Rapamycin Modulates Glucocorticoid Receptor Function, Blocks Atrophogene REDD1, and Protects Skin from Steroid Atrophy

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Glucocorticoids have excellent therapeutic properties; however, they cause significant adverse atrophogenic effects. The mTORC1 inhibitor REDD1 has been recently identified as a key mediator of glucocorticoid-induced atrophy. We performed computational screening of a connectivity map database to identify putative REDD1 inhibitors. The top selected candidates included rapamycin, which was unexpected because it inhibits pro-proliferative mTOR signaling. Indeed, rapamycin inhibited REDD1 induction by glucocorticoids dexamethasone, clobetasol propionate, and fluocinolone acetonide in keratinocytes, lymphoid cells, and mouse skin. We also showed blunting of glucocorticoid-induced REDD1 induction by either catalytic inhibitor of mTORC1/2 (OSI-027) or genetic inhibition of mTORC1, highlighting role of mTOR in glucocorticoid receptor signaling. Moreover, rapamycin inhibited glucocorticoid receptor phosphorylation, nuclear translocation, and loading on glucocorticoid-responsive elements in REDD1 promoter. Using microarrays, we quantified a global effect of rapamycin on gene expression regulation by fluocinolone acetonide in human keratinocytes. Rapamycin inhibited activation of glucocorticoid receptor target genes yet enhanced the repression of pro-proliferative and proinflammatory genes. Remarkably, rapamycin protected skin against glucocorticoid-induced atrophy but had no effect on the glucocorticoid anti-inflammatory activity in different in vivo models, suggesting the clinical potential of combining rapamycin with glucocorticoids for the treatment of inflammatory diseases.

INTRODUCTION

Glucocorticoids are among the most effective anti-inflammatory and anti-lymphoma drugs (Lesovaya et al., 2015). Unfortunately, chronic treatment with glucocorticoids results in multiple metabolic and atrophic adverse effects that reflect glucocorticoid catabolic activity (De Bosscher et al., 2010; Lesovaya et al., 2015). Thus, there is a significant need for safer glucocorticoid receptor (GR)-targeted therapies.

**Abbreviations:** ChIP, chromatin immunoprecipitation; CMAP, connectivity map; FA, fluocinolone acetonide; GR, glucocorticoid receptor; GRE, glucocorticoid responsive element; TF, transcription factor

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Figure 1. Pharmacological or genetic inhibition of mTOR blocks glucocorticoid-induced REDD1 expression. (a–e) HaCaT, NHEK, and CEM cells were pretreated with rapamycin (1 μmol/L x 6 hours) and treated with solvent (control) or glucocorticoid FA (1 μmol/L) for 24 hours. (d, e) HaCaT cells were pretreated with varying doses of rapamycin (as indicated) for 6 hours, followed by treatment with glucocorticoids: (d) dexamethasone or (e) CBP for 24 hours. (f) shRaptor-HaCaT cells with genetically knocked down raptor and control pLKO.1-HaCaT cells were treated with either DMSO or FA (1 μmol/L) for 24 hours. (g, h) HaCaT cells were pretreated with OSI-027 (1 μmol/L x 6 hours) and treated with solvent (control) or glucocorticoid FA (1 μmol/L).
Woodbury and Kligman, 1992). Recently we identified REDD1, a negative regulator of mTOR/Akt signaling (Dennis et al., 2014; Ellisen, 2005; Shoshani et al., 2002), as a central atrophogene in skin (Baida et al., 2015). REDD1 expression is activated by a variety of cellular stresses including hypoxia, depletion of growth factors, DNA damage, and glucocorticoids (Ellisen, 2005; Shimizu et al., 2011; Shoshani et al., 2002).

We and others showed that REDD1 was strongly induced during steroid atrophy in skin and muscle and that REDD1 knockout animals were protected against steroid-induced skin atrophy and muscle waste (Baida et al., 2015; Britto et al., 2014; Wang et al., 2006). We discovered that lack of REDD1 did not alter the anti-inflammatory effects of glucocorticoids (Baida et al., 2015).

