Role of p38 Protein Kinase in the Ligand-independent Ubiquitination and Down-regulation of the IFNAR1 Chain of Type I Interferon Receptor*

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Phosphorylation-dependent ubiquitination and degradation of the IFNAR1 chain of type I interferon (IFN) receptor is a robust and specific mechanism that limits the magnitude and duration of IFNa/β signaling. Besides the ligand-inducible IFNAR1 degradation, the existence of an “inside-out” signaling that accelerates IFNAR1 turnover in the cells undergoing the endoplasmic reticulum (ER) stress and activated unfolded protein response has been recently described. The latter pathway does not require either presence of ligands (IFNa/β) or catalytic activity of Janus kinases (JAK). Instead, this pathway relies on activation of the PKR-like ER kinase (PERK) and ensuing specific priming phosphorylation of IFNAR1. Here, we describe studies that identify the stress activated p38 protein kinase as an important regulator of IFNAR1 that acts downstream of PERK.

Results of the experiments using pharmacologic p38 kinase inhibitors, RNA interference approach, and cells from p38α knock-out mice suggest that p38 kinase activity is required for priming phosphorylation of IFNAR1 in cells undergoing unfolded protein response. We further demonstrate an important role of p38 kinase in the ligand-independent stimulation of IFNAR1 ubiquitination and degradation and ensuing attenuation of IFNα/β signaling and anti-viral defenses. We discuss the distinct importance of p38 kinase in regulating the overall responses to type I IFN in cells that have been already exposed to IFNα/β versus those cells that are yet to encounter these cytokines.

Ubiquitination-mediated down-regulation of signaling receptors plays a key role in restricting the timing of cellular responses to specific ligands (1). This mechanism is especially important for limiting the extent and duration of signaling pathways triggered by those ligands that negatively affect cell proliferation and survival. Such an effect was demonstrated for a number of cytokines, including those that belong to type I interferons (including IFNa and IFNβ) known to elicit antitumorigenic effects and to mount the antiviral defensive mechanisms (2–4).

Effects of IFNa/β within cells are attributed to the induction of the interferon-stimulated genes mediated by signal transducers and activators of transcription (STAT1 and STAT2) proteins (5, 6). The latter become transcriptionally competent upon their phosphorylation on specific tyrosine residues mediated by JAK (TYK2 and JAK1) that are associated with type I IFN receptor chains (IFNAR1 and IFNAR2c,2 respectively). JAK themselves become activated as a result of cross-phosphorylation that occurs upon binding of a ligand (e.g. IFNa or IFNb) to the extracellular domains of IFNAR1 and IFNAR2c (reviewed in Refs. 5–8).

Activation of JAK, particularly of TYK2, is also implicated in activation of the ligand-inducible pathway that leads to the type I IFN receptor down-regulation (9, 10). The latter is driven by the endocytosis of the IFNAR1 chain stimulated by a chain- and site-specific ubiquitination of IFNAR1 (11). Ubiquitination of IFNAR1 is catalyzed by the β-Trcp E3 ubiquitin ligase (12). This ligase can be recruited to IFNAR1 upon its phosphorylation on specific Ser residues such as Ser-535 in human IFNAR1 or analogous Ser-526 in murine IFNAR1 (12, 13). Such phosphorylation is stimulated upon IFNα/β treatment (10, 13) in a manner that depends on kinase activity of TYK2 (9, 10) and activation of the serine/threonine protein kinase D2 (PKD2) (14).

This ligand-inducible pathway mediates IFNAR1 ubiquitination and degradation in cells that have already encountered IFNα/β. Given that activated JAK signals both forward to mediate the functions of IFNα/β (via STAT) and toward IFNAR1 elimination (via PKD2), the JAK- and PKD2-dependent IFNAR1 elimination merely serves to limit the extent of already ongoing IFNα/β signaling. Intriguingly, an existence of a basal pathway that does not require either ligands or JAK activity has been also reported (9). This pathway that relies on Ser-535 phosphorylation by constitutively active casein kinase 1α (CK1α) serves to decrease the basal levels of IFNAR1 and to limit the sensitivity of cells to the future encounters with IFNα/β (15).

