Thiazolidinediones Inhibit Insulin-Like Growth Factor-I–Induced Activation of p70S6 Kinase and Suppress Insulin-Like Growth Factor-I Tumor-Promoting Activity

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Abstract

Thiazolidinediones are a novel class of antidiabetic drugs that improve insulin sensitivity in type 2 diabetic patients. Recently, these compounds have also been shown to suppress tumor development in several animal models. The molecular basis for their antitumor action, however, is largely unknown. We report here that oral administration of thiazolidinediones (rosiglitazone and troglitazone) remarkably inhibited insulin-like growth factor-I (IGF-I)–promoted skin tumor development by 73% in BK5.IGF-1 transgenic mice, although they were previously found to be ineffective in inhibiting UV- or chemically induced mouse skin tumorigenesis. The anti-IGF-I effect of troglitazone in mouse skin keratinocytes was due to, at least partially, inhibition of IGF-I–induced phosphorylation of p70S6 kinase (p70S6K) at Thr389, a site specifically phosphorylated by mammalian target of rapamycin (mTOR). Troglitazone did not directly inhibit mTOR kinase activity as shown by mTOR kinase assay but rapidly activated AMP-activated protein kinase (AMPK) through a yet undefined peroxisome proliferator-activated receptor γ–independent mechanism. Expression of a dominant-negative AMPK reversed the inhibitory effect of troglitazone on IGF-I–induced phosphorylation of p70S6K, suggesting that troglitazone inhibited IGF-I and p70S6K signaling through activation of AMPK. Collectively, these data suggest that thiazolidinediones specifically inhibit IGF-I tumor-promoting activity in mouse skin through activation of AMPK and subsequent inhibition of p70S6K. (Cancer Res 2006; 66(3): 1873-9)

Introduction

Thiazolidinediones are a novel class of antidiabetic drugs that improve insulin sensitivity in patients with type 2 diabetes. Currently, two such drugs (rosiglitazone and pioglitazone) are available for clinical use, although troglitazone, the first thiazolidinedione drug, was removed from the market due to hepatotoxicity. These drugs increase insulin-stimulated glucose uptake in peripheral tissues, particularly in skeletal muscle, reduce glucose production in liver, and suppress free fatty acid concentrations (1–3). Thiazolidinediones are high-affinity ligands for peroxisome proliferator-activated receptor γ (PPARγ), a transcription factor that is highly expressed in adipose tissues (4). PPARγ mutations were found in humans with severe insulin resistance, suggesting that PPARγ plays an important role in insulin signaling in humans (5). It is currently believed that thiazolidinediones enhance insulin action through activating PPARγ (6, 7).

Although thiazolidinediones are currently approved only for treatment of type 2 diabetes, they are known for other potential beneficial effects. It has been shown that they are capable of inhibiting cellular proliferation and inducing apoptosis in a wide variety of tumor cell lines (8). More recently, two groups have reported an inhibitory effect of the thiazolidinediones on azoxymethane-induced colon carcinogenesis in murine animals (9, 10). Both studies showed that oral administration of troglitazone for 4 to 5 weeks significantly inhibited azoxymethane-induced formation of aberrant crypt foci, a type of precancerous lesion of the colon. Osawa et al. further showed that a longer period of feeding (32 weeks) mice with pioglitazone strongly inhibited colon tumor development by 67% (10). In sharp contrast to these findings, two other studies showed that thiazolidinediones did not suppress polyp formation in APC<sup>+/−</sup> min mice, a genetic model of human familial adenomatous polyposis. Instead, thiazolidinediones caused a significant increase in polyp formation in this model (11, 12). We have recently shown that thiazolidinediones did not inhibit UV- or 7,12-dimethylbenz(a)anthracene (DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA)–induced mouse skin tumorigenesis, although they significantly reduced the basal level of keratinocyte proliferation (13). It therefore becomes important to identify the real targets for the potential tumor-preventive effect of the thiazolidinediones.

