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Regulatory Effects of Programmed Cell Death 4 (PDCD4) Protein in Interferon (IFN)-Stimulated Gene Expression and Generation of Type I IFN Responses

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The precise mechanisms by which the activation of interferon (IFN) receptors (IFNRs) ultimately controls mRNA translation of specific target genes to induce IFN-dependent biological responses remain ill defined. We provide evidence that IFN-α induces phosphorylation of programmed cell death 4 (PDCD4) protein on Ser67. This IFN-α-dependent phosphorylation is mediated by either the p70 S6 kinase (S6K) or the p90 ribosomal protein S6K (RSK) in a cell-type-specific manner. IFN-dependent phosphorylation of PDCD4 results in downregulation of PDCD4 protein levels as the phosphorylated form of PDCD4 interacts with the ubiquitin ligase β-TRCP (β-transducin repeat-containing protein) and undergoes degradation. This process facilitates IFN-induced eukaryotic translation initiation factor 4A (eIF4A) activity and binding to translation initiation factor eIF4G to promote mRNA translation. Our data establish that PDCD4 degradation ultimately facilitates expression of several ISG protein products that play important roles in the generation of IFN responses, including IFN-stimulated gene 15 (ISG15), $p21^{WAF1/CIP1}$, and Schlafen 5 (SLFN5). Moreover, engagement of the RSK/PDCD4 pathway by the type I IFNR is required for the suppressive effects of IFN-α on normal $CD34^+$ hematopoietic precursors and for antileukemic effects *in vitro*. Altogether, these findings provide evidence for a unique function of PDCD4 in the type I IFN system and indicate a key regulatory role for this protein in mRNA translation of ISGs and control of IFN responses.

Interferons (IFNs) are a family of pleiotropic cytokines that inhibit viral replication and exhibit important immunomodulatory, antiproliferative, and antitumor properties via their regulatory effects on cell cycle progression, cell proliferation, and, under certain circumstances, apoptosis (5, 35, 47). Because of these activities, IFNs have been examined extensively in clinical trials over the last 3 decades and have found applications in the management of various malignancies, viral syndromes, and autoimmune disorders (35, 49, 57, 59). This broad spectrum of clinical applications for IFNs is a reflection of the diversity of their biological effects.

There are three major IFN groups/families: type I (α, β, ε, κ, and ω), type II (γ), and type III (λ) (8, 35, 47). The mechanisms by which IFNs initiate transcription of target genes have been excessively studied and precisely defined over the years. Engagement of IFN receptors (IFNRs) activates Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathways, leading to transcriptional induction of IFN-stimulated genes (ISGs) and ultimately the generation of ISG products which are critical in order for the IFN biological responses to occur (6, 29, 39, 47). Notably, distinct combinations of JAKs at the receptor complex and inducible STAT complexes are differentially regulated by different types of IFNs, allowing for gene expression specificity via distinct STAT-binding elements in the promoters of ISGs (6, 8, 29, 30, 39, 43, 47).

In previous work we provided evidence that the AKT/mTOR (mammalian target of rapamycin) pathway is activated during engagement of IFNRs and regulates downstream engagement of various effectors of the pathway, including S6 kinase (S6K)/ribosomal protein S6 (rpS6), eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), and eIF4B (19, 20, 22, 23, 27, 28). Moreover, we demonstrated that the functions of AKT and the phosphatidylinositol 3′-kinase (PI3′K) pathway upstream of AKT are essential for engagement of IFN-regulated mTOR effector elements and initiation of mRNA translation of certain ISGs (20, 21).

Programmed cell death 4 (PDCD4) protein is a translational repressor that blocks eIF4A helicase activity by binding to eIF4A and also interfering with eIF4A binding to eIF4G, resulting in negative effects on/control of the initiation of mRNA translation (25, 37, 48, 55). Recent studies have shown a function for PDCD4 as a tumor suppressor that is lost in certain aggressive malignant diseases (11, 12, 24, 31, 33, 44). Interestingly, emerging evidence also suggests that the function of PDCD4 can be altered by the cofactor protein arginine methyltransferase 5 (PRMT5) and that arginine methylation of PDCD4 results in acceleration of tumor growth (41). Thus, there is accumulating evidence that PDCD4 plays an important role in the control of tumorigenesis and that its deregulation has important consequences in cell proliferation and neoplastic transformation.

In the present study, we examined whether engagement of the type I IFNR results in modulation of PDCD4 phosphorylation and assessed the effects of type I IFN treatment on the levels of PDCD4

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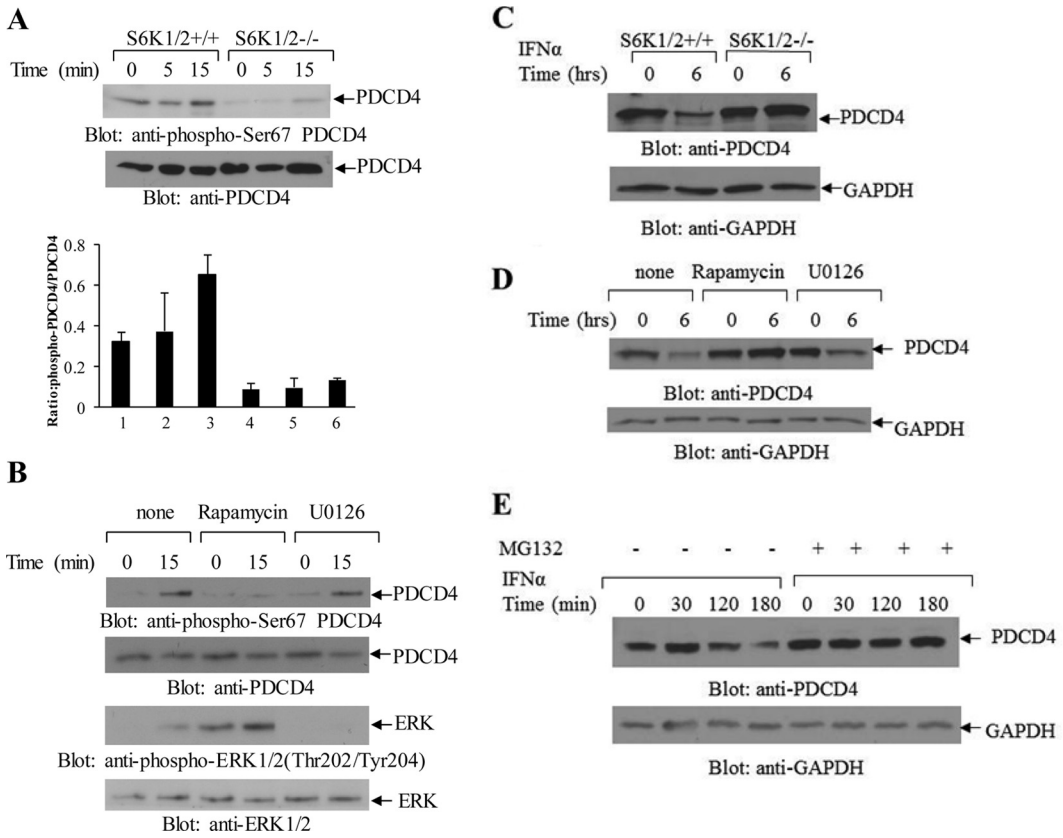

FIG 1 Effects of IFN-α on phosphorylation and degradation of PDCD4 in MEFs. (A) Serum-starved S6K1$^{+/+}$ S6K2$^{+/+}$ (WT) and S6K1$^{-/-}$ S6K2$^{-/-}$ MEFs were treated with IFN-α for the indicated times. Total cell lysates were resolved by SDS-PAGE and immunoblotted with antibodies against the phosphorylated form of PDCD4 on Ser67 or against PDCD4 as indicated (top panel). Signals were quantified by densitometry and used to calculate the intensity of phosphorylated PDCD4 relative to that of total PDCD4 (bottom panel). Data are expressed as ratios of phospho-PDCD4 to PDCD4 for each experimental condition and represent means ± standard error of the results of three experiments, including the one shown in the upper panel. (B) Serum-starved wild-type MEFs were pretreated for 60 min with rapamycin or U0126 and were subsequently treated with IFN-α for the indicated times. Cell lysates were resolved by SDS-PAGE and immunoblotted with anti-phospho-Ser67-PDCD4, anti-PDCD4, anti-phospho-Thr202/Tyr204-ERK1/2 or anti-ERK1/2 antibodies, as indicated. (C) Serum-starved S6K1$^{+/+}$ S6K2$^{+/+}$ (WT) or S6K1$^{-/-}$ S6K2$^{-/-}$ MEFs were incubated with IFN-α for 6 h, as indicated. Total cell lysates were resolved by SDS-PAGE and immunoblotted with antibodies against PDCD4 or against GAPDH, as indicated. (D) Serum-starved WT MEFs were pretreated for 60 min with rapamycin or U0126 and were subsequently treated with IFN-α in the continuous presence or absence of rapamycin or U0126 for 6 h as indicated. The cells were lysed, and total cell lysates were resolved by SDS-PAGE and immunoblotted with antibodies against PDCD4 or against GAPDH, as indicated. (E) Serum-starved S6K1$^{+/+}$ S6K2$^{+/+}$ MEFs were treated with IFN-α for the indicated times in the presence or absence of the proteasome inhibitor MG132. Total cell lysates were resolved by SDS-PAGE and immunoblotted with anti-PDCD4 or anti-GAPDH antibodies, as indicated.

protein expression. Our studies provide evidence that IFN-dependent phosphorylation of PDCD4 on serine 67 results in enhanced interaction of PDCD4 with β-TRCP (β-transducin repeat-containing protein), and this is followed by PDCD4 degradation. We also demonstrate that the activity of the S6 kinase (S6K) is required for phosphorylation of PDCD4 in mouse embryonic fibroblasts (MEFs), while ribosomal S6 kinase 1 (RSK1) is the kinase responsible for this phosphorylation in U266 and KT1 hematopoietic cells. Our data also demonstrate that pharmacological inhibition of RSK or small interfering RNA (siRNA)-mediated RSK1 knockdown results in blocking of IFN-α-inducible expression of ISG15, p21$^{WAF1/CIP1}$, and Schlafen 5 (SLFN5) and results in partial reversal of the antiproliferative effects of IFN-α. Altogether, our data provide the first direct evidence implicating PDCD4 in IFN signaling and suggest a key regulatory role for the RSK/PDCD4 pathway in the generation of the biological properties of type I IFNs.

