on different downstream effectors of the pathway. Initially, we examined the effects of fluvastatin on the phosphorylation of the effector of p70 S6K, rpS6. This protein is a direct target for p70 S6K activity, whose phosphorylation promotes the translation of mRNA that have oligopyrimidine tracts in the 5’ untranslated region (39, 43). Fluvastatin treatment of either 786-0 or Caki-2 cells strongly suppressed phosphorylation of rpS6 (Fig. 3A), establishing that this downstream effector of p70 S6K is statin sensitive. We also examined the effects of fluvastatin treatment on the phosphorylation of the translational repressor 4E-BP1, a protein that plays a key regulatory role on the initiation of cap-dependent mRNA translation by negatively controlling the function of eIF4E (44). Fluvastatin treatment of cells dramatically suppressed phosphorylation of 4E-BP1 (Fig. 3B). We also did studies to understand whether engagement of the regulatory effects of fluvastatin on the Akt/mTOR/p70 S6K pathway occur downstream of its effects on the mevalonate pathway. We determined whether addition of GGPP, a component of the mevalonate pathway whose expression is known to be down-regulated in the presence of statins (8–12), in RCC cells reverses its suppressive effects on the activation of the mTOR pathway. Treatment of 786-0 cells with GGPP reversed the apoptotic effects of fluvastatin (Fig. 3C) and the dephosphorylation of Akt, rpS6, and 4E-BP1 (Fig. 3D), strongly suggesting that the effects of statins on the regulation of the Akt/mTOR pathway in RCC cells occur downstream of regulatory effects on the mevalonate pathway.

As the nonphosphorylated form of 4E-BP1 is the active form that suppresses eIF4E activity, we proceeded to directly determine whether statin treatment of cells enhances the

![Figure 2](https://www.aacrjournals.org/doi/fig/10.1158/1078-0432.CCR-07-1720@apps7)
formation of 4E-BP1-eIF4E complexes. Cells were treated with fluvastatin or atorvastatin, and after immunoprecipitation with an anti-eIF4E antibody, immunoprecipitated complexes were resolved by SDS-PAGE and immunoblotted with an anti-4E-BP1 antibody. As shown in Fig. 4, treatment of either 786-0 (Fig. 4A) or CAKI-2 cells were treated with either solvent control (DMSO) or fluvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated form of rpS6 at Ser240/Ser244. Bottom, the same blot was reprobed with an anti-GAPDH antibody to control for protein loading. Right, top, CAKI-2 cells were treated with either solvent control (DMSO) or atorvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of 4E-BP1 at Ser65. Bottom, the same blot was reprobed with an anti-GAPDH antibody to control for protein loading. C, 786-0 cells were treated with solvent control, GGPP ammonium salt (10 μmol/L), fluvastatin (5 or 10 μmol/L), or the indicated combinations for 72 h. Data are expressed as percent control (solvent-treated) cells. Mean ± SE of three independent experiments. Paired t test analysis for the induction of apoptosis of 786-0 cells treated with fluvastatin (5 μmol/L) versus control DMSO-treated cells showed a two-tailed P = 0.00003. Paired t test analysis for the induction of apoptosis of 786-0 cells treated with fluvastatin (10 μmol/L) versus control DMSO-treated cells showed a two-tailed P = 0.006. Paired t test analysis for the induction of apoptosis of 786-0 cells treated with GGPP (10 μmol/L) and fluvastatin (5 μmol/L) versus control fluvastatin (5 μmol/L)−treated cells showed a two-tailed P = 0.004. Paired t test analysis for the induction of apoptosis of 786-0 cells treated with GGPP (10 μmol/L) and fluvastatin (10 μmol/L) versus control GGPP (10 μmol/L)−treated cells showed a two-tailed P = 0.004.