Insulin-like growth factor-I (IGF-I) and insulin preferentially bind to the IGF-I receptor and insulin receptor, respectively. The two receptors are structurally similar, and many of their downstream molecules are same, including insulin receptor substrate-1 (IRS-1), phosphoinositide 3-kinase (PI3K), Akt, mammalian target of rapamycin (mTOR), and p70S6 kinase (p70S6K), but their physiologic functions are different. Although insulin is important in nutrient metabolism, IGF-I signaling favors cell growth, proliferation, and cell survival in relation to nutrient availability (14). IGF-I also has a well-documented role in cancer development in a number of tissues (reviewed in refs. 15, 16). High circulating levels of IGF-I are associated with increased risk of developing prostate cancer, colorectal cancer, and breast cancer (17–19). Depletion of circulating IGF-I confers resistance to chemically induced murine mammary gland carcinogenesis (20, 21). In addition, epithelium-specific overexpression of IGF-I is sufficient to cause spontaneous tumor development in mouse skin and prostate (22, 23). Inhibition of IGF-I–mediated PI3K/Akt signaling in the epidermis of IGF-I transgenic mice was shown to reduce epidermal proliferation and skin tumor development, suggesting the critical nature of this pathway (24). Interestingly, recent studies have suggested that IGF-I bioavailability is elevated...
in patients with insulin resistance (25). Although it is well known that thiazolidinediones enhance insulin signaling, it is not known what effects they have on IGF-I signaling and its protumor activity. We show here, using mouse skin keratinocytes as a model, that thiazolidinediones inhibited mitogenic signaling of IGF-I and specifically suppressed IGF-I–promoted skin tumor development.

Materials and Methods

Materials. Troglitazone was obtained from Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co. (Ann Arbor, MI). Rosiglitazone was purchased from LKT (St. Paul, MN). IGF-I, 2-deoxy-glucose, and wortmannin were obtained from Sigma (St. Louis, MO). All antibodies and rapamycin were purchased from Cell Signaling (Beverly, MA). Plasmids containing GST-p70S6K and dominant-negative AMP-activated protein kinase (AMPK) α2 (D175A) were kindly provided by Dr. Kuanliang Guan (Department of Biological Chemistry, University of Michigan, Ann Arbor, MI). Plasmids containing wild-type (WT) and kinase-dead mTOR were gifts from Dr. Stuart L. Schreiber (Department of Chemistry and Chemical Biology, Harvard University, Boston, MA) (27). All chemicals for cell culture experiments were dissolved in DMSO.

Tumorigenesis. Two independent tumorigenesis experiments were carried out using BK5:IGF-1 transgenic mice. In one experiment, 60 BK5:IGF-1 male mice, 10 to 12 weeks old, were randomized into two groups and initiated with 100 μg DMBA/mouse on the shaved back skin. After a period of 2 weeks of washout, the mice were fed a powdered AIN-76 diet supplemented with or without 300 ppm rosiglitazone for an additional 15 weeks. Tumors on the back skin were all papillomas and were counted weekly after the first tumor appeared. The experiment was terminated at week 17 due to tumor burden. Tumor sizes were measured by diameter: small, <2 mm; mid, 2 to 7 mm; large, >7 mm. Bromodeoxyuridine (BrdUrd) incorporation after the first tumor appeared. The experiment was terminated at week 17 due to tumor burden. Tumor sizes were measured by diameter: small, <2 mm; mid, 2 to 7 mm; large, >7 mm. Bromodeoxyuridine (BrdUrd) incorporation (within tumor-free skin) was carried out as previously described (28). In the other experiment, two groups each of 30 BK5:IGF-1 male mice, 12 weeks old, were fed a powdered AIN-76 diet supplemented with or without 2,000 ppm (0.2%) troglitazone for 40 weeks. Spontaneous skin tumors were counted at the end of experiment. Tumor type was determined by histology.

Cell culture. C50 cells, a keratinocyte cell line derived from normal mouse keratinocytes, were maintained and plated in Eagle’s MEM (EMEM) as described (29). EMEM was replaced the next day with serum-free KGM-2 medium containing 0.03 mmol/L CaCl2 but without insulin. For insulin starvation, C50 cells were incubated in this insulin-free KGM-2 medium for 48 hours. HEK293 cells were cultured in DMEM with 10% bovine serum. Cell proliferation. Two approaches were employed to measure keratinocyte proliferation in vitro and in vivo. (a) To show that IGF-I induces keratinocyte proliferation in vitro, C50 cells were treated with or without IGF-I in KGM-2 medium for 48 hours. Cells were then trypsinized, and viable cells were counted in trypsin blue. For proliferation inhibition experiments, troglitazone and other inhibitors were added 10 minutes before IGF-I. (b) To assess effect of the thiazolidinediones drugs on skin keratinocyte proliferation in vivo, BrdUrd incorporation assay with mice from the tumorigenesis experiment described above was done (28).