We hypothesized that REDD1 inhibitors may act as anti-atrophogenes and could be combined with glucocorticoids for tissue protection. We used a drug repurposing approach and screened a connectivity map (CMAP) database of transcriptional signatures induced by US Food and Drug Administration-approved and experimental drugs (Lamb et al., 2006) for their potential to reduce REDD1 expression. We identified several putative REDD1 inhibitors, including rapamycin. The potential of rapamycin to display anti-atrophic properties was unexpected, because it is a pharmacological REDD1 analog and a specific mTOR inhibitor (Li et al., 2014).

The goals of this study were to test the effect of rapamycin on basal and glucocorticoid-induced REDD1 expression, its potential effects on GR function, and its effect on therapeutic (anti-inflammatory) and adverse (skin atrophy) effects of glucocorticoids.

RESULTS

Selection of rapamycin as a prospective REDD1 inhibitor

Because pharmacological REDD1 inhibitors are not known, we used a modified connectivity mapping approach and screened a CMAP library representing molecular signatures of approximately 1,300 US Food and Drug Administration-approved and experimental drugs tested in human cancer cells to repurpose them for cancer treatment (Lamb et al., 2006). We selected compounds according to the number of CMAP experiments in which REDD1 was within the top 100 down-regulated genes in cells treated with these compounds (see Supplementary Table S1 online). We identified several putative REDD1 inhibitors, including rapamycin, which displayed consistent negative effects on REDD1 expression in more than 40 tests in multiple cell lines. Thus, we prioritized rapamycin as the top candidate for experimental validation.

mTOR inhibitors rapamycin and OSI-027 down-regulate REDD1 expression induced by diverse glucocorticoids

Rapamycin effects were tested in keratinocytes (human keratinocyte line HaCaT and primary human epidermal keratinocytes) and lymphoid cells (CEM), because glucocorticoid effects in lymphocytes are critically important for steroid anti-inflammatory activity.

Glucocorticoid fluocinolone acetonide (FA) (1 µmol/L) induced REDD1 expression within 2 to 24 hours, with the peak of mRNA and protein induction at 24 hours in all studied cells (Figure 1 a–c, and see Supplementary Figure S1a online). Other glucocorticoids including dexa-methasone, clobetasol propionate (Figure 1d and e, and see Supplementary Figure S2 online) also strongly activated REDD1 expression in keratinocytes and lymphoid cells.

Rapamycin (25 nmol/L–1 µmol/L) inhibited both basal and glucocorticoid-induced REDD1 at the mRNA and protein levels equally efficiently when cells were pretreated with rapamycin for 1–6 hours (6-hour time point was selected to match conditions of CMAP tests) (Figure 1a–e, and see Supplementary Figure S1b–e).

Rapamycin also significantly inhibited mTOR activity assessed by phosphorylation of mTOR downstream substrates 4EBP1, 70S6K kinase, and its substrate rpS6 (Figure 1a–f). We have chosen these markers because of the easily detectable basal phosphorylation level in all studied cells (Figure 1) (Baida et al., 2015). It is known that glucocorticoids inhibit mTOR activity (Baida et al., 2015; Shimizu et al., 2011). Glucocorticoids and rapamycin cooperatively inhibited rpS6 phosphorylation in all cells (Figure 1) and 4EBP1 phosphorylation in keratinocytes but less so in lymphoid cells (Figure 1c).

We included another mTOR inhibitor, OSI-027, to determine whether we can establish REDD1 inhibitory properties for this class of drugs. Although rapamycin is a specific inhibitor of mTORC1, OSI-027 is a catalytic inhibitor that blocks adenosine triphosphate binding to both mTORC1 and mTORC2 complexes (Schenone et al., 2011). OSI-027 (1–5 µmol/L, 6 hours pretreatment) inhibited both basal and glucocorticoid-induced REDD1 in HaCaT and CEM cells (Figure 1g and h).

