S6 Kinase 2 Promotes Breast Cancer Cell Survival via Akt

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Abstract

The 40S ribosomal protein S6 kinase (S6K) acts downstream of mTOR, which plays important roles in cell proliferation, protein translation, and cell survival and is a target for cancer therapy. mTOR inhibitors are, however, of limited success. Although Akt is believed to act upstream of mTOR, persistent inhibition of p70 S6 kinase or S6K1 can activate Akt via a negative feedback loop. S6K exists as two homologues, S6K1 and S6K2, but little is known about the function of S6K2. In the present study, we have examined the effects of S6K2 on Akt activation and cell survival. Silencing of S6K1 caused a modest decrease, whereas knockdown of S6K2 caused a substantial increase in TNF-α and TRAIL (TNF-related apoptosis-inducing ligand)-mediated apoptosis. In contrast to S6K1, depletion of S6K2 by siRNA decreased basal and TNF-induced Akt phosphorylation. Ectopic expression of constitutively active Akt in MCF-7 cells restored cell survival in S6K2-depleted cells. We have previously shown that activation of Akt induces downregulation of Bid via p53. Knockdown of S6K2 caused an increase in p53, and downregulation of p53 by siRNA decreased Bid level. Silencing of Bid blunted the ability of S6K2 deficiency to enhance TNF-induced apoptosis. Taken together, our study shows that the two homologues of S6K have distinct effects on Akt activation and cell survival. Thus, targeting S6K2 may be an effective therapeutic strategy to treat cancers. *Cancer Res; 71(7); 2590–9. ©2011 AACR.*

Introduction

Akt or protein kinase B (PKB), a serine/threonine kinase, is the cellular homologue of the oncogene product *v-Akt* (1). It is activated downstream of phosphoinositide 3-kinase (PI3K) in response to growth factors or cytokines. Akt performs diverse cellular functions, including cell growth, proliferation, and survival (2). It is deregulated in many cancers, including breast cancer, and confers resistance to chemotherapeutic drugs (3). Phosphorylation of Akt at Thr308 and Ser473 sites results in its activation (4).

TNF-α was originally identified as a cytokine that induces necrosis in tumors and regression of cancer in animals (5). It causes selective destruction of tumor tissues but has no effect on normal tissues (6). The presence of antiapoptotic proteins, however, can counteract cell death mediated by TNF. It has been reported that TNF causes activation of Akt through phosphorylation at Ser473 (7). Binding of TNF to its cell surface receptors causes activation of initiator caspase-8, followed by activation of effector caspases such as caspase-3 and caspase-7, resulting in the cleavage of critical cellular proteins and cell death (8, 9). Although caspase-8 is the apical caspase in the death receptor pathway, there is cross-talk between the receptor-initiated and mitochondrial pathways (10–12). The members of the Bcl-2 family proteins play important roles in regulating the intrinsic or mitochondrial cell death pathway (13, 14). Caspase-8 catalyzes the cleavage of the Bcl-2 family protein Bid (10–12). The truncated Bid (tBid) translocates to mitochondria, causing release of cytochrome *c* and activation of caspase-9 (10–12). It has been reported that Akt can exert its antiapoptotic function by inhibiting the function of proapoptotic Bcl-2 family proteins (15–20).

Several cellular functions of Akt are mediated by mTOR, which is considered the master controller of protein synthesis and cell proliferation (21). Activated Akt can phosphorylate and inactivate tuberous sclerosis complex 2 (TSC2), which negatively regulates mTOR (22). mTOR interacts with either raptor or rictor to form mTOR complex I (mTORC1) or mTOR complex 2 (mTORC2), respectively (22). While phosphoinositide-dependent kinase 1 (PDK1), which acts downstream of PI3K, phosphorylates Akt at Thr308 site, rictor complexed with mTORC2 can phosphorylate Akt at Ser473 (22). mTORC1 is inhibited by rapamycin, which is currently being tested for use in cancer therapy, albeit with limited success (23).

The 40S ribosomal protein S6 kinase (S6K) is a downstream target of mTORC1 (24). S6K is represented by 2 homologous cellular proteins, S6K1 and S6K2, both of which act downstream of mTOR and phosphorylate S6 (25). Persistent inhibition of S6K1 has been shown to activate Akt via feedback inhibition of the PI3K pathway wherein S6K1 phosphorylates several sites on IRS-1 (insulin receptor substrate-1) and inhibits it (26–30). The limited therapeutic efficacy of rapamycin and its analogues has been attributed to the activation of Akt via this negative feedback loop due to inhibition of S6K1 (26, 29) and the inability of rapamycin to completely activate

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4E-BP (eIF4E-binding protein), another downstream target of mTORC1 (31–33).

Although there are 2 homologues of S6K (25, 34), most of the studies have been focused on S6K1 and little is known about the function of S6K2. S6K1-deficient mice phosphorylated S6 but had a small body phenotype (35). S6K1/2 double-knockout mice also exhibit normal proliferation and growth reduction (36). Similarly, S6K1/2 double-knockout mouse embryo fibroblasts and myoblasts show defects in size but not proliferation (31, 36, 37). These results suggest that these 2 homologues have redundant as well as nonoverlapping functions. It has been reported that S6K2 but not S6K1 was important for fibroblast growth factor 2 (FGF 2)-induced chemoresistance of small cell lung cancer cells (38). A recent study showed that S6K2 but not S6K1 was important for cell proliferation in response to mTOR activation (39).