Western analysis. To detect phosphorylated proteins, cells or equal amounts of whole skin (epidermis and dermis) were lysed in appropriate volume of 1× sample buffer (62.5 mmol/L Tris-HCl, 2% w/v SDS, 10% glycerol, bromophenol blue, and freshly added 50 mmol/L DTT). Lysates were briefly sonicated and cleared by centrifugation. Samples were used immediately for Western analysis for phosphorylated Akt (Ser473 and Thr308), extracellular signal-regulated kinase (ERK), p70S6K (Thr389), and AMPK (Thr172). The blots were stripped and immunostained with antibodies against corresponding total proteins as loading controls. The procedure for Western analysis was detailed previously (29).

Transient and stable transfection. To generate GST-p70S6K, HEK293 cells were transiently transfected with 15 μg per 100-mm dish pE8G3x-p70S6K plasmid for 48 hours using LipofectAMINE reagent (Invitrogen, Carlsbad, CA) and harvested in extract buffer containing 50 mmol/L HEPES (pH 7.5), 150 mmol/L NaCl, 1% Triton X-100, and one tablet EDTA-free protease inhibitor cocktail (Roche, Indianapolis, IN) per 10 mL. Medium change was avoided after transfection to minimize phosphorylation of p70S6K. Cell extracts were pooled and GST-p70S6K was purified with glutathione S-transferase (GST) beads. The presence of full-length p70S6K was verified with SDS-PAGE and immunostaining with anti-p70S6K antibody. We also transiently transfected HEK293 cells with WT or a kinase-dead mTOR using LipofectAMINE reagent to examine the effect of troglitazone on exogenous mTOR kinase activity. To generate C50 stable cell lines expressing the dominant-negative AMPK, we transfected C50 cells with pcDNA3 empty vector or pcDNA3-AMPKα2 (D175A). Stably transfected cells were selected with 200 μg/mL G418, and positive colonies were pooled.

mTOR in vitro kinase assay. mTOR in vitro kinase assay was done as described (30) and described briefly below. HEK293 cells transiently transfected with a flag-tagged WT or kinase-dead mTOR or untransfected C50 cells in 100-mm dishes were treated with IGF-I for 1 hour and harvested in 500 μL ice-cold kinase lysis buffer. After clearing by centrifugation, supernatants of cell lysates were incubated with 2 μL anti-mTOR or anti-flag antibody and 30 μL of a 50% slurry of protein A-Sepharose on a rocker at 4 °C overnight. Immunoprecipitates were washed four times with ice-cold kinase lysis buffer, twice with wash buffer, and once with kinase buffer. To test if troglitazone directly inhibits mTOR kinase activity, the immunoprecipitates were preincubated with various concentrations of troglitazone in 20 μL kinase buffer containing 50 mmol/L unlabeled ATP at 30 °C for 10 minutes. The reactions were started by adding 200 ng of purified GST-p70S6K fusion protein to each sample, incubating at 30 °C for an additional 15 minutes, and the reactions were stopped by adding 5 μL of 5 × sample buffer. The samples were analyzed by 7% SDS-PAGE and transferred to polyvinylidene difluoride membrane. Phosphorylated p70S6K was detected with an anti-p70S6K (Thr389) antibody. To test if rosiglitazone inhibits mTOR kinase activity in a whole-cell context, troglitazone was added to C50 cell cultures before the mTOR immunoprecipitation.

Statistics. Tumor incidence data were analyzed by χ2. All other data were analyzed by one-way ANOVA (Tukey) using SPSS software (SPSS, Inc., Chicago, IL).