Fig. 3. Fluvastatin-dependent suppression of mTOR-mediated signals. A, left, top, 786-0 cells were treated with either solvent control (DMSO) or fluvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of rpS6 at Ser240/Ser244. Bottom, the same blot was reprobed with an anti-GAPDH antibody to control for protein loading. Right, top, 786-0 cells were treated with either solvent control (DMSO) or fluvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of 4E-BP1 at Ser65. Bottom, the same blot was reprobed with an anti-GAPDH antibody to control for protein loading. B, left, top, 786-0 cells were treated with either solvent control (DMSO) or atorvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of rpS6 at Ser240/Ser244. Bottom, the same blot was reprobed with an anti-GAPDH antibody to control for protein loading. Left, top, CAKI-2 cells were treated with either solvent control (DMSO) or atorvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were subjected to SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of 4E-BP1 at Ser65. Bottom, the same blot was reprobed with an anti-GAPDH antibody to control for protein loading. C, 786-0 cells were treated with solvent control, GGPP ammonium salt (10 μmol/L), fluvastatin (5 or 10 μmol/L), or the indicated combinations for 72 h. Data are expressed as percent control (solvent-treated) cells. Mean ± SE of three independent experiments. Paired t test analysis for the induction of apoptosis of 786-0 cells treated with fluvastatin (5 μmol/L) versus control DMSO-treated cells showed a two-tailed P = 0.00003. Paired t test analysis for the induction of apoptosis of 786-0 cells treated with fluvastatin (10 μmol/L) versus control DMSO-treated cells showed a two-tailed P = 0.006. Paired t test analysis for the induction of apoptosis of 786-0 cells treated with GGPP (10 μmol/L) and fluvastatin (5 μmol/L) versus control fluvastatin (5 μmol/L)−treated cells showed a two-tailed P = 0.004. Paired t test analysis for the induction of apoptosis of 786-0 cells treated with GGPP (10 μmol/L) and fluvastatin (10 μmol/L) versus control GGPP (10 μmol/L)−treated cells showed a two-tailed P = 0.004.
induction of the proapoptotic effects of fluvastatin on target cells. We determined whether overexpression of a constitutively active Akt mutant (Myr-Akt) reverses the proapoptotic effects of fluvastatin. We compared the ability of fluvastatin to induce apoptosis in Rat1a cells stably expressing Myr-Akt (Fig. 6A; ref. 49) to parental Rat1a cells expressing endogenous Akt. As shown in Fig. 6B, there was a dramatic reversal of fluvastatin-induced apoptosis in cells stably overexpressing Myr-Akt (Fig. 6B). Consistent with these results, overexpression of Akt in these Rat1a cells was associated with an inability of fluvastatin to inhibit 4E-BP1 phosphorylation (Fig. 6C). Similarly, in experiments in which the constitutively active Akt mutant was overexpressed in 786-0 cells, fluvastatin failed to suppress mTOR/p70S6K as reflected by the lack of suppression of rpS6 phosphorylation (Fig. 6D). Thus, overexpression of a constitutively active Akt results in reversal of suppression of the mTOR pathway and induction of apoptosis by fluvastatin, establishing that Akt targeting is a critical event in the induction of statin-dependent apoptosis in RCC cells.

**Discussion**

The discovery of statins and their introduction in the treatment of hypercholesterolemia in humans has had a dramatic effect in clinical medicine. Not only did these agents change the natural history of coronary artery disease, but their introduction in clinical practice raised the possibility of potential beneficial effects on other disorders and syndromes. A research area of particular relevance is the identification of potential applications for statins in the prevention and/or treatment of malignancies. A plethora of evidence over the last decade has established that statins induce apoptosis and promote growth suppression of different types of malignant cells in vitro and in vivo (24–27). There is also emerging evidence that statins exhibit such effects when administered to humans (50), raising the prospect of a potential future utility of these agents in the prevention and/or treatment of certain malignancies.

Despite the rapid accumulation of new information on the role of statins against different types of malignant cells, the mechanisms by which these 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors elicit antitumor responses remain unknown. Recent studies have implicated mitogen-activated protein kinase signaling pathways as mediators of statin-dependent proapoptotic effects. There is some evidence showing that atorvastatin and fluvastatin activate the JNK pathway and that such activation is essential for the induction of apoptosis (27). Other work has established that lovastatin blocks engagement of the Raf/MEK/extracellular signal-regulated kinase pathway, and such effects appear to sensitize cells to statin-induced apoptosis (15). On the other hand, statins do not appear to positively or negatively regulate the p38 mitogen-activated protein kinase cascade (15, 27), suggesting that this mitogen-activated protein kinase cascade does not play a role in the induction of statin responses. Thus, coordinated effects of statins on different mitogen-activated protein kinase pathways need to be elucidated to fully understand the molecular mechanisms underlying statin-dependent apoptosis.