Since the Akt/mTOR/S6K axis plays a critical role in cell survival, yet targeting mTOR has been of limited success due to feedback activation of Akt, we have examined whether the 2 homologues of S6 kinase perform distinct functions in mediating breast cancer cell survival. We report for the first time that S6K2 regulates cell survival via the Akt pathway. We have shown that in contrast to S6K1, silencing of S6K2 inhibits Akt and induces cell death via the proapoptotic Bcl-2 family protein Bid. Thus, selective targeting of S6K2 rather than mTOR or S6K1 may be a more effective therapeutic strategy to treat cancers.

Materials and Methods

Materials

Human recombinant TNF and TRAIL were purchased from R&D Systems. Monoclonal antibodies to PARP and p53, and polyclonal antibody to caspase-9, were obtained from Pharmingen. Polyclonal antibody to Akt, phospho-Akt (p-Akt; Ser473), S6K1, and phospho-FOXO3a (p-FOXO3a) were obtained from Cell Signaling Technology. Polyclonal antibody to S6K2 was from Santa Cruz Biotechnology and Bethyl Laboratories. Polyclonal antibody to Bid and monoclonal antibody to caspase-8 were purchased from BioSource. Actin was purchased from Sigma-Aldrich. YO-PRO, Annexin V conjugated to Alexa Fluor 488, and propidium iodide (PI) were purchased from Molecular Probes/Invitrogen. Caspase-3 fluorometric assay kit was obtained from BioVision. Horseradish peroxidase–conjugated goat anti-mouse and donkey anti-rabbit antibodies were obtained from JacksonImmuno Research Lab Inc. Control nontargeting siRNA and siRNA specific for S6K1, S6K2, Bid, Bax, and p53 were obtained from Dharmacon. Polyvinylidene difluoride membrane was from Millipore, and enhanced chemiluminescence detection kit was from Amersham.

Cell culture and transfection

MCF-7 and ZR-75-1 cells were maintained in RPMI 1640 medium and MDA-MB-231 cells were maintained in DMEM supplemented with 10% FBS and 2 mmol/L glutamine. MCF-7 cells were obtained from Dr. Olivera J. Finn. ZR-75-1 and MDA-MB-231 cells were obtained from the University of Texas Southwestern Medical Center, Dallas. Cells were kept in a humidified incubator at $37°C$ with 95% air and 5% CO_2. All these cells were authenticated by DNA fingerprinting at the University of Texas Southwestern Medical Center and the Department of Forensic Genetics at the University of North Texas Health Science Center, Fort Worth. siRNA was transfected using Lipofectamine 2000 transfection reagent according to the manufacturer's protocol (Invitrogen). Cells were infected with adenovirus vector containing green fluorescent protein (GFP) or constitutively active (CA) myristotylated Akt [multiplicity of infection (MOI) = 10].

Immunoblot analysis

Equivalent amounts of total cellular extracts were electrophoresed by SDS-PAGE and transferred electrophoretically to polyvinylidene difluoride membrane. Immunoblot analyses were carried out as described before (40).

Cell death analysis

Cells were labeled with 0.5 μmol/L YO-PRO-1 and 2 μmol/L PI by incubating at $37°C$ for 15 minutes and visualized using a Zeiss Axiovert 40 inverted microscope with the AxioVision Rel 4.6 software (Zeiss).

Annexin V/PI binding assay

Cells were treated with or without TNF as indicated in the text. At the end of the incubation, both detached cells and attached cells were collected and washed with PBS. Cells were then stained with Annexin V–Alexa Fluor 488 conjugate and PI according to the manufacturer's protocol and analyzed using a flow cytometer (Coulter Epics; ref. 41).

Caspase assay

DEVDase activity was determined at $37°C$ by using Ac-DEVD-AFC as the substrate and according to the manufacturer's protocol. The fluorescence liberated from DEVD-AFC was measured using a SpectraMax GeminiXS fluorometer and SOFTmax PRO 3.1.1 software (Molecular Devices), with an excitation wavelength of 400 nm and emission wavelength of 505 nm.

Statistical analysis

Data are presented as the mean $\pm$ SE and $n = 4$. Statistical significance was determined by paired Student's t test by using PASW Statistics (SPSS, Inc.). $P < 0.05$ was considered statistically significant.

Results

S6K homologues exhibit distinct effects on TNF-induced apoptosis in breast cancer MCF-7 cells