Results

Thiazolidinediones specifically suppressed IGF-I–promoted mouse skin tumorigenesis. We examined the potential effect of thiazolidinediones on IGF-I signaling using BK5:IGF-1 transgenic mice in which IGF-I is specifically overexpressed in epithelial tissues, including skin (22). These mice rapidly develop skin tumors after a single topical application of the carcinogen DMBA, whereas WT mice typically need DMBA treatment followed by continuous applications of a tumor promoter (such as TPA) to develop skin tumors. Remarkably, these mice begin to develop spontaneous skin tumors when older than 6 months (22). We fed these mice a powdered diet with or without 300 ppm rosiglitazone 2 weeks after DMBA initiation. All mice (30 of 30) in the control diet bore skin tumors with an average of 11.4 tumors per mouse at the end of the experiment. In contrast, only 67% of the mice (20 of 30; Fig. 1B; χ2, P = 0.001) in the rosiglitazone group developed skin tumors, with an average of only 3.1 skin tumors per mouse (Fig. 1A; 73% reduction versus the control group; one-way ANOVA, P < 0.01). Tumors in the rosiglitazone group were also smaller in size compared with those in the control group (Fig. 1C). No apoptotic cells were found in the epidermis of either group (data not shown); however, skin keratinocyte proliferation in the rosiglitazone group was significantly inhibited compared with the control group, as shown by BrdUrd incorporation assay (Fig. 1D; P < 0.05). Rosiglitazone had no effect on the skin levels of IGF-I or major IGF-I binding proteins (IGFBP), such as IGFBP3 and IGFBP5, in the mouse skin (data not shown), suggesting that the inhibitory effect of
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Figure 1. Dietary thiazolidinediones suppressed skin tumorigenesis in BK5.IGF-1 transgenic mice. Skin tumor multiplicity (A) and tumor incidence (B) in DMBA-initiated BK5.IGF-1 transgenic mice fed a powered diet with or without 300 ppm rosiglitazone. C, categorization of skin tumors (from rosiglitazone experiment) by size (diameter). D, BrdUrd (BrdU) immunohistochemical staining of basal layer cells in the epidermis of mice from the rosiglitazone experiment (n = 4 per group). *, P < 0.05. E, spontaneous skin tumor development in BK5.IGF-1 transgenic mice fed a powered diet with or without 2,000 ppm troglitazone for 40 weeks. *, P = 0.041.

Although thiazolidinediones are ligands for PPARγ, their actions in mouse skin keratinocytes seem not to be mediated by PPARγ because PPARγ expression and function are not detectable in these cells (13, 29). Similar to WT mice, PPARγ mRNA expression in the skin of BK5.IGF-1 transgenic mice is at best very low, and chronic treatment with rosiglitazone did not increase PPARγ expression (Supplementary Fig. S1). In fact, skin keratinocytes of these transgenic mice do not express detectable levels of PPARγ as shown by immunohistochemistry (data not shown), suggesting that the specific inhibition of thiazolidinediones on IGF-I promoted skin tumorigenesis was not PPARγ mediated.

Troglitazone blocked IGF-I mitogenic signaling in cultured keratinocytes. IGF-I is known to be a strong mitogen for cultured human and mouse skin keratinocytes (31, 32). We speculated that thiazolidinediones may interfere with IGF-I receptor signaling pathway in keratinocytes and inhibit the mitogenic activity of IGF-I. We tested this using a mouse skin keratinocyte cell line, C50 cells. As expected, a 48-hour incubation with 25 ng/mL IGF-I nearly doubled the cell number compared with vehicle treatment (Fig. 2A). Troglitazone at ≥1 μM concentrations completely inhibited IGF-I–induced keratinocyte proliferation (Fig. 2B). Interestingly, troglitazone inhibited keratinocyte proliferation even in the absence of IGF-I, suggesting that it may inhibit other mitogenic signals as well (Fig. 2B).

Troglitazone inhibited IGF-I–induced phosphorylation of p70S6K by mTOR. We and others have shown that troglitazone strongly and rapidly inhibited expression of cyclin D1, a sensor of mitogens (refs. 29, 33; Supplementary Fig. S2A). However, keratinocytes obtained from cyclin D1 heterozygous and homozygous null pups were equally sensitive to troglitazone-induced inhibition of proliferation compared with those from cyclin D1 WT pups (Supplementary Fig. S2B), indicating that troglitazone may act independently of cyclin D1. Careful examination of IGF-I signaling allowed us to identify p70S6K as a potential target of troglitazone. p70S6K is a molecular target of at least four kinases in the insulin/IGF-I signaling pathway. IGF-I treatment of C50 cells caused a quick and sustained increase in the phosphorylation of p70S6K at Thr389, a site specifically phosphorylated by mTOR (Fig. 3A). Troglitazone efficiently inhibited this event (Fig. 3A and B). Troglitazone also inhibited phosphorylation of p70S6K in human embryo kidney 293 cells (HEK293), indicating that troglitazone may act independently of cyclin D1.

Thiazolidinediones on mouse skin carcinogenesis in BK5.IGF-1 transgenic mice is not due to their interference with IGF-I expression or bioavailability.