To determine the specificity of FA-induced REDD1 inhibition by these pharmacological inhibitors, we also genetically knocked down raptor, which is one of the important regulators of the mTORC1 complex. Successful raptor knockdown and inhibition of mTORC1 function was confirmed by Western blot analysis (Figure 1f). As expected, we showed that in HaCaT cells infected with short hairpin raptor lentivirus, REDD1 induction at the mRNA and protein levels was blunted (Figure 1f). Taken together, these results show that REDD1 induction in response to glucocorticoids is mTOR dependent.

mTORC1 inhibition by rapamycin shifts GR function toward gene transrepression

REDD1 is a GR target gene. Thus, we tested whether the negative effect of rapamycin on GR transactivation was a general phenomenon, using GRE-luciferase reporter cells.

for 24 hours. Expressions of REDD1 and raptor and activity of mTOR substrates were monitored using Western blotting. Tubulin was used as the loading control. Quantitative PCR results for REDD1 expression were normalized to the housekeeping gene RPL27 and presented as fold change compared with control.

The mean ± standard deviation was calculated for three individual RNA samples/condition. *Statistically significant difference (P < 0.001) compared with control (DMSO). ‡Statistically significant difference (P < 0.001) compared with FA and where reflected. CBP, clobetasol propionate; FA, fluocinolone acetonide; M, mol/L; NHEK, normal human epidermal keratinocyte; NS, not significant compared with control (DMSO) in shRaptor-HaCaT cells; p-, phosphorylated; qPCR, quantitative PCR; Rapa, rapamycin; sh, short hairpin; WB, Western blotting.
Rapamycin (25 nmol/L–1 μmol/L) inhibited luciferase activation by FA in both HaCaT and CEM cells (Figure 2a and c, and see Supplementary Figure S3a online). The effect was dose dependent, with maximum effect on GRE.luciferase at the 1-μmol/L dose (see Supplementary Figure S3a).

Next, we assessed the effect of rapamycin on NF-κB activation. HaCaT cells treated with solvent or rapamycin (1 μmol/L × 6 hours) and treated with solvent or FA (1 μmol/L) for 8 hours (n = 3). Luciferase induction is presented as mean ± standard deviation. aStatistically significant difference (P < 0.001) compared with control. bStatistically significant difference (P < 0.001) compared with FA. (e, f) Effect of rapamycin on NF-κB activation. HaCaT cells treated with solvent or rapamycin (1 μmol/L × 24 hours) were stimulated with TNF-α (50 ng/ml × 15 minutes). The levels of (e) phosphorylated and non-phosphorylated p65 and (f) IκB-α were analyzed by Western blotting (n = 3). GAPDH and lamin B served as loading controls. CE, cytoplasmic; Ctrl, control; FA, fluocinolone acetonide; GR, glucocorticoid receptor; GRE, glucocorticoid responsive element; NE, nuclear; Rapa, rapamycin; WB, western blotting; WC, whole cell.

Rapamycin (25 nmol/L–1 μmol/L) inhibited luciferase activation by FA in both HaCaT and CEM cells (Figure 2a and c, and see Supplementary Figure S3a online). The effect was dose dependent, with maximum effect on GRE.luciferase at the 1-μmol/L dose (see Supplementary Figure S3a). Rapamycin is widely used as an immunosuppressant; however, the underlying molecular mechanisms are not well understood. We found that in HaCaT cells induced with TNF-α, rapamycin inhibited key steps in activation of central proinflammatory factor NF-κB: IκBα phosphorylation and degradation and nuclear translocation of total and phosphorylated NF-κB protein p65/RelA (Figure 2e and f). Because glucocorticoids do not usually affect IκBα degradation and nuclear NF-κB translocation (Ratman et al., 2013), these results provide the molecular mechanisms underlying cooperation between rapamycin and glucocorticoids in negative gene regulation.