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‡ The abbreviations used are: IFNAR1, IFNα/β receptor chain 1; CK1, casein kinase 1; TG, thapsigargin; ER, endoplasmic reticulum; VSV, vesicular stomatitis virus; PERK, pancreatic ER kinase; MEF, mouse embryo fibroblast; PKD2, protein kinase D2; MOI, multiplicity of infection.
p38 Kinase Regulates IFNAR1 Stability and Signaling

CK1α is a constitutively active kinase, yet its ability to phosphorylate diverse substrates can be further augmented via priming phosphorylation of an adjacent proximal Ser/Thr residue (16). IFNAR1 as a CK1 substrate also abides by this rule: phosphorylation of the degron of IFNAR1 by CK1α is robustly increased upon phosphorylation of a conserved priming site (Ser-532 in human IFNAR1, Ser-523 in mouse IFNAR1) (17). Intriguingly, the extent of priming phosphorylation (and, accordingly, of the ligand-independent phosphorylation of IFNAR1 degron that is followed by IFNAR1 ubiquitination and down-regulation) can be increased in cells exposed to stress inducers that cause unfolded protein response (UPR). Among such UPR inducers are pharmacologic agents that target the endoplasmic reticulum (ER) and viruses such as vesicular stomatitis virus (VSV) (17, 18).

UPR-stimulated priming phosphorylation, ensuing down-regulation of IFNAR1 and attenuation of IFNα/β signaling was dependent on activation of PKR-like ER kinase (PERK, (17, 18)). PERK is known to phosphorylate Ser-51 on the eIF2α translational regulator to a decrease in the overall rate of protein synthesis (reviewed in Ref. 19). Accordingly, eIF2α phosphorylation appeared to parallel both degron and priming phosphorylation of IFNAR1 (17, 18). Yet, we were unable to detect any phosphorylation of IFNAR1 by PERK (17), indicating that it is another kinase downstream of PERK that mediates phosphorylation of the priming site of IFNAR1 in response to the UPR inducers. Here, we report the results of pharmacologic and genetic analyses in mouse and human cells, suggesting that stress activated p38 protein kinase is a major regulator of the priming phosphorylation of IFNAR1. Activation of p38 kinase is also required for stimulation of IFNAR1 ubiquitination and degradation and ensuing attenuation of IFNα/β signaling. Furthermore, genetic ablation of p38 kinase augments the antiviral defenses indicating that the modulators of this kinase may be considered for potential use in treatment of viral infections.

EXPERIMENTAL PROCEDURES

Plasmids and Reagents—Vector for bacterial expression of GST-IFNAR1 was described previously (9). shRNA constructs for knockdown of p38α or control shRNA against GFP were purchased from Sigma. Inhibitors of p38 kinase (SB203580) and JNK (SP600125) were purchased from EMD Biosciences. PI3K inhibitor LY 294002 was purchased from LC Laboratories. p38 inhibitor VX-702 was purchased from ChemTek. Thapsigargin (TG) and cycloheximide were purchased from Sigma. Human IFNα2 was purchased from Bio-Sidius S.A., and murine IFNβ was purchased from R&D Systems.

Cell Culture, Treatment, and Viral Infection—Human HeLa and 2fTGH cells were obtained from ATCC. Mouse embryo fibroblasts from p38−/− mice and their wild type counterparts were kindly provided by Angelo Nebreda (Centro Nacional de Investigaciones Oncológicas, Madrid, Spain). All cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum (Hyclone) and various selection antibiotics where indicated. To generate human cells, in which p38α kinase expression is suppressed through the RNAi approach, 2fTGH cells or Hela cells were transduced by lentiviral particles encoding shRNA against GFP or p38α and selected in 2 μg/ml of puromycin for 2 weeks. 2fTGH-derivative 11.1-Tyk2-null cells reconstituted with catalytically inactive Tyk2 (KR2 cells) were a generous gift of S. Pellegrini (Pasteur Institute, Paris, France). VSV (Indiana serotype, a gift from R. Harty, University of Pennsylvania, Philadelphia, PA) was propagated in HeLa cells. For infections, the cells were inoculated with a multiplicity of infection 0.01 of VSV for 1 h, washed, and incubated with fresh medium for 14 h for observing phosphorylation of IFNAR1. Cells were incubated for 18 h post-infection for assays that evaluated IFNAR1 down-regulation or the extent of type I IFN signaling.