Since S6K1 is overexpressed in MCF-7 breast cancer cells (42) and has been associated with chemoresistance (43, 44), we examined whether S6K1 confers resistance to TNF in MCF-7 breast cancer cells. Figure 1A shows that silencing of S6K1 by siRNA caused a modest decrease rather than an increase in the cleavage of PARP in response to TNF. Since there are 2 S6K homologues, we examined the effect of S6K2 knockdown on TNF-induced cell death. As shown in Figure 1B, depletion of
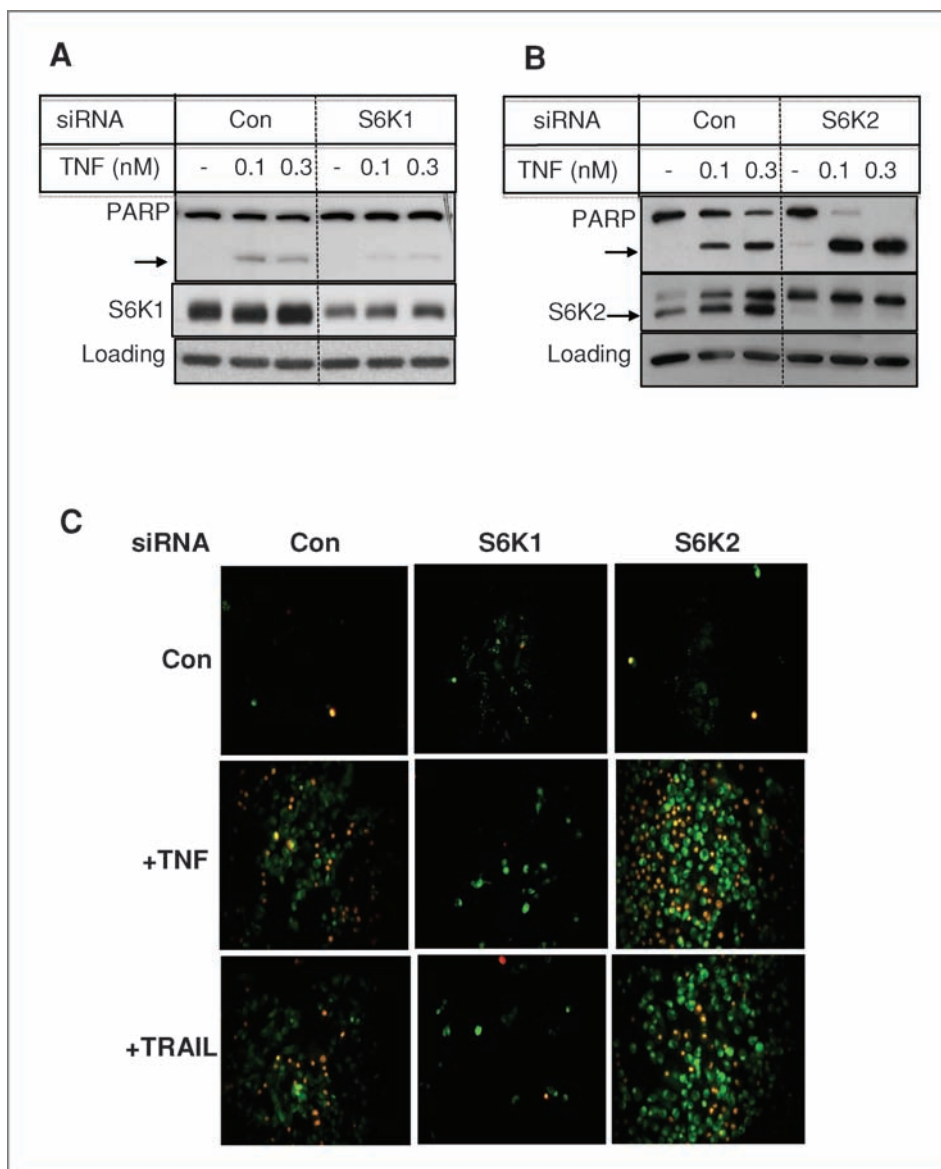
Figure 1. S6K1 and S6K2 have distinct effects on breast cancer cell survival. MCF-7 cells were transfected with control (Con) nontargeting siRNA and either S6K1 (A) or S6K2 (B) siRNA and then treated with indicated concentrations of TNF. Western blot analysis was carried out with indicated antibodies. Actin was used to control for loading differences. The arrow indicates 85-kDa cleaved PARP. C, MCF-7 cells transfected with control, S6K1, or S6K2 siRNA were treated without or with TNF or TRAIL. Cells were then stained with YO-PRO-1 (green) to detect apoptotic cells and PI (red) to detect necrotic cells, using a fluorescence microscope. Merged figures are shown. Results are representative of 3 independent experiments.

S6K2 caused a substantial increase in TNF-induced cleavage of the 116-kDa full-length PARP to the 85-kDa form. We also monitored the effect of S6K1 and S6K2 knockdown on cell death by staining cells with YO-PRO-1 and PI (Fig. 1C). Apoptotic cells are permeable to the green fluorescent dye YO-PRO-1, whereas PI (red) is taken up only by necrotic and late apoptotic cells. S6K2 depletion increased the number of YO-PRO-1/PI-stained cells in response to TNF and TRAIL (TNF-related apoptosis-inducing ligand), whereas S6K1 depletion seems to decrease it. Thus, the 2 S6K homologues had distinct effects on TNF- and TRAIL-induced cell death.

S6K homologues exert opposite effects on TNF-induced Akt phosphorylation

Since silencing of S6K1 caused a modest inhibition of TNF- and TRAIL-induced apoptosis (Fig. 1A and C), and S6K1 was

shown to negatively regulate Akt via a feedback loop (26, 28–30), we examined whether knockdown of S6K1 enhances TNF-induced activation of Akt in MCF-7 cells. Figure 2 shows that depletion of S6K1 in MCF-7 breast cancer cells enhanced phosphorylation of Akt. In contrast to S6K1, knockdown of S6K2 decreased basal and TNF-induced Akt phosphorylation (Fig. 3A). On the basis of densitometric scanning of 4 independent experiments, knockdown of S6K2 decreased basal and TNF-induced Akt phosphorylation at Ser473 by 40% and 60%, respectively (Fig. 3B).