It is unlikely that rosiglitazone inhibited tumor development in this model through interfering with the DMBA metabolism process. We applied rosiglitazone 2 weeks after DMBA initiation, by which time metabolism of DMBA and DMBA-induced damage to DNA is complete. Indeed, spontaneous skin tumor development was also significantly reduced when these mice were fed troglitazone-containing diet for 40 weeks (Fig. 1E; 31.6%, 12 of 38 incidence in the control versus 12.5%, 5 of 40 in the troglitazone group; Pearson χ², P = 0.041). Because we previously showed that neither oral nor topically administrated thiazolidinediones had an effect on DMBA/TPA- or UV-induced mouse skin tumorigenesis (13), it seemed that thiazolidinediones specifically suppressed IGF-I–promoted mouse skin tumorigenesis.

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were fed a diet with or without 2,000 ppm troglitazone for 1 week. Transgenic mice showed elevated levels of p70S6K phosphorylation in skin epidermis when compared with WT mice. Troglitazone significantly inhibited p70S6K phosphorylation in the epidermis of transgenic mice (Fig. 3D). To assess the significance of the inhibitory effect of troglitazone on mTOR signaling to p70S6K, we determined the role of mTOR signaling in IGF-I-induced cell proliferation in keratinocytes. Inhibition of mTOR signaling by rapamycin (a specific mTOR inhibitor) completely blocked IGF-I–induced cell proliferation (Fig. 3E), suggesting that troglitazone inhibits keratinocyte proliferation at least partially through inhibiting mTOR phosphorylation of p70S6K.

Akt lies at the crossroad of growth factor signaling and upstream of mTOR. Troglitazone did not inhibit IGF-I–induced phosphorylation of Akt at Ser473 or Thr308 in C50 cells; rather, it slightly enhanced the effect of IGF-I in a dose-dependent manner (Fig. 4A and B), suggesting that thiazolidinedione inhibition of mTOR kinase activity is independent of Akt. Troglitazone slightly increased phosphorylation of ERK as well (Fig. 4A). It has been recently reported that p70S6K can phosphorylate IRS-1, an initiator of insulin/IGF-I receptor signaling at Ser636/Ser639 and inactivate it (34). Constitutive activation of p70S6K causes mouse embryo fibroblasts to be resistant to insulin and IGF-I signaling due to inactivation of IRS-1 (35). This negative feedback was observed in keratinocytes as well and was completely shut down by troglitazone treatment (Fig. 4C). Because IRS-1 lies upstream of Akt and ERK, alleviating this negative feedback loop with troglitazone likely explains the increased phosphorylation of Akt and ERK.

Troglitazone inhibited mTOR kinase activity indirectly. To determine whether troglitazone directly inhibited mTOR kinase activity, we transfected HEK293 cells with a flag-tagged WT or kinase-dead mTOR construct. Only WT mTOR immunoprecipitates phosphorylated full-length p70S6K; kinase-dead mTOR did not (Fig. 5A). Addition of troglitazone in vitro did not result in decreased mTOR kinase activity, suggesting that troglitazone does not inhibit mTOR kinase activity directly (Fig. 5A). We also repeatedly found that troglitazone did not directly inhibit kinase activity of endogenous mTOR from C50 cells, whereas 0.5 μmol/L wortmannin did, indicating that the assay was working (Fig. 5B).
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However, when we applied troglitazone to the keratinocyte culture, it inhibited IGF-I–induced mTOR kinase activity in a dose-dependent manner (Fig. 5C), which is consistent with our Western blot results.

Activation of p70S6K can also be regulated by protein phosphatase 2A (PP2A; ref. 36). Calyculin, a specific PP2A inhibitor, completely reversed troglitazone’s effect (data not shown). Troglitazone, however, did not enhance PP2A activity in keratinocytes (data not shown). Together, these data suggest that troglitazone inhibited IGF-I–induced phosphorylation of p70S6K by indirectly inhibiting mTOR kinase activity in keratinocytes.

**Thiazolidinedione inhibited mTOR signaling by activating AMPK.** It has been proposed that full activation of mTOR needs coordination of growth factors and amino acid sufficiency (reviewed in ref. 37). To test this, starved keratinocytes were stimulated with IGF-I or amino acids or both. IGF-I and amino acids showed an additive effect in inducing mTOR activity as shown by phosphorylation of p70S6K (Supplementary Fig. S3A). IGF-I and amino acids also showed an additive effect in inducing cyclin D1 expression in keratinocytes (Supplementary Fig. S3B). These results suggest that mTOR activity can be effectively regulated by cellular energy status in addition to growth factors.