Antibodies and Immunotechniques—Monoclonal antibodies against human IFNAR1 that were used for immunoprecipitation (EA12) or immunoblotting (GB8) were described in detail elsewhere (20). Commercially available antibodies against pSTAT1, p-eIF2α, p-p38 kinase, STAT1 (Cell Signaling), eIF2α (Biosources), hIFNAR1, p38 (Santa Cruz Biotechnology), β-actin (Sigma), mouse IFNAR1 (Leinco), and ubiquitin (P4D1, Santa Cruz Biotechnology) were purchased. Monoclonal 23H12 antibody against the M protein of VSV (VSV-M) was a generous gift from D. S. Lyles (Wake Forest University School of Medicine, Winston-Salem, NC). Antibodies against IFNAR1 phosphorylated on Ser-535 (Ser-526 in mice) and Ser-532 (Ser-523 in mice) were described previously (13, 17). Immunoprecipitations, immunoblotting, in vivo ubiquitination assay using denaturing immunoprecipitation, and assessment of the kinetics of IFNAR1 degradation by cycloheximide chase were carried out as described previously (12, 13, 17, 18, 21).

In Vitro Kinase Assay—Lysates from untreated or VSV-infected cells (4 μg) were cleared of CK1α by immunodepletion as described previously (15). Bacterially expressed and purified GST-IFNAR1 (2 μg) used as a substrate was incubated with these lysates in kinase buffer (25 mM Tris HCl, pH 7.4, 10 mM MgCl2, 1 mM NaF, 1 mM NaVO3) and ATP (1 mM). The reactions were carried out at 30 °C for 30 min with shaking at 600 rpm on the tabletop incubator. The products were analyzed by immunoblotting using phosphospecific antibody and antibodies against GST as described previously (15).

RESULTS

We aimed to characterize a kinase activity that is activated in cells undergoing UPR and is responsible for the priming phosphorylation of IFNAR1. PERK-dependent activation of diverse kinases, including Jun N-terminal kinase (JNK), p38 kinase, and phosphoinositide 3-kinase (PI3K)-Akt was demonstrated in cells undergoing UPR (22, 23). As UPR inducers, we used a pharmacologic agent that inhibits ER Ca2+ ATPase TG or infection with VSV. In the latter case, we used a low dose of virus (MOI of 0.01) and timed the harvesting of infected cells to a point where viral proteins are already synthesized and UPR is already activated (~14 h post-infection as judged by expression of VSV-M and phosphorylation of Ser-51 of eIF2α), and we are capable of detecting phosphorylation of IFNAR1 just before a dramatic down-regulation in the levels of this protein (~17~18 h post-infection, see below).

We initially used human KR2 cells that harbor catalytically inactive form of TYK2 (24, 25); these cells were shown incapа-
ble of inducing IFNAR1 degron phosphorylation and stimulating IFNAR1 ubiquitination and down-regulation in response to IFNα (10) or IFNβ.3 Consistent with previous reports (17, 18) infection with VSV robustly stimulated phosphorylation of both the IFNAR1 degron (Ser-535) and priming site (Ser-532). We then examined the effect of pharmacologic inhibitors of several UPR-induced kinases including p38 kinase, LY294002, JNK inhibitor SP600125, and p38 inhibitor VX702. Pre-treatment of KR2 cells with the latter two agents after VSV infection yet prior to cell harvesting noticeably decreased phosphorylation of IFNAR1 without affecting either expression of viral protein VSV-M or specific phosphorylation of eIF2α (Fig. 1A). These results suggest that activation of stress activated protein kinases downstream of the UPR pathway may contribute to phosphorylation of IFNAR1.

The effects of p38 inhibitor VX702 were more robust (Fig. 1A); furthermore, the JNK inhibitor SP600125 was reported to also attenuate p38 kinase activity (26). Given that, we hypothesized that p38 kinase might be a regulator of IFNAR1 phosphorylation. Either VX702 or another inhibitor of p38 kinase SB203580 prevented induction of priming phosphorylation of IFNAR1 on Ser532 in response to either VSV infection or TG treatment in HeLa cells (Fig. 1B). We switched to HeLa cells or KR2-isogenic wild type fibrosarcoma 2fTGH cells because they were shown to respond to IFNα by stimulated phosphorylation of IFNAR1 degron (13, 17) and, therefore, enabled us to compare the effects of UPR with those of IFNα. Unlike VSV or TG, treatment with IFNα did not induce phosphorylation of eIF2α (Fig. 1B). Consistent with previous reports (11, 13, 17), IFNα was not efficient in stimulating the priming phosphorylation on Ser-532, yet it robustly induced IFNAR1 degron phosphorylation on Ser-535. Neither VX702 nor SB203580 prevented Ser-535 phosphorylation by IFNα, whereas being efficient in decreasing this phosphorylation in cells that received VSV or TG (Fig. 1B). These results further implicate p38 kinase activity in IFNAR1 phosphorylation induced by the stimuli that trigger the ligand-independent pathway.