We also examined the consequence of S6K2 knockdown on Akt phosphorylation in ZR-75-1 and MDA-MB-231 breast cancer cells (Fig. 3C and D). Knockdown of S6K2 decreased Akt phosphorylation and enhanced PARP cleavage and caspase activation in ZR-75-1 cells (Fig. 3C). TNF had little effect on cell death in MDA-MB-231 cells (data not shown). However,
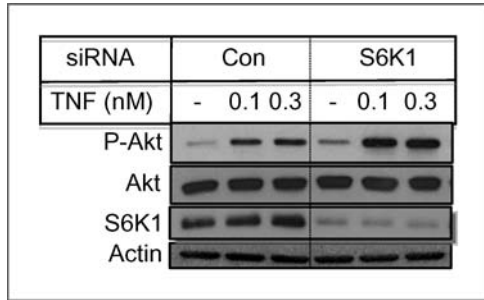
Figure 2. Knockdown of S6K1 increased Akt phosphorylation. MCF-7 cells transfected with control (Con) nontargeting or S6K1 siRNA were treated with indicated concentrations of TNF. Western blot analysis was carried out with indicated antibodies.

S6K2 depletion failed to enhance cell death in response to TRAIL in MDA-MB-231 cells (Fig. 3D). In contrast to MCF-7 cells, which lack caspase-3, ZR-75-1 and MDA-MB-231 cells contain functional caspase-3. Since Akt is a substrate for caspase-3, apoptotic stimuli can also induce cleavage of Akt and this may contribute to decrease in Akt level in response to TNF or TRAIL.
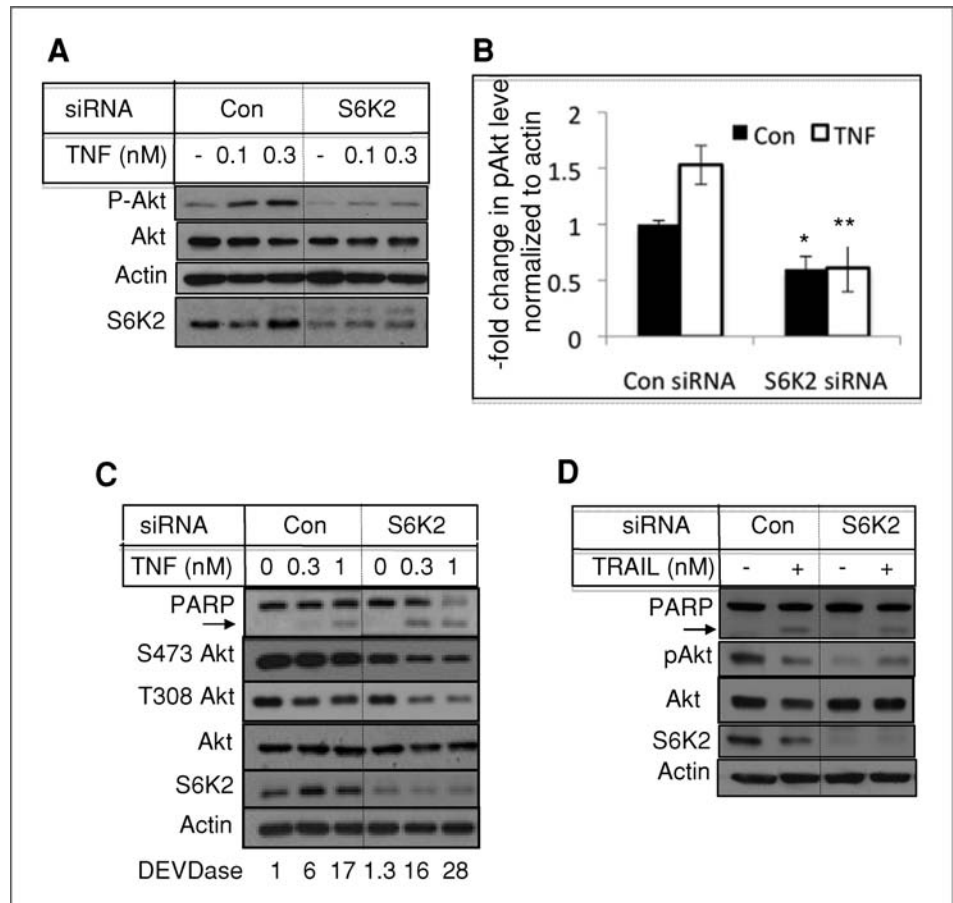
S6K2 promotes MCF-7 cell survival via Akt