MATERIALS AND METHODS

Cells and reagents. The human KT1 and U266 cell lines were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and antibiotics. In some experiments, the cells were serum starved for 24 h prior to the indicated treatments. Immortalized S6K1$^{+/+}$ S6K2$^{+/+}$ and S6K1$^{-/-}$ S6K2$^{-/-}$ MEF cells (38) were grown in Dulbecco's modified Eagle's medium (DMEM) medium supplemented with 10% FBS and antibiotics. In some experiments, the MEF cells were serum starved in 0.5% FBS for 24 h prior to the treatments shown in the figures and/or legends. Antibodies against p21$^{WAF1/CIP1}$ and against the phosphorylated form of PDCD4 and RSK1 were purchased from Abcam (Cambridge, MA). An antibody against PDCD4 was purchased from Rockland (Gilbertsville, PA). Antibodies against eIF4A, β-TRCP, and PDCD4 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and were used in immunoprecipitations. Control siRNA, siRNA targeting PDCD4 or RSK1, control short hairpin RNA (shRNA), shRNA targeting PDCD4, and puromycin were from Santa Cruz Biotechnology (Santa Cruz, CA). An antibody against SLFN5 was obtained from Sigma-Aldrich (Steinheim, Germany). A monoclonal antibody recognizing human ISG15 was kindly provided

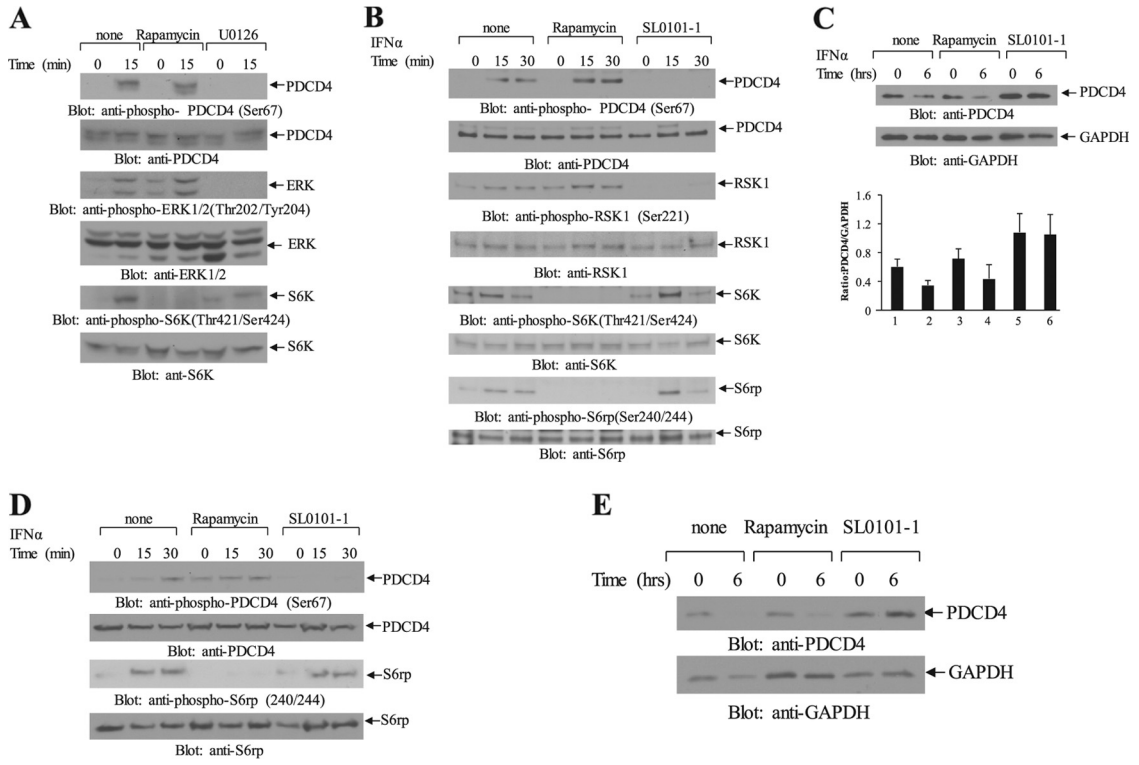

FIG 2 IFN-dependent regulation of PDCD4 phosphorylation and protein expression in KT1 hematopoietic cells. (A) Serum-starved KT1 cells were pretreated for 60 min with rapamycin or the MEK inhibitor U0126 and were subsequently treated with IFN-α in the continuous presence or absence of rapamycin or U0126 for the indicated times. Cell lysates were resolved by SDS-PAGE and immunoblotted with anti-phospho-Ser67-PDCD4, anti-PDCD4, anti-phospho-Thr202/Tyr204-ERK1/2, anti-ERK1/2, anti-phospho-S6K, and anti-S6K antibodies as indicated. (B) Similar experiment as shown in panel A, except that cells were pretreated for 60 min with rapamycin or for 180 min with SL0101-1. Total cell lysates were resolved by SDS-PAGE and immunoblotted witFh anti-phospho-Ser67-PDCD4, anti-phospho-Ser221-RSK1, anti-RSK1, and anti-S6K, as indicated. Equal cell lysates from the same experiment were analyzed separately by SDS-PAGE and immunoblotted with anti-PDCD4, anti-phospho-Thr421/Ser424-S6K, anti-phospho-Ser240/244-rpS6, and anti-rpS6. (C) The upper panel shows an experiment similar to that in panel B, except that the cells were treated for 6 h as indicated. Protein lysates were analyzed by immunoblotting with antibodies against PDCD4 or against GAPDH. Signals were quantified by densitometry and used to calculate the intensity of expression of PDCD4 relative to that of GAPDH (lower panel). Data are expressed as ratios of PDCD4 to GAPDH for each experimental condition and represent means ± standard error of the results of three experiments, including the experiment shown in the upper panel. (D) Serum-starved U266 cells were pretreated for 60 min with rapamycin or for 3 h with SL0101-1 and were subsequently treated with IFN-α in the continuous presence or absence of rapamycin or SL0101-1, as indicated. Cell lysates were resolved by SDS-PAGE and immunoblotted with anti-phospho-Ser67-PDCD4, anti-PDCD4, anti-phospho-Ser240/244-rpS6, or anti-rpS6, as indicated. (E) Serum-starved U266 cells were pretreated for 60 min with rapamycin or for 3 h with SL0101-1, as indicated, and were subsequently treated with IFN-α in the continuous presence or absence of the indicated inhibitors for 6 h. Cell lysates were resolved by SDS-PAGE and immunoblotted with anti-PDCD4 or anti-GAPDH antibodies, as indicated.

by Ernest Borden (Taussing Cancer Center, Cleveland, OH). An antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was from Chemicon (Billerica, MA). Antibodies against phospho-Thr202/Tyr204–extracellular signal-regulated kinase 1 and 2 (ERK1/2), ERK1/2, phospho-Ser221-RSK1, RSK1, phospho-Thr421/Ser424-p70^{S6K}, anti-p70^{S6K}, phospho-Ser240/244-rpS6, rpS6, eIF4A, eIF4E, eIF4G, 4E-BP1, and hemagglutinin (HA) were obtained from Cell Signaling Technology (Beverly, MA). Normal CD34^{+} bone marrow-derived cells were obtained from Lonza (Basel, Switzerland). Recombinant human IFN-α was provided by Hoffman La Roche, Inc. The FRAP (FKBP12-rapamycin-associated protein)/mTOR inhibitor rapamycin and the MEK1 and MEK2 (MEK1/2) inhibitor U0126, the proteosomal inhibitor MG132, phosphatase, and protease inhibitor were obtained from Calbiochem Inc. (La Jolla, CA). The RSK inhibitor SL0101-1 was from Symansis (Auckland, New Zealand). 7-Methyl GTP-Sepharose was from GE Healthcare UK, Ltd. (Little Chalfont Buckinghamshire, United Kingdom). The siRNA transfection reagent TransIT-TKO and plasmid DNA transfection reagent TransIT-LT1 were from Mirus Bio Corporation (Madison, WI). Nucleofector solution was from Lonza Cologne AG (Cologne, Germany). Recombinant PDCD4 protein was from Prospec (Rehovot, Israel). Clean-Blot immunoprecipitation (IP) detection reagent was from Thermo Scientific. HA-tagged wild-type (WT) PDCD4 and the HA-tagged PDCD4(S67/71A) mutant (10) were a gift from Michele Pagano (Department of Pathology, New York University).

Immunoprecipitations and immunoblotting. Cells were treated with IFN-α for the indicated times and lysed in phosphorylation lysis buffer (PLB) as in previous studies (19–23). Cells were serum starved for 24 h prior to the treatments shown in the figures and/or legends. Immunoprecipitations and immunoblotting using an enhanced chemiluminescence (ECL) method were performed as previously described (19–23). In some experiments the cells were preincubated with rapamycin (20 nM) or U0126 (10 μM) for 60 min or SL0101-1 (60 μM) for 3 h prior to IFN-treatment.

Cap binding assays. Cap binding assays were performed as previously described (22). Briefly, KT1 cells were incubated for 24 h in serum-free medium and then pretreated for 60 min with rapamycin (20 nM) or SL0101-1 (60 μM), followed by IFN-α treatment for the indicated times. Cell lysates were incubated with 7-methyl-GTP Sepharose (Amersham) for 4 h and then washed with lysis buffer. Proteins were resolved by SDS-PAGE, transferred onto Immobilon membranes (Millipore), and probed with the antibodies shown in the figures and/or legends.