The unexpected finding of rapamycin’s potential to modify GR signaling prompted us to use Illumina (San Diego, CA) gene expression array to assess the global effect of rapamycin on glucocorticoid molecular signature in HaCaT keratinocytes. (Cells were treated with solvent, FA only, or rapamycin plus FA). We used rapamycin at a 1-μmol/L dose that was optimal in luciferase assays. We identified 130 differentially expressed genes (adjusted P-value < 0.1) affected by FA: 78 genes were up-regulated, and 52 were down-regulated. We observed multiple pathways and biological processes consistent with known glucocorticoid effects on inflammation-, steroid hormone synthesis-, cell adhesion- and extracellular matrix-related genes, as well as genes involved in development, epithelial-mesenchymal transition, and apoptosis (see Supplementary Table S2 online) (Sarkar et al., 2017; Stojadinovic et al., 2007; Wu et al., 2004). The analysis of gene categories also provided evidence of intensive crosstalk between GR and signaling, mediated by TGF-α, Ras, and Notch, which are central for differentiation and proliferation of skin cells. Some of the revealed pathway interactions, such as GR-Notch, are not known to occur in skin.

Approximately 90% of FA differentially expressed genes remained differentially expressed after combined FA plus rapamycin treatment. At the same time, rapamycin significantly affected the amplitude of FA effects according to the gene expression fold changes (logarithm of fold change) for these differentially expressed genes and even for a larger...
Rapamycin inhibits GR phosphorylation, nuclear translocation, and promoter loading

To delineate the mechanism of rapamycin-mediated blunting of GR transactivation, we assessed whether rapamycin affects key steps in GR activation: phosphorylation, nuclear import, and loading on GREs in human REDD1 gene promoter.

In FA-stimulated HaCaT cells, GR nuclear translocation occurred within 10 minutes, and GR remained in the nucleus for 30 minutes to 2 hours (Figure 4c). In CEM cells, GR nuclear localization was observed within 30 minutes to 2 hours after FA (Figure 4d). Pretreatment with rapamycin delayed nuclear translocation of GR and accelerated GR nuclear export in both cell types (Figure 4c and d).

GR phosphorylation at Ser211 in response to glucocorticoids is critical for GR transactivation (Chen et al., 2008; Galliher-Beckley and Cidlowski, 2009). GR phosphorylation was almost completely blocked by rapamycin in both keratinocytes and lymphoid CEM cells; even though the kinetics were cell type specific (Figure 4e and f). At the same time, rapamycin, even after prolonged treatment (30 hours), did not significantly alter GR expression (Figure 4a and b).

Furthermore, we evaluated GR loading on seven putative GREs in REDD1 gene promoter (see Supplementary Tables S4 and S5 online) using chromatin immunoprecipitation (ChIP). We found that FA induced recruitment of GR on six out of seven GREs in HaCaT cells. However, in the presence of rapamycin, FA did not induce and even inhibited GR-DNA binding compared with the control level, possibly because of the decreased GR nuclear translocation in the presence of rapamycin. This finding is consistent with the observed negative effect of rapamycin on basal REDD1 expression (Figure 1a–c).

Rapamycin protects skin against steroid-induced atrophy but does not affect anti-inflammatory effects of glucocorticoids

Next, we assessed whether rapamycin prevents REDD1 induction by glucocorticoids in vivo in F1 C57Bl×129 mice. In agreement with previous results (Baida et al., 2015), FA (2 μg/animal) strongly induced REDD1 expression in epidermis. Pretreatment with rapamycin blocked REDD1 mRNA and protein induction (Figure 5a and b).

Chronic topical treatment of mice with FA (every 72 hours for 2 weeks) induced severe skin atrophy (Figure 5c and d). The rapamycin spared epidermis from steroid atrophy: in mice treated with FA only, the epidermal thickness was...
reduced by 62%, compared with less than 30% in mice treated with FA plus rapamycin (Figure 5d). Subcutaneous adipose tissue was not significantly protected (Figure 5c), possibly because of a limited rapamycin penetration deeper through the skin barrier when applied in solvent.