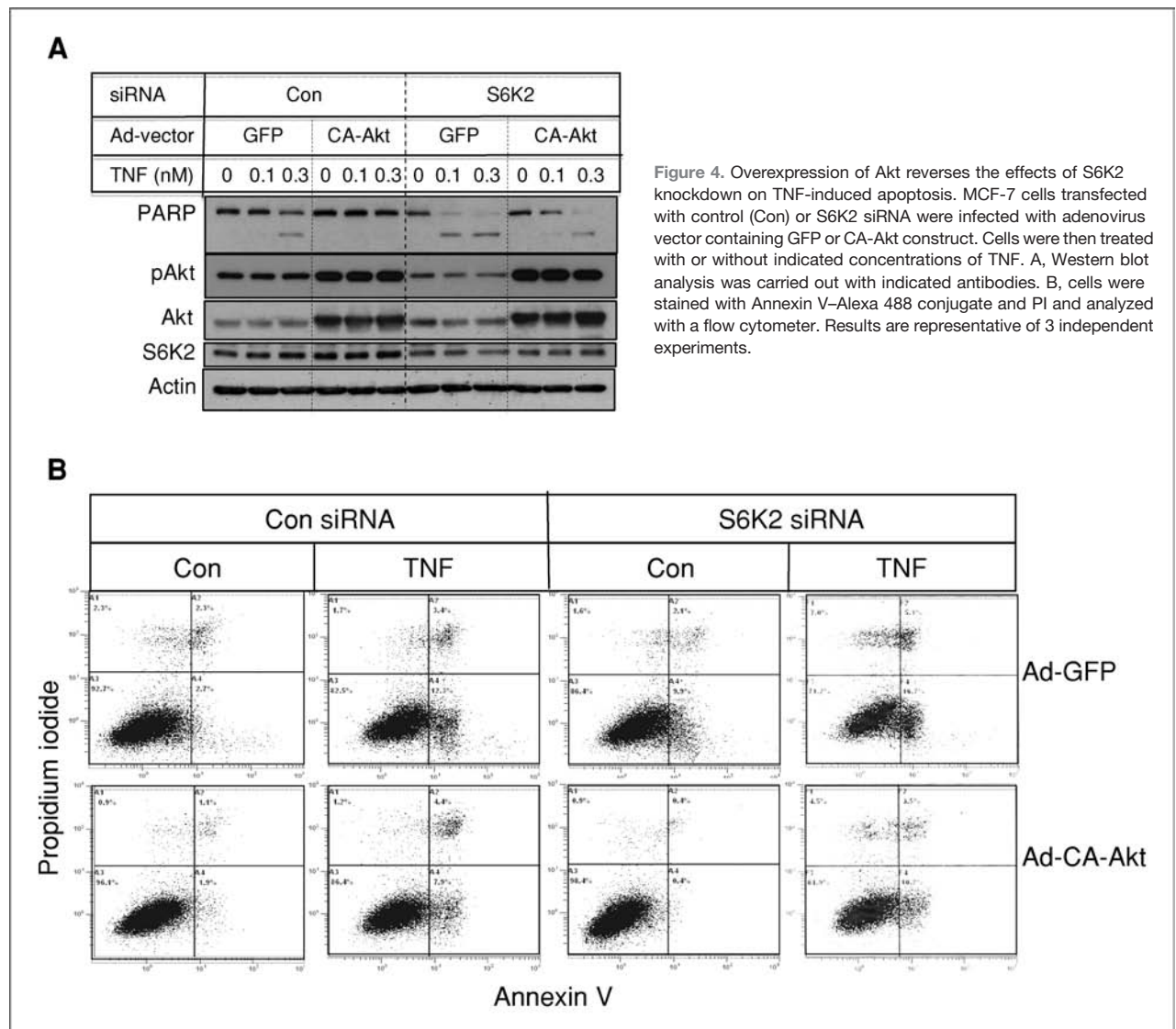Since knockdown of S6K2 inhibits Akt phosphorylation, we examined whether S6K2 promotes cell survival via Akt. We examined the ability of CA-Akt to reverse the potentiation of cell death caused by S6K2 depletion. Figure 4A shows that the adenoviral vector-mediated delivery of CA-Akt in MCF-7 cells decreased TNF-induced PARP cleavage compared with cells transfected with adeno-GFP. While knockdown of S6K2 caused a substantial increase in TNF-induced PARP cleavage, overexpression of CA-Akt inhibited TNF-induced PARP cleavage in S6K2-depleted cells. Similar results were obtained when we monitored cell death by staining cells with Annexin V and PI (Fig. 4B). These results suggest that S6K2 mediates its prosurvival effect via Akt.

Knockdown of S6K2 enhanced cell death via Bid

Although TNF and TRAIL trigger cell death via the receptor-initiated pathway, they can also amplify cell death via the mitochondrial pathway (10–12). To determine the mechanism(s) by which depletion of S6K2 potentiates TNF-induced cell death, we monitored TNF-induced caspase activation and processing of Bid. Figure 5A shows that TNF caused an increase in p-Akt, which was attenuated by S6K2 knockdown. Depletion of S6K2 was associated with enhanced processing of PARP and procaspase-8 in response to TNF. This was accompanied by an increase in the cleavage of Bid, a substrate for caspase-8 (10), and increased processing of procaspase-9, the apical caspase of the mitochondrial cell death pathway. We also compared the effects

Figure 3. Knockdown of S6K2 decreased Akt phosphorylation. MCF-7 (A), ZR-75-1 (C), and MDA-MB-231 (D) cells were transfected with control (Con) nontargeting or S6K2 siRNA. Cells were treated with indicated concentrations of TNF and Western blot analysis was carried out with indicated antibodies. B, intensity of p-Akt was determined by densitometry and standardized by loading. The data represent the fold decrease in Akt phosphorylation in S6K2-depleted cells compared with control siRNA-transfected cells. Each bar represents the mean ± SE of 4 independent experiments. The solid bar represents untreated cells and the open bar represents TNF treatment. *, $P < 0.05$; **, $P < 0.01$, using paired Student's t test.

Figure 4. Overexpression of Akt reverses the effects of S6K2 knockdown on TNF-induced apoptosis. MCF-7 cells transfected with control (Con) or S6K2 siRNA were infected with adenovirus vector containing GFP or CA-Akt construct. Cells were then treated with or without indicated concentrations of TNF. A, Western blot analysis was carried out with indicated antibodies. B, cells were stained with Annexin V–Alexa 488 conjugate and PI and analyzed with a flow cytometer. Results are representative of 3 independent experiments.

of S6K1 and S6K2 knockdown on cellular responses with TRAIL (Fig. 5B). Knockdown of S6K2 had little effect on caspase-8 inhibitor c-FLIP (Flice-like inhibitory protein), but it enhanced processing of procaspase-8, procaspase-9, and Bid (Fig. 5B).