We next used a genetic approach to complement these pharmacologic analyses. Various tissues are known to express four related yet distinct isoforms of p38 (α, β, γ, and δ) of which p38α and p38β are sensitive to SB203580 (27), and p38α protein is a ubiquitously expressed isoform in all tissues (28). We used shRNA against p38α kinase, which decreased the levels of endogenous protein by 60–90% in either HeLa or 2fTGH cells (Figs. 2, A and B, 3A, and 4B). Knockdown of p38α kinase in 2fTGH cells did not affect VSV- or TG-induced phosphorylation of eIF2α (Fig. 2A). Yet, shRNA against p38α noticeably decreased the priming phosphorylation of IFNAR1 on Ser-532 in cells that received either TG or VSV. Importantly, whereas a similar effect of p38α kinase knockdown was detected on the IFNAR1 degron phosphorylation on Ser-535, this phosphorylation was not affected in cells treated with IFNα (Fig. 2A). Similar results were obtained in HeLa cells (Fig. 2B). These results implicate p38α in phosphorylation of IFNAR1 that occurs through the ligand-independent pathway.

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We sought to corroborate these conclusions in mouse cells. Induction of the phosphorylation of the priming site (Ser-523 in mouse IFNAR1) was observed in mouse embryo fibroblasts (MEFs) derived from the wild type animals but not from p38α knock-out mice (Fig. 2C). Activation of PERK evident by eIF2α phosphorylation was not affected in these fibroblasts suggesting that the role of p38α in priming phosphorylation occurs downstream of PERK. When lysates from these MEFs were used as a source of kinase activity to phosphorylate bacterially expressed GST-IFNAR1 protein on Ser-532 downstream of PERK, we detected a marked increase in activity of a priming kinase in IFNAR1 ubiquitination induced by UPR. (Fig. 2D). Furthermore, whereas phosphorylation of IFNAR1 degron (Ser-526 in mouse IFNAR1) was observed in mouse embryo fibroblasts (MEFs) derived from the wild type animals but not from p38α knock-out mice. Mouse IFNβ (5000 international units/ml) was used instead of human IFNα. Analyses of ubiquitination and levels of IFNAR1 and of phosphorylation and levels of p38α are shown.

We next sought to determine whether p38 kinase plays a role in the regulation of IFNAR1 stability and IFNAR1-mediated signaling. Consistent with previous reports (18), infection of HeLa cells with VSV accelerated the rate of turnover of IFNAR1 assessed via a cycloheximide chase assay (Fig. 4A). A similar effect was observed either in 2fTGH cells that received irrelevant control shRNA against GFP (Fig. 4B) or in wild type MEFs (Fig. 4C). Under these conditions, the acceleration of the rate of proteolytic turnover of human or murine IFNAR1 could be reverted by either inhibition of p38 kinase using treatment with VX702 inhibitor (Fig. 4A) or knockdown of p38α using shRNA (Fig. 4B) or genetic ablation of p38α in MEFs (Fig. 4C). These data implicate p38 kinase in general (and its α-isofrom in particular) in the regulation of IFNAR1 degradation in cells exposed to VSV.

Infection with low doses of VSV was shown to induce IFNAR1 phosphorylation and down-regulation in a manner that required PERK activation (18). We carried out a time course for PERK and p38 activation after a pulse infection of HeLa cells. Activation of PERK (assessed by eIF2α phosphorylation) preceded activation of p38 kinase as evidenced from its phosphorylation; both events occurred before noticeable decrease in the levels of endogenous IFNAR1 (Fig. 5A). This
result suggests that sequential activation of PERK and p38 may promote down-regulation of IFNAR1. Whereas the role of PERK in IFNAR1 down-regulation in VSV-infected cells has been established previously (18), here, we focused on the importance of p38 kinase. Either inhibition of p38 kinase using VX702 or knockdown of p38α kinase using RNAi prevented down-regulation of IFNAR1 in VSV-infected human cells (Fig. 5, B and C). In addition, treatment with TG did not efficiently decrease IFNAR1 levels in cells that received shRNA against p38α kinase (Fig. 5D). Furthermore, neither VSV infection nor TG treatment caused a robust down-regulation of murine IFNAR1 in MEFs from p38α-null mice (Fig. 5, E and F). These data strongly suggest that p38 kinase plays an important role in regulating IFNAR1 levels.