***In vitro* kinase assays.** Immune complex kinase assays to detect RSK1 kinase activity in anti-RSK1 immunoprecipitates were performed essentially as previously described (23). PDCD4 was used as an exogenous substrate.

Isolation of polysomal RNA and quantitative RT-PCR. KT1 cells transduced with control shRNA or shRNA targeting human PDCD4 were serum starved for 24 h and then left untreated or treated with IFN-α for 24 h. Isolation of polysomal RNA and quantitative reverse transcription-PCR (RT-PCR) of the polysomal fractions were performed as previously described (16). Real-time PCR for the *Isg15*, *p21*, and *slfn5* genes was conducted using commercially available 6-carboxyfluorescein (FAM)-labeled probes and primers (Applied Biosystems), and *gapdh* was used for normalization. mRNA amplification was determined as previously described (15, 16), and relative quantitation of mRNA levels was plotted as fold increase compared to untreated samples.

Hematopoietic progenitor assays in methylcellulose. Clonogenic assays in methylcellulose to detect leukemic CFU-blast (CFU-L) colony formation from KT1 cells were performed essentially as previously described (3). The effects of IFN-α on CFU-L colony formation from KT1 cells transfected with control siRNA or siRNAs specific for PDCD4 or HA-tagged PDCD4(S67/71A) were determined essentially as previously described (3). Myeloid progenitor (granulocyte-macrophage CFU [CFU-GM]) colony formation from normal $CD34^+$ cells was assessed in clonogenic assays in methylcellulose, as previously described (45).

RESULTS

There is evidence that PDCD4 is a target for the kinase activity of S6K in other systems (9), and our previous work has demonstrated that S6K is phosphorylated/activated in a type I IFN-dependent manner in different cell types (23, 28, 36). We examined whether IFN-α induces phosphorylation of PDCD4 and, if so, whether such phosphorylation occurs in an S6K-dependent manner. For this purpose, experiments were performed using immortalized MEFs with targeted disruption of both the *S6k1* and *S6k2* genes (38). Serum-starved MEFs were incubated in the presence or absence of mouse IFN-α, and total cell lysates were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with an antibody against the phosphorylated form of PDCD4 on serine 67. IFN-α treatment resulted in induction of phosphorylation of PDCD4 on Ser67 in $S6K1^{+/+}$ $S6K2^{+/+}$ MEFs, but this phosphorylation was defective in S6K1 S6K2 double-knockout MEFs (Fig. 1A). Notably, when wild-type MEFs were preincubated with the mTOR inhibitor rapamycin, the phosphorylation of PDCD4 on Ser67 was blocked (Fig. 1B), consistent with the lack of phosphorylation seen in the S6K1 S6K2 double-knockout MEFs. On the other hand, IFN-α-dependent PDCD4 phosphorylation was still detectable in cells treated with the MEK inhibitor U0126 (Fig. 1B).

PDCD4 phosphorylation on Ser67 results in the degradation of the protein via the ubiquitin ligase β-TRCP, as reported in other systems (9, 10). Accordingly, we examined the effects of IFN-α on PDCD4 protein expression in the S6K1 S6K2 double knockout cells. Serum starved $S6K1^{+/+}$ $S6K2^{+/+}$ or $S6K1^{-/-}$ $S6K2^{-/-}$ MEFs were treated with IFN-α for 6 h, and PDCD4 expression was assessed. After 6 h of IFN treatment, detectable PDCD4 protein levels decreased in $S6K1^{+/+}$ $S6K2^{+/+}$ MEFs while they remained unchanged in $S6K1^{-/-}$ $S6K2^{-/-}$ cells (Fig. 1C). Similarly, pretreatment of the cells with rapamycin, which blocks PDCD4 phosphorylation, reversed the IFN-α-dependent decrease in PDCD4 expression (Fig. 1D). In contrast, the addition of the MEK inhibitor U0126 did not reverse the suppressive effects of IFN-α (Fig. 1D). It should be noted that the downregulation of PDCD4 was

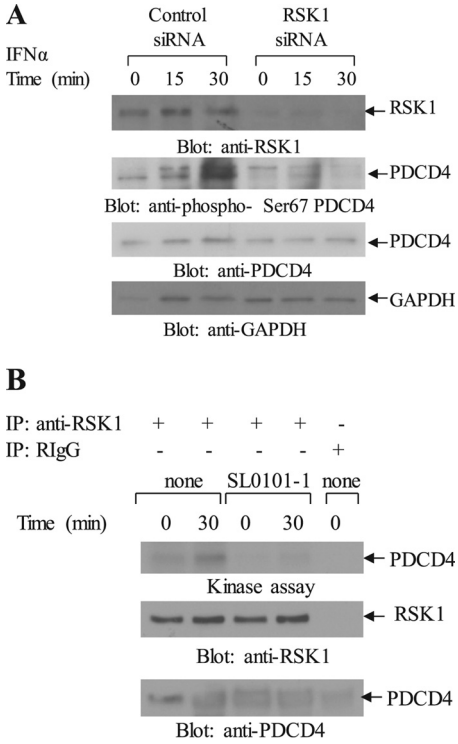

FIG 3 RSK1 activity mediates IFN-α-dependent PDCD4 phosphorylation in hematopoietic cells. (A) KT1 cells were transfected with either control siRNA or siRNAs specifically targeting RSK1 and after serum starvation were treated with IFN-α, as indicated. Total cell lysates were resolved by SDS-PAGE and immunoblotted with anti-RSK1, anti-phospho-Ser67-PDCD4, anti-PDCD4, or anti-GAPDH antibodies, as indicated. (B) Serum-starved KT1 cells were pretreated with SL0101-1 for 3 h and then treated with IFN-α for the indicated times. The cells were lysed, and equal amounts of protein were immunoprecipitated (IP) with an anti-RSK1 antibody. In *vitro* kinase assays to detect RSK1 activity were subsequently carried out on the immunoprecipitates, using PDCD4 protein as an exogenous substrate.

time dependent, commencing after 120 min of IFN treatment and reaching a maximum at 180 min (Fig. 1E). To directly examine whether the decrease in protein levels seen after IFN treatment reflects degradation of the protein, the effects of the proteasomal inhibitor MG132 were assessed. MG132 treatment of the cells resulted in reversal of the IFN-α-dependent suppression of PDCD4 (Fig. 1E), suggesting a mechanism involving proteasomal degradation. Altogether, these studies establish that IFN-α induces S6K-mediated phosphorylation of PDCD4 and that this phosphorylation ultimately promotes degradation of PDCD4 protein.

To further define the mechanisms of the IFN-dependent regulation of PDCD4 phosphorylation, similar studies were performed in cells of hematopoietic origin. In previous work we have shown that IFN-inducible phosphorylation of the mTORC1 effector eIF4B is differentially regulated by $p70^{S6K}$ or RSK in a cell-type-specific manner, with RSK1 being the predominant kinase in cells of hematopoietic origin (23). As there is also evidence from other investigators that RSK and $p70^{S6K}$ can phosphorylate substrates at the RXRXXS/T motif (26, 32, 52), we examined whether PDCD4 phosphorylation can be regulated in hematopoietic cells by RSK, whose activation in the IFN system is MEK/ERK dependent (23). As shown in Fig. 2A, IFN-α-dependent phosphorylation of PDCD4 and ERK in KT1 cells was blocked by the MEK