The effect of rapamycin in preserving therapeutic/anti-inflammatory potential of FA was assessed using two different models: acute croton oil-induced ear edema and chronic contact dermatitis induced by irritant 12-O-tetradecanoyl-phorbol-13-acetate. As expected, croton oil-induced inflammation resulted in ear swelling and weight increase, and FA completely prevented ear edema (Figure 5e). In the second model, 12-O-tetradecanoylphorbol-13-acetate induced both significant inflammation and epidermal hyperplasia. FA significantly reduced skin hyperplasia measured by epidermal thickness and inflammation measured by dermal cellularity and quantitative PCR of inflammation- and proliferation-related genes (cytokines IL-1β, IL-22, and S100 calcium binding proteins 8 and 9; MMP3, and p65/RelA- and p50-encoding major NF-kB subunits) (see

Figure 4. Rapamycin inhibits GR phosphorylation, nuclear translocation, and binding to GREs in REDD1 promoter. (a, b) Western blot analysis of GR expression in (a) HaCaT and (b) CEM cells treated with rapamycin (1 μmol/L). (c–f) Western blot and immunofluorescence analysis of GR translocation and phosphorylation in (c, e) HaCaT and (d, f) CEM cells pretreated with rapamycin (1 μmol/L x 6 hours) and treated with FA (1 μmol/L). GAPDH and HDAC1 served as protein loading controls. Scale bars = 10 μm. (g) GR loading on seven GREs in REDD1 promoter was assessed in HaCaT cells treated as described by ChIP. Statistically significant difference (P<0.001) compared with control and FA. Data are mean ± standard deviation, n = 3. ChIP, chromatin immunoprecipitation; Ctrl, control; FA, fluocinolone acetonide; GR, glucocorticoid receptor; GRE, glucocorticoid responsive element; h, hour; min, minute; p-, phosphorylation.
These genes were selected from the database of 12-O-tetradecanoylphorbol-13-acetate–induced transcriptome in mouse skin (Riggs et al., 2005). Pretreatment with rapamycin did not affect the anti-inflammatory effects of FA in both models (Figure 5e, and see Supplementary Figure S5).

**DISCUSSION**

The long-term goal of this work is to develop safer GR-targeted therapies using glucocorticoids combined with compounds protecting tissues against steroid catabolic effects. Our recent findings that, in a cell milieu lacking REDD1, the anti-inflammatory and atrophogenic effects of glucocorticoids are dissociated (Baida et al., 2015) prompted us to search for REDD1 pharmacological inhibitors using a drug repurposing approach (Dudley et al., 2011; Sirota et al., 2011). We identified rapamycin as a top candidate.

The capability of mTOR inhibitor rapamycin to down-regulate REDD1 expression suggested the existence of a feedback loop in mTOR signaling. This was further supported by experiments with genetic mTORC1 blockade by short hairpin raptor. REDD1 is a short-lived protein tightly controlled by proteasome degradation (Katiyar et al., 2009; Kimball et al., 2008). It was shown that mTOR regulates REDD1 proteasomal degradation and that mTOR inhibitors reduce REDD1 protein half-life (Tan and Hagen, 2013). Here, we report that pharmacological mTOR inhibitors rapamycin and OSI-027 resulted in inhibition of both basal and glucocorticoid-induced REDD1 expression at the mRNA and protein levels in different cell types and in skin in vivo. These findings indicate the physiological importance of mTOR/REDD1 feedback loop and imply its role in tuning the negative crosstalk between catabolic GR effects and anabolic effects of mTOR signaling (Figure 6).

Unexpectedly, we also found that rapamycin significantly modulated GR activity, shifting it toward transrepression. Overall, our results suggest that in the presence of rapamycin, glucocorticoids behave as selective GR activators designed to preserve therapeutic effects but decrease adverse effects of GR signaling (De Bosscher et al., 2010; Lesovaya et al., 2015).

GR nuclear import and transactivation function strongly depend on GR phosphorylation (Lesovaya et al., 2015; Vandeheyver et al., 2013), which is mediated by MAPKs, CDKs, and GSK-3β (Chen et al., 2008; Galliher-Beckley and Cidlowski, 2009). We report here that rapamycin inhibited GR phosphorylation at activating Ser211 and slowed GR nuclear import. It is conceivable that rapamycin affected GR phosphorlating kinases as mTOR crosstalk with p38, GSK-3β signaling pathways is known (Kitagishi et al., 2012; Mendoza et al., 2011; Nader et al., 2010). The diverse mechanisms of negative gene regulation include negative protein–protein interaction between GR and other TFs, which could occur in the nucleus (tethering) or, as we showed, in the cytoplasm, blocking TF nuclear import (Yemelyanov et al., 2008). Our results clearly indicate that exaggeration...
of GR transrepression by rapamycin at least partially occurs via NF-kB blockage (Figure 2, and see Supplementary Table S3). However, the detailed mechanisms of cooperation between glucocorticoids and rapamycin in transrepression regulation remain to be investigated.