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**Fig. 3** Continued. D, top left, top, 786-0 cells were treated with solvent control, GGPP (10 μmol/L), fluvastatin (5 μmol/L), or a combination of GGPP (10 μmol/L) and fluvastatin (5 μmol/L). Equal amounts of total cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of Akt at Ser473. Middle, the same blot was stripped and reprobed with an antibody against Akt; bottom, the blot was subsequently reprobed with an anti-GAPDH antibody to control for protein loading. Top right, top, 786-0 cells were treated with solvent control, GGPP (10 μmol/L), fluvastatin (5 μmol/L), or a combination of GGPP (10 μmol/L) and fluvastatin (5 μmol/L). Equal amounts of total cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of rpS6 at Ser240/244. Middle, the same blot was stripped and reprobed with an antibody against rpS6; bottom, the blot was subsequently reprobed with an anti-GAPDH antibody to control for protein loading.
protein kinase signaling pathways may account for their balanced action on target cells and optimal generation of growth inhibition and/or apoptosis.

A cellular pathway of critical importance in the regulation of cell proliferation, growth, and cell survival is the Akt/mTOR signaling cascade (37–39, 44). This pathway is of particular relevance in the pathogenesis and pathophysiology of renal cancer, as it appears to be a major regulator of the hypoxia-inducible factor 1α and 2α (HIF-1α and HIF-2α) proteins (51). It is well established that loss of function of the von Hippel-Lindau gene occurs in RCC and is associated with increased accumulations of HIF-1α and HIF-2α, whose mRNA contain oligopyrimidine tracts in the 5′ untranslated region (52, 53). It is also established that the downstream effector of the mTOR/p70 S6K pathway, rpS6, regulates translation of such mRNA (39), consistent with an important role of this pathway in the control of HIF-1α/HIF-2α expression in this malignancy. It should be, however, noted that in some RCC cells, which do not have the characteristic loss of function of the von Hippel-Lindau gene, including CAKI-2, there is very little HIF-1α and HIF-2α (54). The key role of the mTOR pathway in the control of cell growth and survival of RCC cells has been documented extensively by the work of many research groups (52) and has led to several clinical trials over the years that have provided evidence for important clinical activities of temsirolimus and everolimus in the treatment of this disease (41, 52).

![Fig. 4. Statin-dependent formation of 4E-BP1-eIF4E complexes.](image)

**A**, top, 786-0 cells were treated with solvent control (DMSO), fluvastatin (5 μmol/L), or atorvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were immunoprecipitated with agarose-eIF4E beads or with control nonimmune mouse immunoglobulin (IgG). The immunoprecipitates were then subjected to SDS-PAGE and immunoblotted with an antibody against 4E-BP1. **Bottom**, the same blot was stripped and reprobed with an antibody against eIF4E.

**B**, top, CAKI-2 cells were treated with solvent control (DMSO), fluvastatin (2 μmol/L), or atorvastatin (2 μmol/L) for 48 h. Equal amounts of total cell lysates were immunoprecipitated with agarose-eIF4E beads or with control nonimmune mouse immunoglobulin. The immunoprecipitates were then subjected to SDS-PAGE and immunoblotted with an antibody against 4E-BP1. **Bottom**, the same blot was stripped and reprobed with an antibody against eIF4E.

![Fig. 5. Effect of fluvastatin on PDCD4 protein expression in RCC cells.](image)

**A**, top, 786-0 cells were treated with solvent control (DMSO), rapamycin (10 nmol/L), or fluvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against PDCD4. **Bottom**, the same immunoblot was reprobed with an anti-GAPDH antibody to control for protein loading.

**B**, top, CAKI-2 cells were treated with solvent control (DMSO) or fluvastatin (2 μmol/L) for 48 h. Equal amounts of total cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against PDCD4. **Bottom**, the same immunoblot was reprobed with an anti-GAPDH antibody to control for protein loading.

**C**, top, 786-0 cells were treated with solvent control (DMSO) or atorvastatin (5 μmol/L) for 48 h. Equal amounts of total cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against PDCD4. **Bottom**, the same immunoblot was reprobed with an anti-GAPDH antibody to control for protein loading.