To further validate our observation that S6K2 depletion decreases Akt phosphorylation and increases cell death via the mitochondrial pathway, we used 4 different siRNA constructs against S6K2. Figure 5C shows that siRNAs 1, 3 and 4 against S6K2 decreased Akt phosphorylation, enhanced PARP cleavage, and increased processing of pro-caspase-8 and procaspase-9 similar to S6K2 SMARTpool siRNA. In contrast, siRNA 2 was less effective in attenuating Akt phosphorylation and cleavage of PARP, caspase-8, and caspase-9. Thus, a decrease in Akt phosphorylation by S6K2 depletion was associated with an increase in PARP cleavage.

Since programmed cell death 4 (PDCD4) has been impli-cated in TNF-induced apoptosis and acts as a tumor sup-pressor (45, 46), we have also examined the effects of S6K1 and S6K-2 knockdown on the level of PDCD4. Silencing of S6K1 or S6K2 effectively depleted the homologue and attenuated phosphorylation of the substrate S6. However, while knock-down of S6K1 consistently increased PDCD4 level, depletion of S6K2 had either no effect or decreased the level of PDCD4 modestly (Fig. 5D and data not shown). Thus, it is unlikely that a decrease in the level of PDCD4 was responsible for the potentiation of cell death caused by S6K2 knockdown.

We have previously shown that activation of Akt promotes cell survival by downregulating Bid via p53 (17). We therefore examined whether S6K2 knockdown affects p53 level. Figure 6 shows that knockdown of S6K2 enhanced TNF-induced p53 level and silencing of p53 decreased Bid level, suggesting that S6K2 may regulate Bid via p53. Finally, to determine whether
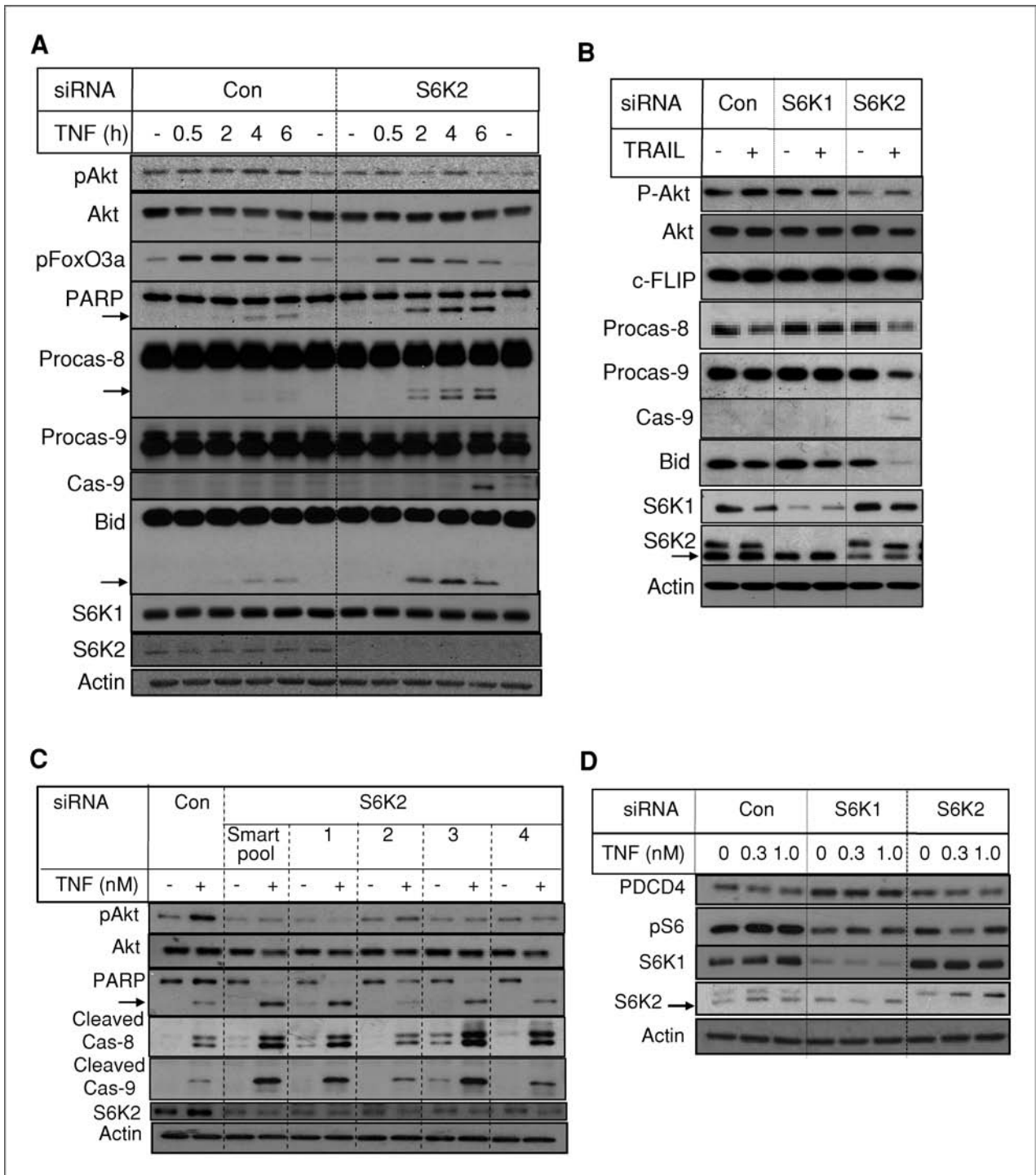
Figure 5. Knockdown of S6K2 induced apoptosis via the mitochondrial pathway. MCF-7 cells were transfected with control (Con) or S6K2 siRNA. A, cells were treated with 1 nmol/L TNF for indicated periods of time. B, cells were treated with or without TRAIL. C, cells were transfected with control, siRNA SMARTpool, or 4 different S6K2 siRNAs and then treated with 0.3 nmol/L TNF. D, cells were transfected with control, S6K1, or S6K2 siRNA. Western blot analyses were carried out with indicated antibodies. The top band in the S6K2 blot is likely to be S6K1. Results are representative of 3 independent experiments. The arrows indicate the processed forms of PARP, caspase-8, caspase-9, and Bid.
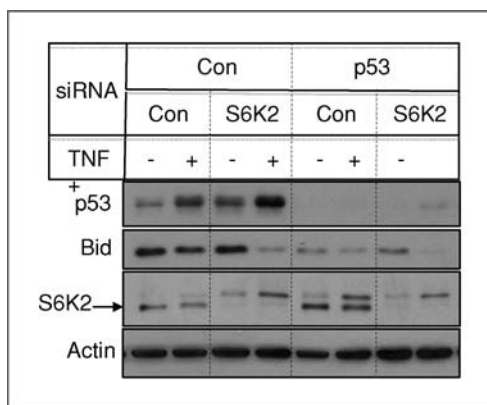
Figure 6. Knockdown of p53 attenuates Bid level. MCF-7 cells were transfected with indicated siRNAs and then treated with or without TNF. Cell lysates were analyzed by Western blotting by using the indicated antibodies. Actin was used to control for loading differences. Results are representative of 2 independent experiments. Con, control.