Glucocorticoids induce atrophy in many organs besides skin (Lesovaya et al., 2015). We previously showed a strong similarity between GR molecular signatures in epidermis, muscle, and subcutaneous adipose tissue undergoing steroid-induced hypoplasia (Baida et al., 2015). We and others also showed that REDD1 plays a central role as an atrophogene in those tissues (Baida et al., 2015; Britto et al., 2014). Thus, rapamycin represents a promising therapeutic candidate for safer combination GR-targeting therapies for the treatment of inflammatory skin conditions and other inflammatory/ autoimmune diseases (such as rheumatoid arthritis, asthma, and inflammatory bowel disease), as well as blood cancer.

MATERIALS AND METHODS

Chemicals
OSI-027 was from Selleck Chemicals (Houston, TX), rapamycin from LC Laboratories (Woburn, MA), TNF-α from Thermo Fisher Scientific (Waltham, MA), and puromycin dihydrochloride and all other chemicals from Sigma-Aldrich (St. Louis, MO).

Computational screen of REDD1 inhibitors
Putative REDD1 inhibitors were identified by computational screening of the CMAP database (https://www.broadinstitute.org/cmap/) (Lamb et al., 2006). The selection of top prospective REDD1 inhibitors was based on the number of experiments in which REDD1 was within the top 100 down-regulated genes in CMAP experiments. Computational screening was performed using the R project for statistical computing, version 3.2.5 (https://www.r-project.org/).

Animals and treatments
Seven-week-old F1 C57Bl/129 female mice (Taconic, Germantown, NY) in the telogene stage of hair cycle were shaved 3 days before treatments.

Both rapamycin and FA were dissolved in acetone and applied in 200 μl of acetone to the mouse back skin, as described (Baida et al., 2015; Checkley et al., 2011). The treated area was approximate 10 cm². The concentration of rapamycin in animal experiments was 2.5 mg/ml of solvent, and FA was applied at 10 μg/ml of solvent.

Acute treatment. Animals were pretreated topically with vehicle or rapamycin for 6 hours and then treated with FA for 8–24 hours. Skin was harvested, and epidermis mechanically separated from dermis was used for RNA and protein isolation, as described (Baida et al., 2015).

Skin atrophy test. Animals were treated topically with vehicle, FA, or FA plus rapamycin (doses as above) every 72 hours for 2 weeks. This FA regimen of treatment induces profound skin atrophy. Skin was harvested 24 hours after the last FA application.

Contact dermatitis. Animals were pretreated topically with rapamycin (as described) and treated with contact irritant 12-O-tetradecanoylphorbol-13-acetate (4 μg/mouse) with or without FA (1 μg/mouse) every 72 hours for 2 weeks. Control animals were treated with vehicle only. Skin was harvested 24 hours after the last treatment. RNA was extracted from epidermis.

Ear edema test. Test was performed as described (Baida et al., 2015). Mouse ear lobes were pretreated with either vehicle (20 μl acetone) or rapamycin (0.25 mg/ear) for 6 hours and then treated with nonspecific contact irritant croton oil (5% in acetone) with or without FA (1 μg/ear) for 9 hours, a time point at which we observed maximum ear edema in B6×129 animals (Baida et al., 2015).
4-mm ear punch biopsy samples were weighted to measure ear swelling as a readout for inflammation.

All animal experiments were performed according to protocols approved by the Northwestern University Animal Care and Use Committee (#IS00005246).