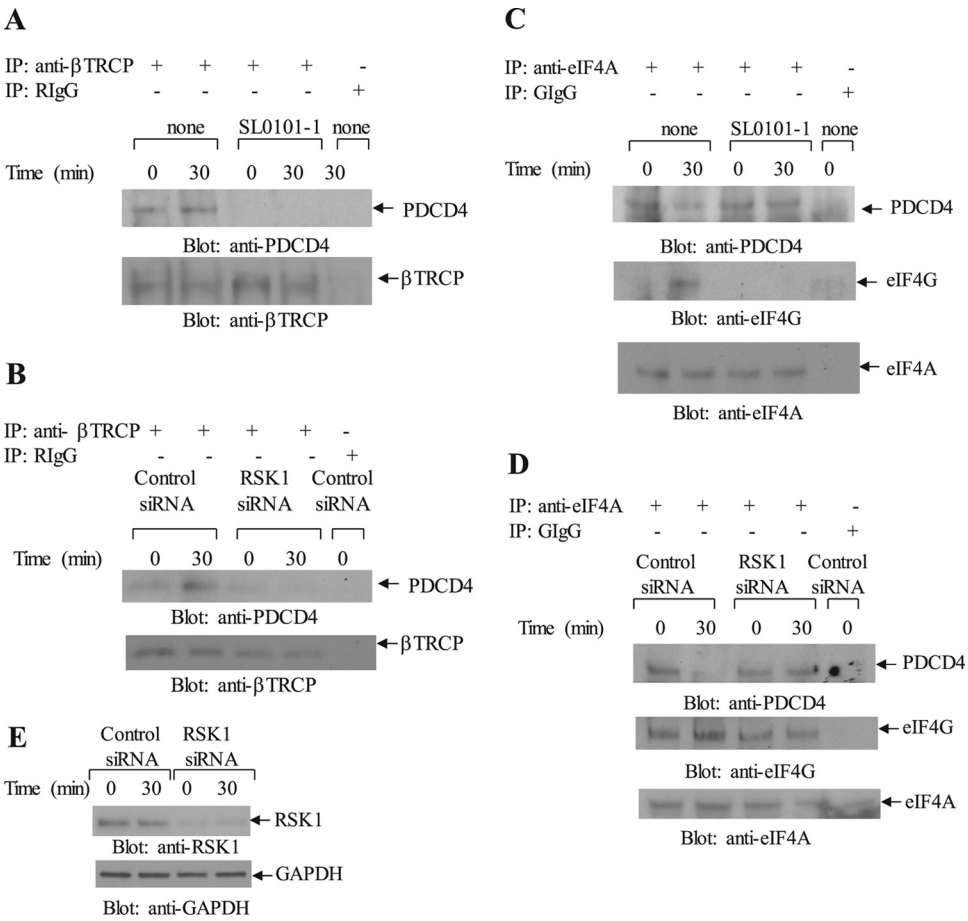

FIG 4 IFN-α-inducible Ser67 PDCD4 phosphorylation results in its interaction with β-TRCP. (A) Serum-starved KT1 cells were pretreated for 3 h with SL0101-1 and were left untreated or treated with IFN-α in the continuous presence or absence of SL0101-1, as indicated. Equal amounts of cell lysates were immunoprecipitated with an anti-β-TRCP antibody or control nonimmune rabbit IgG (RIgG). Immune complexes were resolved by SDS-PAGE for analysis of PDCD4 and β-TRCP, as indicated. (B) KT1 cells were transfected with either control siRNA or RSK1 siRNA and after serum starvation were either left untreated or treated with IFN-α for 30 min as indicated. Equal amounts of cell lysates were immunoprecipitated with an anti-β-TRCP antibody or control RIgG. Immune complexes were resolved by SDS-PAGE for analysis of PDCD4 and β-TRCP, as indicated. (C) The experiment is similar to that shown in panel A, except that equal amounts of cell lysates were immunoprecipitated with an anti-eIF4A antibody or control nonimmune goat IgG (GIgG). Immune complexes were resolved by SDS-PAGE for analysis of PDCD4, eIF4G, and eIF4A, as indicated. (D) The experiment is similar to that shown in panel B, except that equal amounts of cell lysates were immunoprecipitated with an anti-eIF4A antibody or control GIgG. Immune complexes were resolved by SDS-PAGE for analysis of PDCD4, eIF4G, and eIF4A, as indicated. (E) Lysates for the different experimental conditions from the experiment shown in panel D were resolved by SDS-PAGE and immunoblotted by anti-RSK1 or anti-GAPDH antibodies to establish RSK1 knockdown in cells transfected with siRNA against RSK1.

inhibitor U0126 (Fig. 2A), which regulates upstream IFN-dependent engagement of RSK1 in these cells. Next, the effects of RSK inhibition on IFN-dependent phosphorylation of PDCD4 and downregulation of PDCD4 protein levels were determined in these cells. When KT1 cells were treated with the RSK1 inhibitor SL0101-1, we found that phosphorylation of PDCD4 on Ser67 and phosphorylation of RSK1 on Ser221 were blocked (Fig. 2B), while phosphorylation of $p70^{S6K}$ and rpS6 was not (Fig. 2B). Addition of the RSK inhibitor, but not rapamycin, also prevented the decrease in PDCD4 protein levels seen in these cells after prolonged IFN treatment (Fig. 2C). Similar results were obtained when studies were performed using the U266 hematopoietic cell line. As in the case of KT1 cells, IFN-α also induced phosphorylation of PDCD4, and this phosphorylation was SL0101-1 sensitive but rapamycin insensitive (Fig. 2D). In addition, the suppression of PDCD4 protein levels seen after IFN treatment was reversible by concomitant SL0101-1 treatment of cells (Fig. 2E).

To further define the involvement of RSK1 in PDCD4 phosphorylation in hematopoietic cells, experiments were carried out in which RSK1 was knocked down in KT1 cells. As shown in Fig. 3A, siRNA-mediated inhibition of RSK1 expression blocked PDCD4 phosphorylation, indicating that in these cells IFN-α-induced RSK1 is the dominant kinase controlling PDCD4 phosphorylation (Fig. 3A). In studies in which immune complex kinase assays were conducted on anti-RSK immunoprecipitates, using PDCD4 as an exogenous substrate, we found strong IFN-α-inducible phosphorylation of PDCD4, which was blocked by SL0101-1, suggesting that PDCD4 is a substrate for the kinase activity of RSK1 (Fig. 3B).

Previous studies have suggested a model in which, after undergoing S6K-mediated phosphorylation on Ser67, PDCD4 interacts with the ubiquitin ligase β-TRCP and undergoes proteolytic degradation (9, 10). To determine whether IFN treatment results in the formation of β-TRCP/PDCD4 complexes, coimmunoprecipi-

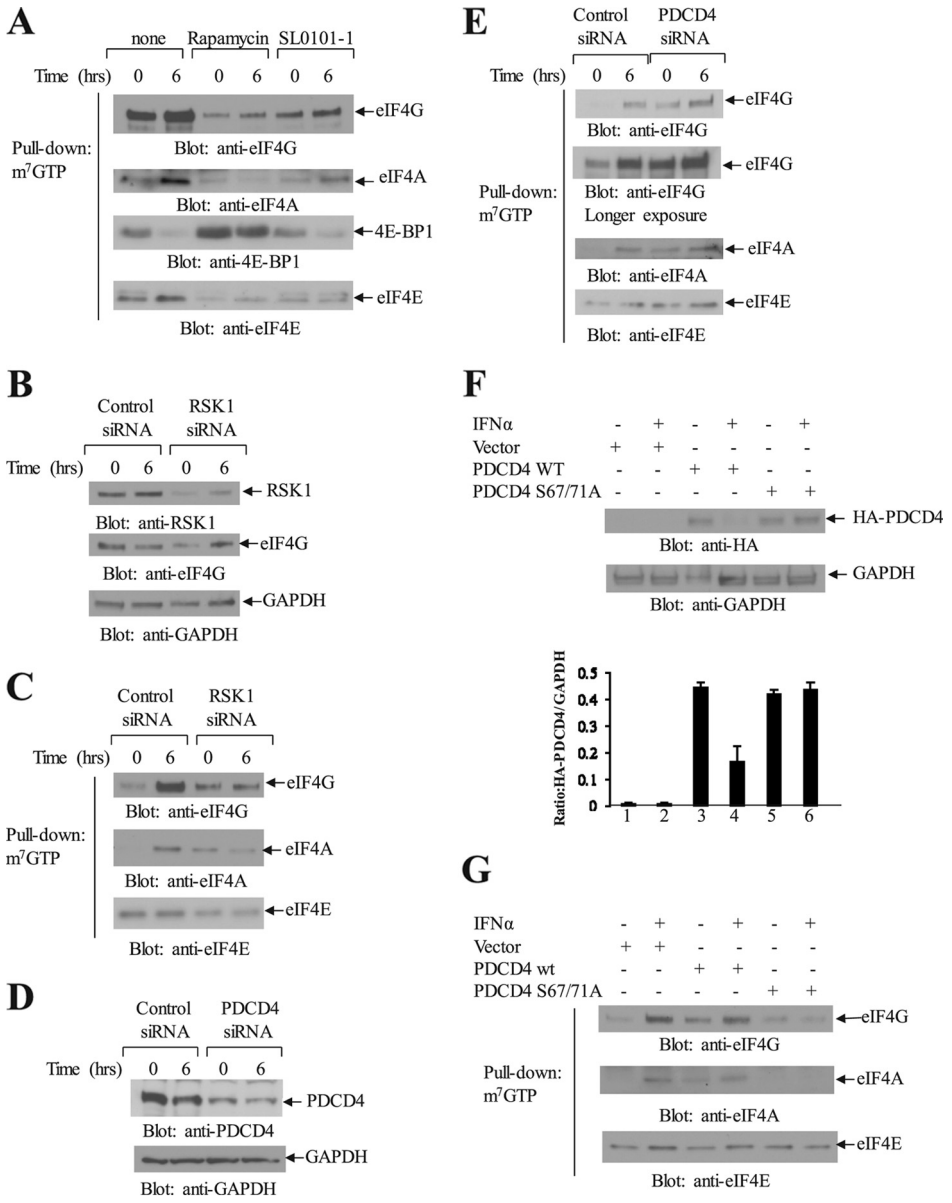

FIG 5 Regulation of type I IFN-inducible binding of eIF4G and eIF4A to the 7-methylguanosine cap complex by PDCD4. (A) Serum-starved KT1 cells were pretreated for 60 min with rapamycin or for 3 h with SL0101-1 and were subsequently treated with IFN-α in the continuous presence or absence of SL0101-1 for the indicated times. Total cell lysates were bound to the cap analog m^7GTP conjugated to beads, and bound proteins were resolved by SDS-PAGE and immunoblotted with the indicated antibodies. The lysates used were the same from the experiment shown in Fig. 2C. (B) KT1 cells were transfected with either control siRNA or RSK1 siRNA and after serum starvation were either left untreated or treated with IFN-α for 6 h as indicated. Total cell lysates were resolved by SDS-PAGE and immunoblotted with the indicated antibodies. (C) Cell lysates from the experiment shown in panel B were bound to the cap analog m^7GTP conjugated to beads, and after extensive washing, bound proteins were resolved by SDS-PAGE and immunoblotted with the indicated antibodies. (D) KT1 cells were transfected with either control siRNA or PDCD4 siRNA and after serum starvation were either left untreated or treated with IFN-α for 6 h, as indicated. Total cell lysates were resolved by SDS-PAGE and immunoblotted with the indicated antibodies. (E) Cell lysates from the experiment shown in panel D were bound to the cap analog m^7GTP conjugated to beads, and after extensive washing, bound proteins were resolved by SDS-PAGE and immunoblotted with the indicated antibodies. For the anti-eIF4G blot, two different exposures of the same blot (a shorter and a longer exposure) are shown in the upper two panels. (F) KT1 cells were transfected with HA-tagged PDCD4 WT, the PDCD4(S67/71A) mutant, or empty vector, as indicated, serum starved, and treated with IFN-α as indicated. Total cell lysates were resolved by SDS-PAGE and immunoblotted with the indicated antibodies (upper panel). Signals were quantified by densitometry and used to calculate the intensity of expression of HA-PDCD4 relative to that of GAPDH (lower panel). Data are expressed as ratios of HA-PDCD4 to GAPDH for each experimental condition and represent means ± standard error of the results of three experiments, including the one shown in the upper panel. (G) Cell lysates from the experiment shown in panel F were bound to the cap analog m^7GTP conjugated to beads, and after extensive washing, bound proteins were resolved by SDS-PAGE and immunoblotted with the indicated antibodies. (H) The experiment shown in the upper panel is similar to that shown in panel A, except that equal amounts of cell lysates were immunoprecipitated with an anti-eIF4E antibody or control mouse IgG1 (mIgG1). Immune complexes were resolved by SDS-PAGE for analysis of eIF4G, eIF4A, and eIF4E as indicated. The signals were quantified by densitometry and used to calculate the intensity of binding of eIF4G and eIF4A to eIF4E (lower panel). Data are expressed as ratios of eIF4G or eIF4A to eIF4E for each experimental condition and represent means ± standard error of the results of three experiments, including the one shown in the upper panel. (I) The experiment is similar to that shown in panel C, except that equal amounts of cell lysates were immunoprecipitated with an anti-eIF4E antibody. Immune complexes were resolved by SDS-PAGE for analysis of eIF4G, eIF4A, and eIF4E, as indicated. (J) The experiment is similar to that shown in panel E, except that equal amounts of cell lysates were immunoprecipitated with an anti-eIF4E antibody or control mIgG1. Immune complexes were resolved by SDS-PAGE for analysis of eIF4G, eIF4A, 4E-BP1, and eIF4E, as indicated. (K) The experiment is similar to that shown in panel G, except that equal amounts of cell lysates were immunoprecipitated with an anti-eIF4E antibody or control mIgG1. Immune complexes were resolved by SDS-PAGE for analysis of eIF4G, eIF4A, and eIF4E, as indicated.