Bid is indeed involved in the potentiation of cell death caused by S6K2 knockdown, we examined whether S6K2 depletion sensitizes cells to TNF when Bid is depleted. We compared the effect of Bid with another proapoptotic Bcl-2 family member Bax. Figure 7 shows that knockdown of Bid abolished TNF-induced PARP cleavage. In addition, knockdown of Bid but not Bax attenuated the ability of S6K2 to enhance TNF-induced PARP cleavage. These results suggest that the mechanism by which S6K2 potentiates receptor-mediated apoptosis involves the proapoptotic protein Bid.

Discussion

The results of our present study show that the 2 S6K homologues S6K1 and S6K2 exhibit distinct functions on breast cancer cell survival. While it has been reported that
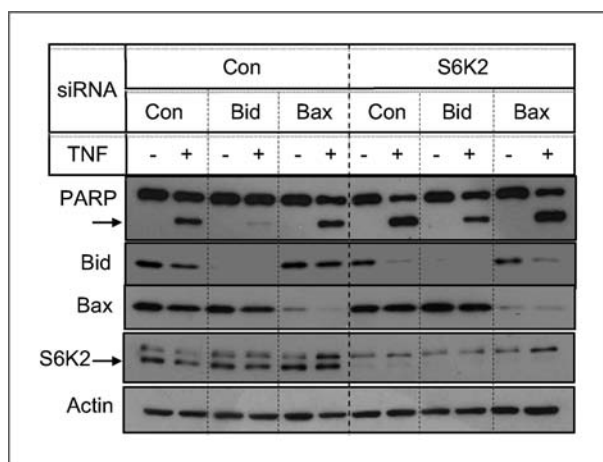
Figure 7. Knockdown of Bid counteracts increase in TNF-induced apoptosis caused by S6K2 depletion. MCF-7 cells transfected with indicated siRNAs were treated with or without TNF, and Western blot analysis was carried out with indicated antibodies. Results are representative of 2 independent experiments. Con, control.

S6K1 can negatively regulate Akt via a negative feedback loop, we report for the first time that depletion of S6K2 inhibits Akt activity and promotes breast cancer cell death via the mitochondrial cell death pathway that involves the Bcl-2 family protein Bid.

It is generally believed that activation of PI3K/Akt stimulates the mTOR pathway by phosphorylating and inactivating the tumor suppressor protein TSC2, which negatively regulates mTOR activity. mTOR is required for estrogen-induced breast tumor cell proliferation (47), and constitutive signaling through the mTOR pathway is a cause of treatment failure in breast cancer patients (48). S6K1, a downstream target of mTOR, is an important mediator of mTOR function (49). Elevation/activation of S6K has been associated with several cancers and resistance to chemotherapeutic drugs (42, 44, 50, 51). The *S6K1* gene is amplified in approximately 9% of primary breast cancers (52), and *S6K1* mRNA is elevated in almost 40% of the tumors (42). The status of the activated S6K1 was shown to be a predictor of patient's survival and treatment response (42, 50, 53). Recently, it has been reported that S6K1 promotes breast cancer cell proliferation by phosphorylating ERα (estrogen receptor α), leading to its transcriptional activation (54). Thus, we anticipated that knockdown of S6K1 would enhance cell death in breast cancer cells. To our surprise, depletion of S6K1 caused a modest decrease in cell death in response to TNF. Our results are, however, consistent with the recent reports that S6K1 deficiency protects against death receptor–mediated apoptosis in hepatocytes (55) and mTOR/S6K1 activates p53-dependent cell death in response to DNA damage (56). As has been reported earlier that persistent inhibition of mTOR/S6K1 can activate Akt via a negative feedback loop (26, 28–30), we also found that depletion of S6K1 resulted in an increase in TNF-induced Akt phosphorylation and this may explain why S6K1 knockdown inhibits rather than potentiates TNF-induced cell death.