AMPK is a cellular sensor of energy status and has been implicated as a potential target for type 2 diabetes (38). Thiazolidinediones and other antidiabetic drugs have been recently shown to activate AMPK in muscle cells (39, 40). As expected, troglitazone treatment rapidly (as early as 10 minutes) induced phosphorylation of AMPK in keratinocytes, at a dose-dependent fashion (Fig. 6A and B). Rosiglitazone at 5 μmol/L showed a similar effect. Thiazolidinediones activated AMPK in keratinocytes most likely through a PPARγ-independent mechanism, because these cells do not express functional levels of PPARγ (29). This is supported by the observation that 10 μmol/L 15-deoxy-Δ12,14-prostaglandin J2, a potent but nonthiazolidinedione ligand of PPARγ, only slightly activates AMPK (Fig. 6B).

**Discussion**

We show that thiazolidinediones activate AMPK through an as yet undefined PPARγ-independent mechanism, which subsequently inhibits IGF-I–induced phosphorylation of p70S6K (Fig. 6C) and partially alleviated troglitazone’s inhibition of cellular proliferation (Fig. 6D). Another consequence of inhibited mTOR and p70S6K by thiazolidinediones is that Akt activity is elevated due to suppression of the p70S6K-to-IRS-1 negative feedback (Fig. 6E).

In summary, our data support a model in which thiazolidinediones activate AMPK through an as yet undefined PPARγ-independent mechanism, which subsequently inhibits IGF-I–induced cell proliferation by inhibiting mTOR kinase activity and phosphorylation of p70S6K (Fig. 6E). Another consequence of inhibited mTOR and p70S6K by thiazolidinediones is that Akt activity is elevated due to suppression of the p70S6K-to-IRS-1 negative feedback (Fig. 6E).
Although thiazolidinediones may have systemic effects, inhibition of IGF-I signaling following activation of AMPK in keratinocytes by thiazolidinediones most likely accounts for their inhibitory effect on skin tumorigenesis in BK5.IGF-1 transgenic mice. The reason why thiazolidinediones do not inhibit UV- or DMBA/TPA-induced skin tumorigenesis remains to be determined. A possible explanation is that TPA or UV may activate a signaling pathway(s) that enables keratinocytes to bypass a requirement for IGF-I signaling. Interestingly, a recent study showed that activation of AMPK by glucose depletion caused cell cycle arrest through a mechanism independent of mTOR but involving p53 (41). This is consistent with our finding that thiazolidinediones inhibited keratinocyte proliferation even in the absence of IGF-I.

Insulin resistance, which is the cause of type 2 diabetes and other metabolism disorders, is associated with an increased risk of developing tumors in several tissues (42). Compensatory elevation of IGF-I signaling in insulin resistance is believed to be responsible for this association (25). Thiazolidinediones are currently widely used in the treatment of type 2 diabetes. Our findings now show that thiazolidinediones are capable of directly inhibiting the tumor-promoting activity of IGF-I signaling. In addition, by ameliorating insulin resistance, thiazolidinediones also have the potential to inhibit the development of skin tumors in BK5.IGF-1 transgenic mice (42). Compensatory elevation of IGF-I signaling through inhibiting IGF-I–induced activation of p70S6K (40). We therefore suggest that activation of AMPK and inhibition of p70S6K by thiazolidinediones may contribute partly to their insulin-sensitizing action, although it remains to be determined whether thiazolidinediones also inhibit activation of p70S6K in muscle, fat, and liver, the major target organs of insulin. Indeed, thiazolidinediones inhibited IRS-1 phosphorylation and enhanced IGF-I–induced phosphorylation of Akt in mouse skin keratinocytes. In addition, activation of AMPK by thiazolidinediones has been proposed to mediate the acute glucose-lowering effect of thiazolidinediones through increasing glucose uptake into the muscle (40). It is not yet clear how thiazolidinediones rapidly activate AMPK, although disruption of the mitochondrial membrane has been implicated (40). We therefore suggest that activation of AMPK and subsequent inhibition of p70S6K may provide a link between the antitumor effect of the thiazolidinediones and their insulin-sensitizing action.

In summary, we found that thiazolidinediones inhibited IGF-I signaling through inhibiting IGF-1–induced activation of p70S6K via rapidly activating AMPK. This seems to account for at least part of the mechanism by which thiazolidinediones inhibit the development of skin tumors in BK5.IGF-1 transgenic mice and suggests that thiazolidinediones may have chemopreventive activity in individuals with elevated IGF-I signaling.

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References


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