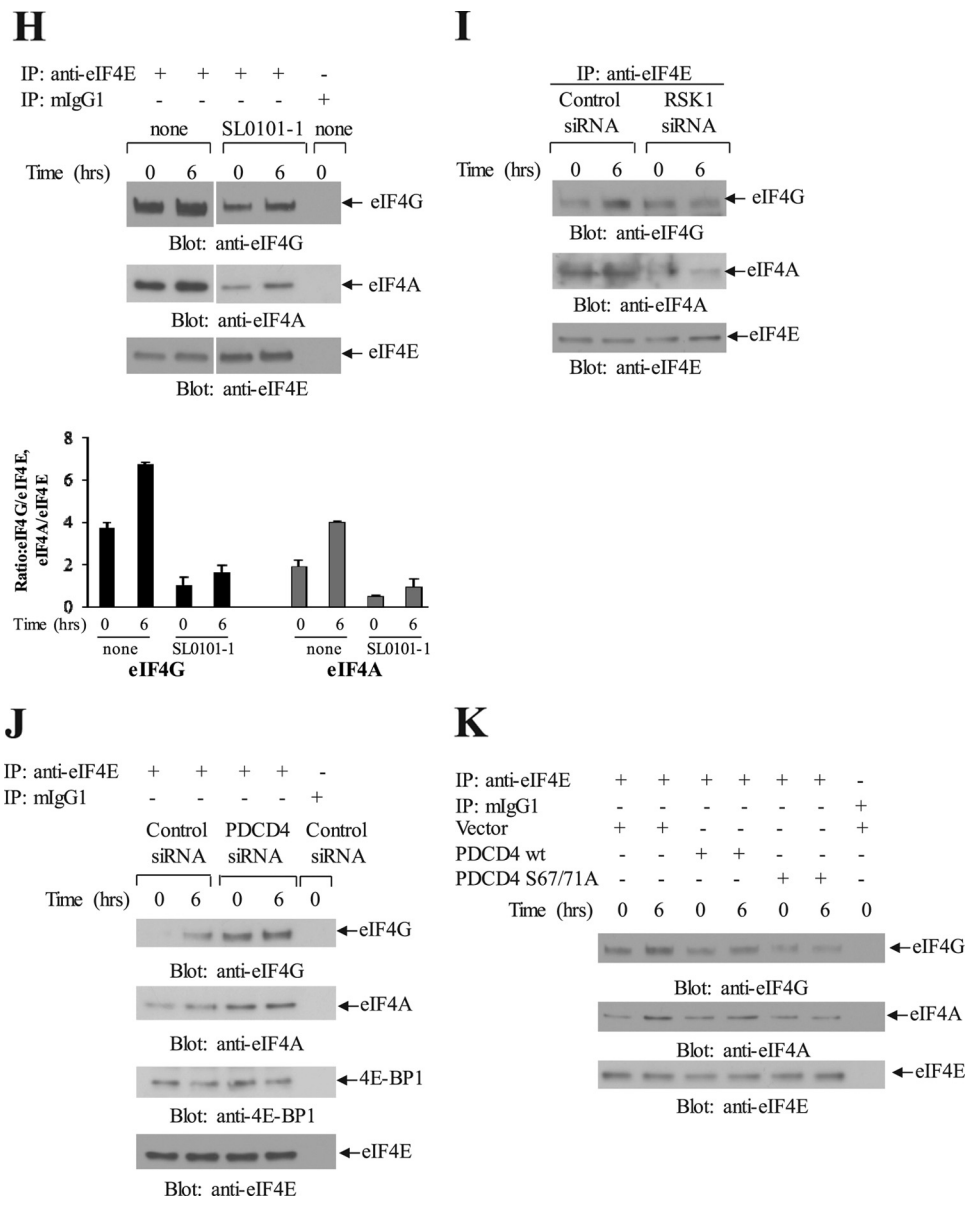

FIG 5 continued

tation experiments were carried out. Serum-starved KT1 cells were treated with IFN-α in the presence or absence of SL0101-1, and lysates were immunoprecipitated with an anti-β-TRCP antibody, followed by immunoblotting with an antibody against PDCD4. As shown in Fig. 4A, IFN-α treatment induced an association of β-TRCP with PDCD4 (Fig. 4A). This IFN-α-dependent β-TRCP–PDCD4 interaction was not detectable in cells pretreated with the RSK inhibitor or in cells transfected with siRNA targeting RSK1 (Fig. 4B). In parallel studies, we examined whether IFN-dependent phosphorylation of PDCD4 modulates its interaction with eIF4A. Cells were treated with IFN-α in the presence or absence of SL0101-1, and lysates were immunoprecipitated with an anti-eIF4A antibody and immunoblotted with an anti-PDCD4 antibody. IFN-α treatment resulted in decreased amounts of detectable PDCD4 in association with eIF4A, but this decrease was reversed by treatment of cells with SL0101-1 (Fig. 4C). IFN-α treatment resulted in a significant increase in the amount of eIF4G interacting with eIF4A, but the association was suppressed by treatment of cells with SL0101-1 (Fig. 4C), suggesting involvement of RSK1 activity in the process. In other studies, lysates from cells transfected with control siRNA or an siRNA specifically targeting RSK1 were immunoprecipitated with an anti-eIF4A antibody and immunoblotted with anti-PDCD4, anti-eIF4G, or anti-eIF4A antibody (Fig. 4D). IFN-α treatment did not decrease the amount of PDCD4 associated with eIF4A in cells in which RSK1 was knocked down (Fig. 4D and E).

As the recruitment of eIF4F to the 5′ cap structure of mRNA is an important step in the translation initiation process, we sought to determine the regulatory effects of IFN-α-dependent engagement of RSK and mTOR pathways in KT1 cells on binding of the translation initiation factors to the 7-methylguanosine cap complex. Cells were pretreated with rapamycin or SL0101-1, and the

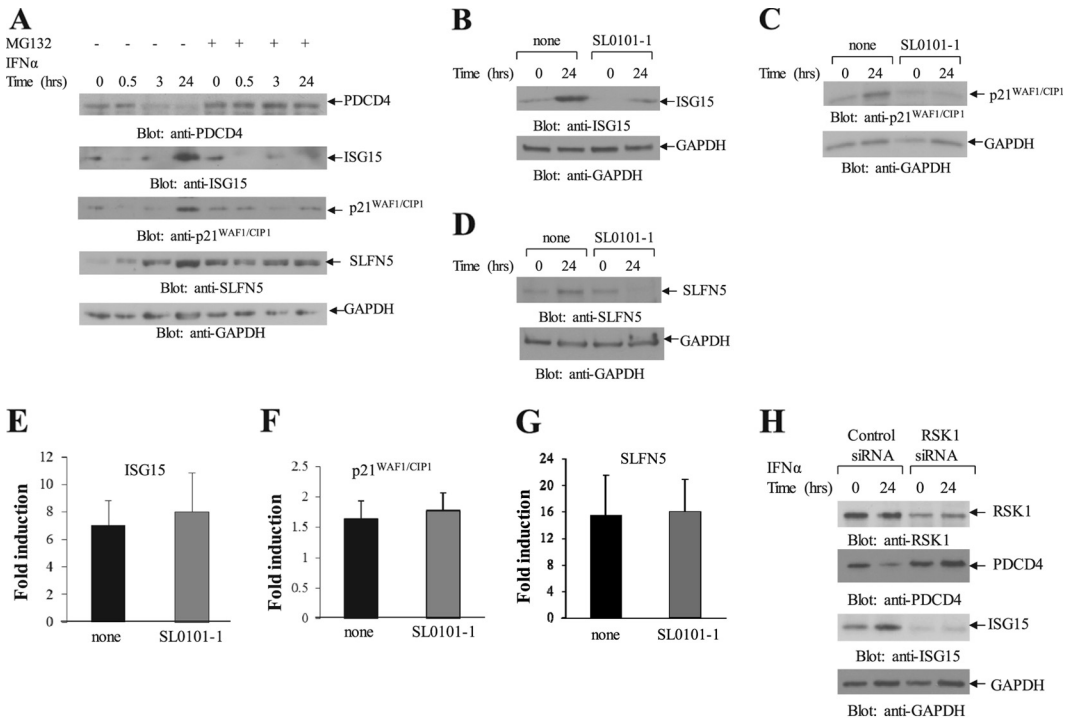

FIG 6 Effects of RSK1-mediated phosphorylation and degradation of PDCD4 on IFN-α dependent expression of ISG protein products. (A) Serum-starved KT1 cells were either left untreated or were treated with IFN-α for the indicated times, in the presence or absence of MG132 or diluent for MG132 (dimethyl sulfoxide). Cell lysates were resolved by SDS-PAGE and immunoblotted with anti-PDCD4, -p21$^{WAF1/CIP1}$, -ISG15, -SLFN5, or anti-GAPDH antibody. (B to D) Serum-starved KT1 cells were pretreated with SL0101-1 for 3 h and then treated with IFN-α for the indicated times. The cells were lysed, and equal amounts of protein were resolved by SDS-PAGE and immunoblotted with the indicated antibodies. (E to G) Serum-starved KT1 cells were treated with IFN-α for 6 h in presence or absence of SL0101-1. Expression of mRNA for *Isg15* (E), *p21$^{WAF1/CIP1}$* (F), and *slfn5* (G) genes was assessed by quantitative real-time RT-PCR. The GAPDH transcript was used for normalization. Data are expressed as fold increase over IFN-α-untreated samples and represent means ± standard error of three experiments. (H) KT1 cells were transfected with either control siRNA or siRNA specifically targeting RSK1 and after serum starvation were either left untreated or treated with IFN-α, as indicated. The cells were lysed, and equal amounts of protein were resolved by SDS-PAGE and immunoblotted with anti-RSK1, anti-PDCD4, anti-ISG15, or anti-GAPDH antibody, as indicated.