Although most of the published reports have focused on S6K1, there are 2 homologues of S6K, S6K1 and S6K2, that act downstream of mTOR (25, 34). While these homologues share overall similarity in structure and exhibit redundant functions, there are also important differences. S6K2 has been shown to potentiate IL3 (interleukin 3)-mediated mitogenic response (57). A recent study has shown that S6K2 but not S6K1 interacts with heterogeneous ribonucleoproteins (hnRNP) F/H to drive cell proliferation (39). We have consistently found that in contrast to S6K1, depletion of S6K2 caused a dramatic increase in TNF- and TRAIL-induced apoptosis, suggesting that S6K2 functions as a prosurvival protein. TNF has been shown to activate mTOR signaling (58), and we have found that TNF preferentially activates S6K1 (data not shown), presumably because the abundance of S6K1 is much greater than that of S6K2 in MCF-7 cells. We made a novel observation that in contrast to S6K1, S6K2 positively regulates Akt. Knockdown of S6K2 caused a decrease in basal and TNF-induced Akt phosphorylation, which is indicative of its activation status, suggesting that S6K2 promotes cell survival via activation of Akt. In fact, overexpression of CA-Akt blocked increase in cell death caused by S6K2 depletion, suggesting that S6K2 acts upstream of Akt, although

we cannot rule out the possibility that Akt and S6K2 act in parallel pathways where Akt has a dominant role over S6K2.

There are several potential mechanisms by which S6K2 affects phosphorylation/activity of Akt. Since mTORC2 activates Akt by phosphorylating at the hydrophobic site, it is conceivable that knockdown of S6K2 decreases Akt phosphorylation by inhibiting mTORC2. Others and we have also shown that Ser473 phosphorylation of Akt is also regulated by DNA-dependent protein kinase (41). Since PTEN inhibits PI3K/Akt, another possibility is that S6K2 knockdown increases PTEN level, resulting in inhibition of Akt. It has been reported that a major kinase downstream of mTORC2 is serum- and gluco-corticoid-inducible kinase (SGK1; ref. 59). Thus, it is also important to determine whether S6K2 regulates cell survival via SGK1. Moreover, since activation of Akt would lead to the activation of mTORC1, there may be a positive feedback loop between S6K2 and Akt. Thus, mTORC1 and its downstream targets may mediate some of the effects of the potential functional interaction between S6K2 and Akt. Future studies should discern the mechanisms by which S6K2 regulates Akt and the functional interaction between S6K2 and Akt.

Our results suggest that the mechanism by which S6K2 promotes cell survival via Akt involves the proapoptotic Bcl-2 family protein Bid. We have previously shown that activation of Akt can cause a decrease in p53 levels in MCF-7 cells by phosphorylating and stabilizing Hdm2 [(human homologue of Mdm2 (murine double minute 2)], which degrades p53 via the ubiquitin proteasome–mediated pathway (17). We have also shown that Bid is a transcriptional target of p53 and Akt can decrease Bid expression by inducing downregulation of p53 (17). The results of our present study show that knockdown of S6K2 increased p53 and silencing of p53 was associated with a decrease in Bid level. However, depletion of S6K2 was not associated with upregulation of Bid. We have previously shown that overexpression of Bid is sufficient to cause cell death (19). Since Bid is a proapoptotic protein, an increase in Bid level may also lead to its cleavage. Therefore, it may be difficult to show an increase in Bid level. Nevertheless, knockdown of S6K2 had little effect on enhancing TNF-induced cell death when Bid was depleted by siRNA silencing. Moreover, knockdown of S6K2 failed to enhance cell death in MDA-MB-231 cells, which express mutant *p53*. Thus, the mechanism by which S6K2 promotes cell survival via Akt may involve down-regulation of Bid.

S6K2 has also been implicated in FGF-mediated chemoresistance of small cell lung cancer H69 cells (38). It has been reported that protein kinase C-ε (PKCε) interacts with S6K2 and mediates the prosurvival effects of S6K2 via Raf/MAPK (mitogen-activated protein kinase) signaling pathway by increasing the levels of antiapoptotic proteins XIAP and Bcl-xL (38). We were unable to detect a decrease in XIAP and Bcl-xL levels in S6K-2–depleted MCF-7 cells (data not shown), although we cannot rule out the possibility of other Bcl-2 family members. Interestingly, we have previously shown that PKCε also acts upstream of Akt during TNF-induced apoptosis in MCF-7 breast cancer cells (41) and inhibits TNF- and TRAIL-mediated apoptosis by increasing antiapoptotic Bcl-2 and decreasing proapoptotic Bid levels (19). Moreover, PKCε caused a decrease in Bid levels via Akt (17). Thus, depending on the cellular context and apoptotic stimulus, PKCε may promote cell survival either via the Raf/MEK/ERK pathway or via the Akt signaling pathway.

Aberrations in Akt/mTOR/S6K pathway have been associated with many cancers. Consequently, this pathway is an important target for cancer therapy. Rapamycin and its analogues that inhibit mTOR, however, were of limited success (26–30). Since S6K1 and S6K2 seem to have opposite effects on cell death, targeting mTOR, which acts upstream of both S6K1 and S6K2, may not be effective. Our observation that S6K2 rather than S6K1 is needed for the survival of breast cancer cells has significant implications in the treatment of the disease. Inhibition of S6K2 rather than of S6K1 should sensitize cancer cells to chemotherapeutic agents, providing a basis for rational combination chemotherapy. Since Akt signaling pathway is often deregulated in cancer, the observation that knockdown of S6K2 results in inhibition of Akt shows positive feedback regulation of Akt by S6K2 and has significant impact in cancer therapy.

Disclosure of Potential Conflicts of Interest

Acknowledgment

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