**D**, 786-0 cells were treated with either solvent control (DMSO) or fluvastatin (5 μmol/L) for the indicated times and PDCD4 mRNA expression was assessed by quantitative real-time PCR using GAPDH as control. Mean ± SE of three independent experiments.
tensirolimus (41) was recently approved by the Food and Drug Administration for the treatment of RCC and appears to be the single most effective agent identified thus far for the treatment of this malignancy. Because of the high relevance of the mTOR pathway in survival and growth of RCC cells, identification of other novel inhibitors of this pathway and determination of their precise mechanisms of action are of high interest, as it may ultimately lead to the development of new combinations and/or targeted therapies for the treatment of this malignancy.

In the present study, we provide evidence that statins suppress activation of the mTOR pathway in RCC cells. Our studies show that fluvastatin and/or atorvastatin block phosphorylation/activation of Akt, resulting in downstream inhibition of mTOR and the p70 S6K. In addition, treatment of RCC cells with fluvastatin inhibits phosphorylation of 4E-BP1 and enhances the binding of 4E-BP1 to eIF4E, events that are well known to result in blocking of eIF4E activity and suppression of cap-dependent mRNA translation (44). These data provide the first evidence linking statin-dependent suppression of Akt activity to inhibition of the mTOR pathway in malignant cells. Although there has been some recent evidence suggesting that statins may be modifying activation of the mTOR pathway, the mechanisms by which such effects occur are not known, as there has also been some conflicting information. One study (55) recently showed that atorvastatin induces phosphorylation of mTOR on Ser2448 as well as mTOR-dependent phosphorylation of insulin receptor substrate-1 in hepatocellular and lung carcinoma cell lines. In addition, that study had suggested that statin-mediated mTOR activation results in inhibition of Akt phosphorylation and nuclear translocation and sensitizes cells to cytostatic drugs (55). On the other hand, another very recent study showed that atorvastatin inhibits the growth of Tsc2 knockout mouse embryonic fibroblasts and smooth muscle cells by blocking Rheb and Rho GTPase function and reducing mTOR/p70 S6K activity (56). These studies, which were done using nonmalignant cells (mouse embryonic fibroblasts), suggested that Tsc2 may be playing a regulatory role on the effects of statins in normal cells, as there was enhanced suppression of growth in cells with targeted disruption of the Tsc2 gene (56). Interestingly, in that study, the authors failed to observe significant suppression of Akt phosphorylation by atorvastatin in Tsc2−/− nonmalignant mouse embryonic fibroblasts (56). Our data establishing that in malignant RCC cells statins suppress phosphorylation/activation of Akt, followed by inhibition of mTOR activity, suggest a different mechanism of regulation of mTOR and its effectors in malignant renal cells,
with Akt being the primary target for the suppressive effects of statins. Consistent with this, our studies showed that induction of fluvastatin-dependent apoptosis is reversible by overexpression of a constitutively active Akt mutant, whereas such overexpression also reversed the fluvastatin-dependent suppression of activation of the mTOR pathway.

In efforts to identify downstream effectors of the mTOR/p70 S6K pathway that may mediate antitumor responses, we found that PDCD4 is induced in a fluvastatin-dependent manner in malignant cells. PDCD4 is known to exhibit tumor suppressor activity and appears to act by inhibiting eIF4A, a RNA helicase whose function is important in the secondary structure at the 5’ untranslated regions of mRNA (45–48). A recent study has established that PDCD4 is a substrate for the kinase activity of p70 S6K1 and that its S6K1-phosphorylated form on Ser24 undergoes degradation by the ubiquitin ligase SCP (JTRCP; ref. 45). Our findings that fluvastatin up-regulates PDCD4 expression, which is associated with inhibition of p70 S6K activity, suggest that the primary mechanism of statin-dependent induction of this tumor suppressor protein is inhibition of the Akt/mTOR pathway. This is further supported by the fact that rapamycin also up-regulates the expression of this protein in RCC cells and further suggests that Akt/mTOR inhibition is a major mechanism by which statins mediate their effects.

Taken altogether, our studies suggest that statins have activity against RCC cells, which is associated with suppression of Akt/mTOR activity and downstream formation of complexes that suppress cap-dependent mRNA translation as well as up-regulation of the tumor suppressor PDCD4. They also raise the possibility that combinations of statins with mTOR inhibitors or other drugs with activity in RCC may be an effective approach in the treatment of RCC and provide the basis for future clinicotranslational efforts in that direction.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

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Statin-Dependent Suppression of the Akt/Mammalian Target of Rapamycin Signaling Cascade and Programmed Cell Death 4 Up-Regulation in Renal Cell Carcinoma

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