IFN-inducible recruitment of eIF4G and eIF4A to the complex was assessed. IFN-α-inducible binding of eIF4G, eIF4A, and eIF4E was clearly detectable (Fig. 5A). As expected, there was no dissociation of unphosphorylated 4E-BP1 in rapamycin-pretreated cell lysates, leading to decreased levels of eIF4G and eIF4A in the complex, which reflects decreased eIF4E binding to eIF4G and associated eIF4A. Interestingly, in cells pretreated with RSK inhibitor, there was normal 4E-BP1 dissociation but also decreased levels of eIF4A and eIF4G in the complex compared to cells not pretreated with the inhibitor (Fig. 5A). Similarly, in experiments in which RSK1 was knocked down (Fig. 5B), we found reversal of IFN-dependent enhanced binding of eIF4G, eIF4A, and eIF4E to the 7-methylguanosine cap complex (Fig. 5C), raising the possibility that phosphorylation of PDCD4 mediated by RSK1 increases IFN-inducible eIF4F assembly. To further define the potential role of PDCD4 in the process, we used lysates from cells transfected with control siRNA or siRNA specifically targeting PDCD4 (Fig. 5D). IFN-α treatment resulted in enhanced binding of eIF4G, eIF4A, and eIF4E to the 7-methylguanosine cap complex (Fig. 5E), but knockdown of PDCD4 further increased eIF4G, eIF4A, and eIF4E binding. Moreover, ectopic expression of a PDCD4(S67/71A) mutant (Fig. 5F), which cannot undergo phosphorylation at Ser67 and subsequent degradation (9), decreased the binding of eIF4A and eIF4G to the 5′ cap complex (Fig. 5G).

To further delineate the involvement of PDCD4 in eIF4F complex formation, coimmunoprecipitation experiments were carried out. Serum-starved KT1 cells were treated with IFN-α in the presence or absence of SL0101-1, and lysates were immunoprecipitated with an anti-eIF4E antibody, followed by immunoblotting with antibodies against eIF4G, eIF4A, and eIF4E. As shown in Fig. 5H, IFN-α treatment induced an association of eIF4E with eIF4G and eIF4A. This IFN-dependent interaction was less pronounced in cells pretreated with SL0101-1 (Fig. 5H). We also performed similar analyses using lysates from cells transfected with control siRNA or siRNA specifically targeting RSK1. As expected, IFN-α treatment resulted in enhanced binding of eIF4G and eIF4A with eIF4E in lysates from cells transfected with control siRNA, but knockdown of RSK1 decreased the amount of eIF4G and eIF4A binding to eIF4E (Fig. 5I). Consistent with a role for PDCD4 downstream of RSK1 in the process, IFN-α treatment resulted in enhanced binding of eIF4G and eIF4A with eIF4E, but knockdown of PDCD4 further increased the amount of eIF4G and eIF4A binding to the eIF4E (Fig. 5J). On the other hand, there was no effect on the dissociation of 4E-BP1 from eIF4E in cells treated with IFN-α, suggesting that PDCD4 exhibits negative effects in IFN-inducible eIF4F complex formation in a 4E-BP1-independent manner (Fig. 5J). Consistent with these findings, ectopic expression of a PDCD4(S67/71A) mutant also decreased the association of eIF4G and eIF4A with eIF4E (Fig. 5K).

As our data suggested a negative regulatory effect of PDCD4 on IFN-α-activated eIF4F complexes, likely via inhibitory effects on eIF4A, we sought to determine whether this mechanism participates in the control of IFN-inducible gene (ISG) protein products that mediate generation of IFN-α responses. For this purpose, serum-starved KT1 cells were treated with IFN-α in the presence or absence of the proteasomal inhibitor MG132. As expected, treatment with MG132 stabilized PDCD4 and reversed IFN-α-inducible degradation of the protein (Fig. 6A). This stabilization of PDCD4 protein correlated with suppression of induction of gene products with important roles in the generation of the biological effects of IFNs, including ISG15 (58), p21$^{WAF1/CIP1}$ (17), and SLFN5 (18) (Fig. 6A). To determine whether RSK1-mediated phosphorylation of PDCD4 is important for these responses, we assessed the effects of the RSK inhibitor SL0101-1 on IFN-inducible expression of ISG15, p21$^{WAF1/CIP1}$, and SLFN5. IFN-α-dependent induction of expression of ISG15 (Fig. 6B), p21$^{WAF1/CIP1}$ (Fig. 6C), and SLFN5 (Fig. 6D) was partially reversed by pretreatment of cells with SL0101-1. This defective ISG15, p21$^{WAF1/CIP1}$, and SLFN5 protein expression in cells pretreated with SL0101-1 was not associated with decreased IFN-dependent mRNA induction/transcription of the *Isg15* (Fig. 6E), *p21*$^{WAF1/CIP1}$ (Fig. 6F), or *slfn5* (Fig. 6G) gene, suggesting that this may be the result of defective mRNA translation/protein expression. In experiments in which RSK1 expression was knocked down using siRNAs, there was also defective IFN-dependent ISG15 protein expression (Fig. 6H). Altogether, these studies suggest that the IFN-α-induced RSK1 kinase activity is required for phosphorylation and subsequent degradation of PDCD4, possibly by promoting initiation of cap-dependent mRNA translation of ISGs.

We also performed studies in which a PDCD4 wild type or mutant for the RSK1 phosphorylation site [PDCD4(S67/71A)] was overexpressed in KT1 cells, and IFN-α-dependent ISG protein expression was examined. As shown in Fig. 7A, induction of ISG15, p21$^{WAF1/CIP1}$, and SLFN5 protein was defective in cells in which PDCD4(S67/71A) was expressed, establishing potent negative regulatory effects of unphosphorylated PDCD4 on ISG expression. Thus, IFN-α-dependent, RSK1-mediated, phosphorylation of PDCD4 on Ser67 is essential for proteasomal degradation of the protein, and this regulatory mechanism is critical for expression of ISG proteins that mediate the biological effects of IFNs. Notably, in experiments in which PDCD4 was knocked down by specific siRNAs, IFN-dependent expression of several key ISG proteins, including ISG15 (Fig. 7B), p21$^{WAF1/CIP1}$ (Fig. 7C), and SLFN5 (Fig. 7C) was further enhanced. In addition, an increase in the basal levels of expression of SLFN5 and p21$^{WAF1/CIP1}$ was noticeable (Fig. 7C).

In subsequent studies, we sought to directly determine whether mRNA translation for ISG15, p21$^{WAF1/CIP1}$, and SLFN5 genes is enhanced in cells in which PDCD4 is knocked down. For this purpose, polysomal mRNA from KT1 cells transduced with a control shRNA or shRNA specifically targeting human PDCD4 was isolated and PDCD4 mRNA expression prior to and after IFN treatment was assessed in polysomal fractions. The polysomal profiles from KT1 cells transduced with a control shRNA or with shRNA targeting the PDCD4, before and after IFN-α treatment, are shown in Fig. 8A. There was a significant increase of *Isg15* (Fig. 8B) and *slfn5* (Fig. 8C) mRNAs in polysomes isolated from cells that were IFN-α treated and transduced with shRNA targeted against PDCD4 compared to cells that were IFN-α treated and transduced with the control shRNA (Fig. 8 B and C), establishing regulatory effects of PDCD4 on ISG mRNA translation.