**Morphometric analysis**

Sections of formalin-fixed, paraffin-embedded skin were stained with hematoxylin and eosin. The quantification of the epidermal width and dermal cellularity was performed as described (Baida et al., 2015). Ten individual fields of view per slide in three individual skin samples per treatment (at least 30 images/treatment group) were analyzed.

**Cell cultures**

Human keratinocyte cell line HaCaT was kindly provided by Kathleen Green (Northwestern University, Chicago, IL). T-leukemic cell line CEM was from ATCC (ATCC-CCL-119; Manassas, VA). The cells were cultured as described (Baida et al., 2015; Lesovaya et al., 2013). Primary newborn human epidermal keratinocytes were obtained from Northwestern University Skin Disease Research Center (Chicago, IL) and cultured in M154CF serum-free medium with 70 μmol/L calcium supplemented with human keratinocyte growth supplement (Thermo Fisher Scientific, Waltham, MA). mTOR inhibitors and glucocorticoids were dissolved in DMSO and added to cell culture medium (final DMSO concentration < 0.1%).

**Western blot analysis**

Western blot analysis of whole-cell, cytoplasmic, and nuclear protein extracts was performed as described (Baida et al., 2015). Proteins were resolved by SDS-PAGE and transferred to Odyssey membrane. Western blot analysis of whole-cell, cytoplasmic, and nuclear proteins was performed as described (Baida et al., 2015). Primary newborn human epidermal keratinocytes were treated with solvent only (control); rapamycin (1 μmol/L), with FA (1 μmol/L), and with FA after 6 hours pretreatment with rapamycin. The experiment was repeated twice. RNA amplification, labeling, and hybridization with the Illumina Whole-Genome Gene Expression BeadChip array (HumanHT-12) were performed at University of Chicago Genomics Facility according to Illumina protocols. Array results were submitted to the Gene Expression Omnibus (GSE97279). Details of the array analysis are presented in the Supplementary Materials online.

**Luciferase assay**

We generated GRE.Luc, NF-κB.Luc and control mCMV.Luc reporter cells by lentiviral infection as described using viral stocks obtained from Northwestern University Skin Disease Research Center DNA/RNA Delivery Core. Reporter cells were pretreated for 6 hours with rapamycin (1 μmol/L) or vehicle followed by FA (1 μmol/L) or vehicle treatment for 8 hours. Luciferase activity was measured as described by Baida et al. (2015).

**Immunofluorescence**

GR nuclear translocation was determined by immunofluorescence using anti-GR antibody in HaCaT cells fixed and permeabilized as described (Baida et al., 2015). Cell nuclei were counterstained with DAPI (Thermo Fisher Scientific, Waltham, MA).

**Chromatin immunoprecipitation (ChIP)**

ChIP was performed to assess GR loading on REDD1 GREs using EZ-Magna ChIP A/G ChIP Kit (Millipore, Darmstadt, Germany) per the manufacturer’s recommendations. The GR binding sites in human REDD1 promoter were predicted based on HOCOMOCO models of TF binding motifs using ChIP sequencing and high-throughput systematic evolution of ligands by exponential enrichment (i.e., HT-SELEX) data (Yevshin et al., 2017).

HaCaT cells were either pretreated with 1 μmol/L rapamycin for 6 hours followed by addition of FA for 24 hours or treated with solvent and FA (1 μmol/L) alone. Primers are listed in Supplementary Table S5. Fold enrichment was reported as 2^{ΔΔCt}, where ΔCt = Ct(IP) – Ct(Input × DF) and ΔΔCt = ΔCt(IP) – ΔCt(NS). (DF indicates dilution factor; IP indicates immunoprecipitation; and NS indicates IP with nonspecific IgG.) Data are mean ± standard deviation (n = 3).

**Statistical analysis**

Mean and standard deviation values were calculated using Microsoft Excel software (Microsoft, Redmond, WA). The treatment effects in each experiment were compared by one-way analysis of variance or t test. Differences between groups were considered significant at P < 0.05. All experiments were repeated two or three times. In animal experiments, we used three or four animals per experimental group.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.
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SUPPLEMENTARY MATERIALS
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.02.045.

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