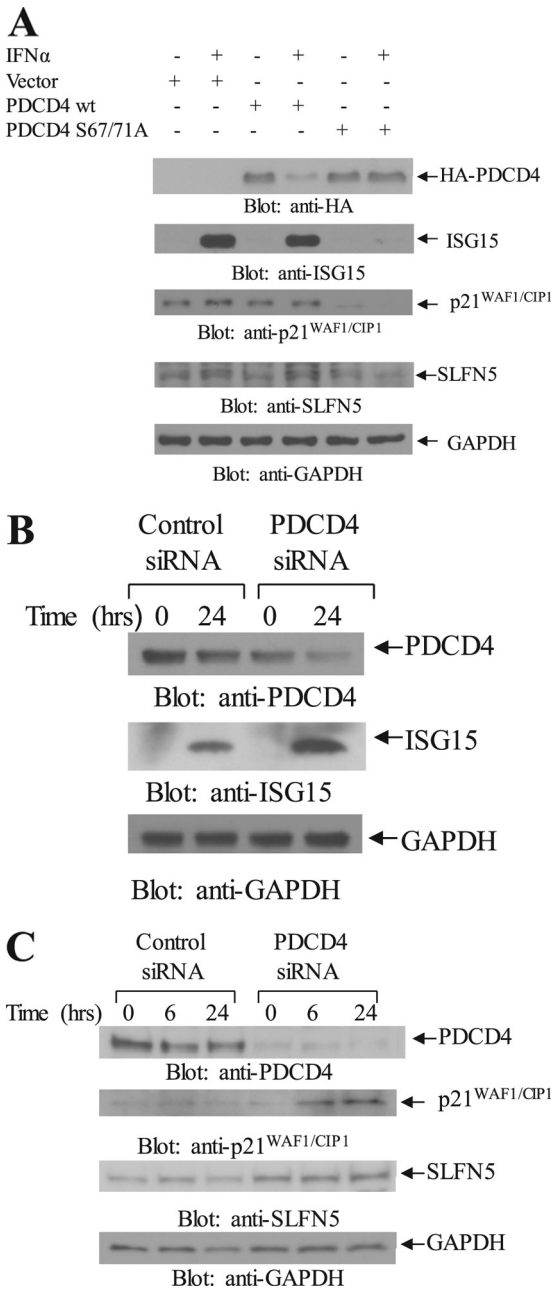

FIG 7 Phosphorylation of PDCD4 on Ser67 is required for expression of ISG protein products by IFN-α. (A) KT1 cells were transfected with either empty vector, HA-tagged wild-type PDCD4, or HA-tagged PDCD4(S67/71A) mutant, serum starved, and left untreated or treated with IFN-α. Total cell lysates were resolved by SDS-PAGE and immunoblotted with the indicated antibodies. (B and C) KT1 cells were transfected with either control siRNA or siRNA specifically targeting PDCD4 and after serum starvation were either left untreated or were treated with IFN-α for the indicated times. Total cell lysates were resolved by SDS-PAGE and immunoblotted with the indicated antibodies.

We have previously shown that RSK1 plays an essential role in the generation of the suppressive effects of IFN-α on primitive leukemic precursors (CFU-L) and have identified eIF4B as a downstream phosphorylation target and mediator of these effects

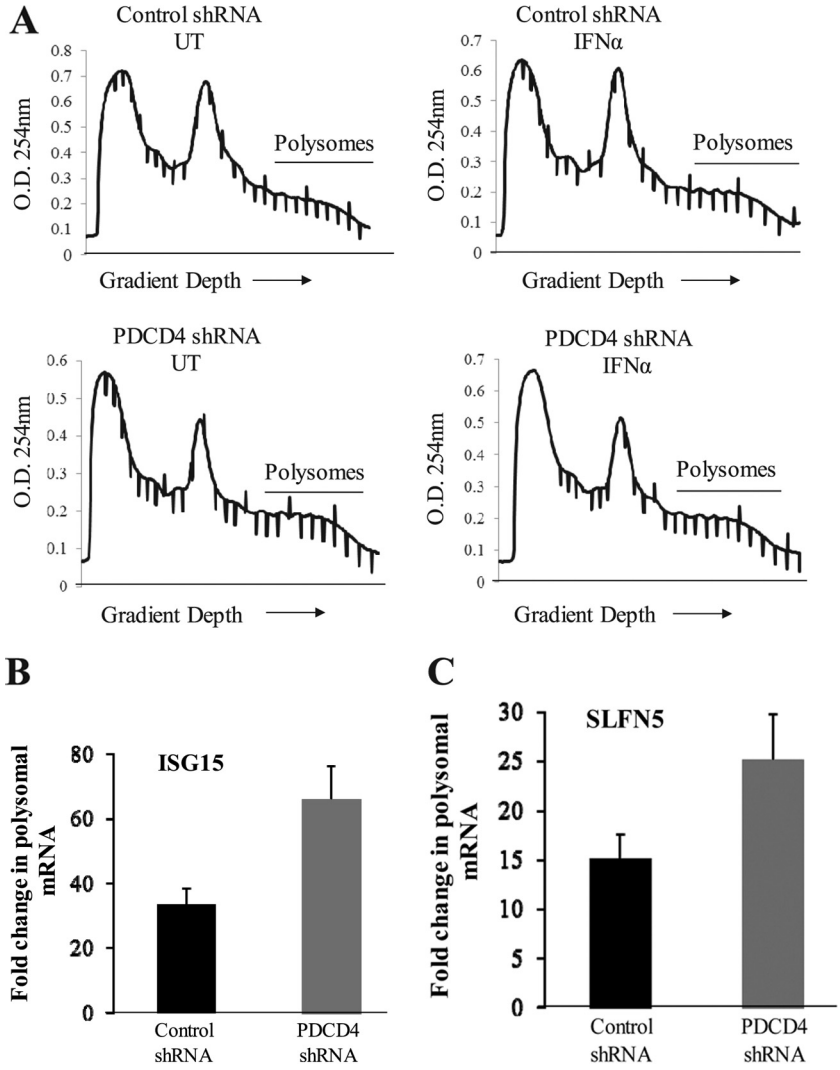

FIG 8 Regulatory effects of PDCD4 ISG mRNA translation. (A) KT1 cells transduced with control shRNA or shRNA-targeted human PDCD4 were either left untreated (UT) or treated with IFN-α for 24 h. Cell lysates were separated on 10 to 50% sucrose gradient, and the optical density (OD) at 254 nm was recorded. The OD at 254 nm is shown as a function of gradient depth for each treatment. (B and C) Polysomal fractions were collected as indicated in panel A, and RNA was isolated. Quantitative real-time RT-PCR assays to determine *Isg15* (B) and *slfn5* (C) mRNA expression in polysomal fractions were conducted using *gapdh* for normalization. Data are expressed as fold increase over IFN-α-untreated samples and represent means ± standard deviations of three independent experiments.

(23). We examined whether phosphorylation of PDCD4 by activated RSK1 plays a role in the generation of the antileukemic effects of IFN-α. For this purpose, the effects of PDCD4 knockdown or expression of the mutant PDCD4(S67/71A) on the generation of the effects of IFN-α on primitive KT1-derived leukemic progenitors were assessed by clonogenic assays in methylcellulose. Suppression of CFU-L growth after IFN-α treatment was further enhanced in PDCD4 knockdown cells compared to suppression in control siRNA-transfected cells (Fig. 9A). On the other hand, expression of the PDCD4(S67/71A) mutant significantly reversed the inhibitory effects of IFN-α on CFU-L colony formation compared with a control empty vector or ectopic expression of wild-type PDCD4 (Fig. 9B). In other studies, we assessed the potential regulatory roles of RSK1 and PDCD4 on normal bone marrow-derived (CFU-GM) progenitor colony formation. As shown in Fig. 10A, siRNA-mediated PDCD4 knockdown resulted in enhancement of the suppressive effects of IFN-α on normal CFU-GM colonies, while RSK1 knockdown had opposing effects and partially reversed the inhibitory effects of IFN-α (Fig. 10A). These findings suggest that RSK1-mediated phosphorylation and subsequent degradation of PDCD4 relieve the inhibitory effects of the protein on the generation of IFN biological responses. Consistent with this, expression of the PDCD4(S67/71A) mutant in $CD34^+$ cells resulted in reversal of the effects of IFN-α on normal CFU-GM (Fig. 10B), underscoring the functional relevance of IFN-dependent PDCD4 phosphorylation.

DISCUSSION

IFNs are pleiotropic cytokines with important biological properties that were originally defined as key elements of the host defense against viral infections (35, 47). Beyond eliciting antiviral responses, IFNs exhibit important immuno-modulatory and anti-

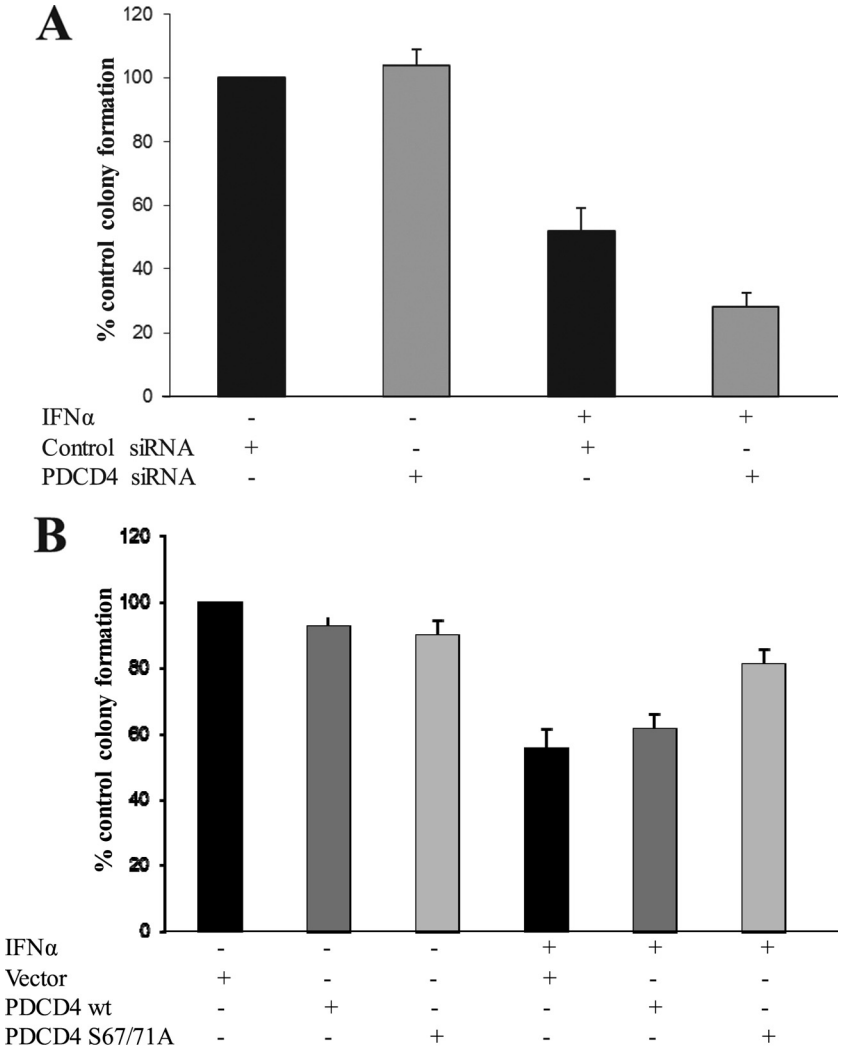

FIG 9 Regulatory effects of PDCD4 in the generation of the antileukemic effects of IFN-α. (A) KT1 cells were transfected with either control siRNA or siRNA specifically targeting PDCD4, as indicated. The cells were subsequently plated in methylcellulose, in the absence or presence of IFN-α, and leukemic CFU-L colony formation was assessed. Data are expressed as the percentage of control colony formation of untreated samples for each condition and represent means ± standard error of four experiments. (B) KT1 cells were transfected with the empty vector, PDCD4 WT, or PDCD4 S67/71A mutant, as indicated. The cells were subsequently plated in methylcellulose, in the absence or presence of IFN-α, and leukemic CFU-L colony formation was assessed. Data are expressed as the percentage of control colony formation of untreated samples for each condition and represent means ± standard error of six experiments.

neoplastic activities and play key roles in the immune surveillance against malignancies (35, 47). IFNs generate their biological effects by engaging JAK-STAT signaling pathways, leading to the transcriptional activation of ISGs and the expression of their protein products, which promote diverse IFN responses (29, 30, 39, 43, 47). Beyond activation of classical JAK-STAT pathways by IFNs, there has been emerging evidence in recent years for the existence of several additional "nonclassical" IFNR-activated cellular cascades which complement the function of the JAK-STAT pathways and optimize expression of ISGs (40). Engagement of the p38 mitogen-activated protein kinase (MAPK) pathway is required for optimal ISG transcription and induction of IFN responses (50), while members of the protein kinase C (PKC) family of proteins play key roles in complementing transcriptional activation of ISGs by modulating STAT serine phosphorylation and/or acting as regulators of other IFN-induced cellular responses (42, 51). We previously demonstrated that the PI3′-kinase/Akt/mTOR/S6K pathway activated by IFNs is essential for IFN-dependent mRNA translation of key ISGs (19–23). Importantly, we recently provided evidence for coordination between the mTOR pathway and MAPK-regulated cascades in ISG mRNA translation, as shown by the requirement for IFN-induced, MEK/ERK-dependent, MAPK-interacting kinase (MNK) kinases in eIF4E phosphorylation and mRNA translation of ISGs (15, 16). Such coordination of MAPK and mTOR pathways in the regulation of mRNA translation of IFN-induced genes is also evident by the cell-type-specific involvement in the process of the kinase RSK1, an effector of the MEK/ERK cascade (23). In previous work we demonstrated that phosphorylation/activation of eIF4B in the IFN system occurs either by the activity of the S6K downstream of mTOR or via RSK1, an effector of the MEK/ERK cascade (23). In addition, in the type III (IFN-λ) system, RSK1 appears to be the primary kinase that controls 4E-BP1 phosphorylation in HT-29 colorectal cells (22).

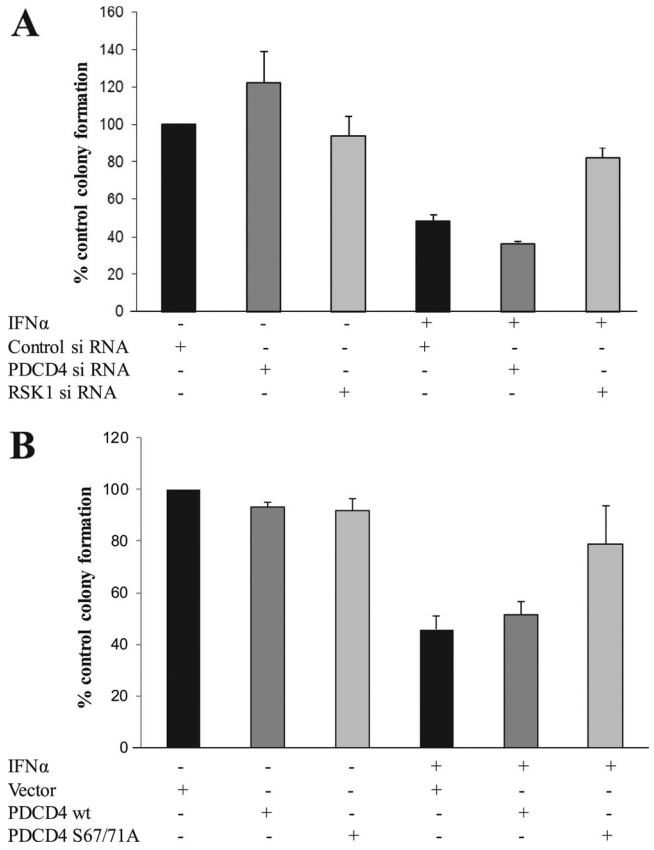

FIG 10 Regulatory effects of RSK1 and PDCD4 in the inhibitory properties of IFN-α on normal bone marrow-derived myeloid precursors. (A) Normal $CD34^+$ bone marrow-derived cells were transfected with either control siRNA or siRNA specifically targeting PDCD4 or siRNA specifically targeting RSK1, as indicated. The cells were subsequently plated in methylcellulose, in the absence or presence of IFN-α. CFU-GM progenitor colonies were scored after 14 days in culture. Data are expressed as percent control colony formation from untreated cells and represent means ± standard error of three independent experiments. (B) Normal $CD34^+$ bone marrow-derived cells were transfected with either the plasmid PCDNA3, HA-tagged PDCD4 WT, or PDCD4(S67/71A) mutant and incubated in the absence or presence of IFN in clonogenic assays in methylcellulose, as indicated. CFU-GM progenitor colonies were scored after 14 days in culture. Data are expressed as percent control colony formation from untreated cells and represent means ± standard error of four independent experiments.

PDCD4, a tumor suppressor protein for which the human gene is located on chromosome band 10q24 (46), has important regulatory functions in mammalian cells (4, 14, 24, 25, 33, 54, 56). This protein interacts with eIF4A (25, 48, 53, 55) and negatively controls the helicase activity of eIF4A and cap-dependent translation (37, 48, 55). Notably, beyond free eIF4A, PDCD4 inhibits the function of eIF4F-bound eIF4A (24, 53, 55), providing a mechanism by which it may be acting as a suppressor of mRNA translation (48, 55). There is also evidence that PDCD4 binds RNA and interacts with eIF4G *in vitro* (2, 48, 55). PDCD4 expression is regulated by multiple mechanisms (25). Previous studies have shown that an increase in microRNA-21 (miR-21) results in PDCD4 downregulation (7). Another mechanism for regulation of PDCD4 involves its phosphorylation by Akt and S6K1, leading to proteosomal degradation (9, 34). This mechanism may also apply in the context of constitutive mTOR/S6K activation, as seen in response to transformation of cells by oncogene proteins such as BCR-ABL (3).

As IFNRs are known to activate the mTOR/S6K pathway, we sought to determine the effects of IFN treatment on PDCD4 phosphorylation. Our data demonstrate that treatment of cells with IFN-α results in phosphorylation of PDCD4 on serine 67. Importantly, this IFN-dependent phosphorylation of PDCD4 results in an association of the protein with the ubiquitin ligase $SCF^{\beta\text{-}TrCP}$ (9, 10), ultimately leading to its degradation. IFN-dependent phosphorylation of PDCD4 occurred in an S6K-dependent manner in MEF cells, but it was found to be MEK/ERK dependent and RSK1 mediated in the malignant hematopoietic cell lines KT1 and U266. Thus, as in the case of eIF4B (23), RSK1 appears to be the dominant kinase that regulates phosphorylation of PDCD4 in hematopoietic cells. Remarkably, our data demonstrate that PDCD4 exhibits negative regulatory effects on the eIF4F complex and on the expression of ISGs with important functions in the generation of IFN responses, including ISG15, $p21^{WAF1/CIP1}$, and SLFN5 (17, 18, 58). Such PDCD4-mediated effects on the expression of these ISG products appear to reflect inhibitory effects of PDCD4 on cap-dependent mRNA translation, which, based on previous work, is required for ISG expression. In studies to define the functional relevance of PDCD4 in the generation of the biological effects of IFN-α, we found that siRNA-mediated knockdown of PDCD4 enhanced the suppressive effects of IFN-α on primitive hematopoietic precursors from normal marrows and leukemic CFU-L progenitors. On the other hand, ectopic expression of the PDCD4(S67/71A) mutant reversed the suppressive effects of IFN-α on normal myeloid and leukemic progenitor colony formation, suggesting that PDCD4 inhibits IFN responses, likely via suppressive effects on ISG mRNA translation.

There is extensive previous work demonstrating that PDCD4 is a tumor suppressor (4, 12, 53) and that its expression is decreased in several malignancies (11, 12, 14, 31, 33, 44, 53). PDCD4 has also been shown to regulate cell cycle progression in a cell-type-specific manner and to inhibit cell proliferation (13). The finding that this protein antagonizes and negatively controls generation of the growth-suppressive effects of IFN-α is, at first glance, surprising and somewhat unexpected. However, it should be noted that there is previous evidence for some opposing cellular regulatory roles of PDCD4, depending on the cell type and context. For instance, PDCD4 increases $p21^{WAF1/CIP1}$ and suppresses CDK1 and CDC2 expression in neuroendocrine cells (13), while in HCT116 colon adenocarcinoma cells it appears to negatively control $p21^{WAF1/CIP1}$ levels (1). The findings of the current study indicate that in the case of type I IFNs, PDCD4 negatively controls mRNA translation/protein expression of several IFN-induced genes with antiproliferative properties, including $p21^{WAF1/CIP1}$, SLFN5, and ISG15. Importantly, such a role for PDCD4 in the IFN system is consistent with the positive regulatory effects of mTOR- and RSK-mediated signals in the mRNA translation/expression of ISG products (19–23). In future studies it will be important to define the functional role of PDCD4 in the signaling pathways of other cytokines with antineoplastic properties as this may lead to the identification of unexpected novel targets and new approaches to block malignant cell growth, with potentially important therapeutic implications.

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