Given that cells from heterozygous Ifnar1+/− mice display a partially decreased response to type I IFN, it has been proposed that levels of IFNAR1 play an important role in IFN signaling (29). Indeed, pre-exposure of human or murine cells to PERK inducers such as VSV was shown to attenuate their responses to the future encounter with IFNα/β (18). In line with these data, IFNα-induced phosphorylation of STAT1 was decreased in VSV-infected cells (Fig. 6A). Under these conditions, treatment of cells with p38 kinase inhibitor VX702 noticeably restored the extent of IFNα signaling (Fig. 6A). Similar results were observed when modulation of p38α status was achieved using the RNAi approach (Fig. 6B). Furthermore, VSV-mediated inhibition of STAT1 phosphorylation induced by exogenous IFNβ was seen in wild type MEFs but not in MEFs from p38α knockout mice (Fig. 6C). These results implicate p38 kinase in controlling IFNα/β signaling in infected cells.

We further sought to corroborate the role of p38 kinase in cells that undergo UPR using a non-viral UPR inducer. Whereas pre-treatment of either human or mouse cells with TG noticeably decreased the efficacy of STAT1 activation by VSV (MOI of 0.01 for 1 h), washed, and incubated for 8 h. Then, cells were treated (or not) with VX702 (1 μM) and harvested at indicated times post-infection. Levels of IFNAR1 and β-actin were determined as described in 4A. C2GH cells that received indicated shRNA were infected with VSV as described in B and harvested at the indicated times post-infection. Levels of IFNAR1 and β-actin were described as in B, D, down-regulation of IFNAR1 in indicated C2GH cells treated with TG (1 μM, 3 h) was analyzed as in C. E, wild type or p38α−/− MEFs were infected with VSV (MOI of 0.01) and harvested 0, 16, and 20 h following infection. IFNAR1 was immunoprecipitated, and levels were detected by immunoblotting. F, down-regulation of murine IFNAR1 in indicated MEFs treated with TG (1 μM, 3 h) was analyzed as described in E.
macologic inhibitors of p38. Knockdown of p38α kinase in human 2TGH cells has dramatically decreased the efficacy of VSV infection as evident from a decreased viral titer and expression of VSV-M protein (Fig. 7A). Similarly, replication of VSV was much less evident in MEFs from p38α knockout mice compared with their wild type counterparts (Fig. 7B). These data strongly suggest that p38 kinase plays an important role in regulation the anti-viral defenses.

**DISCUSSION**

In this study, we aimed to characterize the PERK-dependent pathway that leads to the ligand-independent priming phosphorylation of IFNAR1 followed by phosphorylation of IFNAR1 degron, ubiquitination, and degradation of IFNAR1. Pharmacologic analyses using inhibitors against protein kinases known to be activated by UPR downstream of PERK implicated p38 kinase in the ligand-independent induction of IFNAR1 phosphorylation (Fig. 1). Activation of p38 kinase by inducers of UPR has been long established in the literature (22, 30). Given that p38 kinase can be activated by tumor necrosis factor α (TNFα) (31, 32), identification of TNF receptor associated factor-2 as an interacting protein for IRE1 transmembrane kinase/endoribonuclease (33), a known sensor of UPR (19), initially suggested the role of IRE1 in UPR-induced activation of stress-activated protein kinases (e.g. JNK (33)). However, subsequent analysis using genetically defined cell system firmly placed p38 kinase activation by UPR inducers downstream of PERK (22). In our study, we further observed that modulation of p38α kinase by knockdown or knock-out approaches had a profound negative effect on IFNAR1 phosphorylation stimulated by UPR inducers but not by IFNα/β (Fig. 2).

Subsequent pharmacologic and genetic analyses revealed p38 kinase as a key regulator of IFNAR1 ubiquitination, degradation, down-regulation, and downstream signaling (Figs. 3–6). As VSV infection presents a convenient and robust yet inexpensive tool for inducing UPR and PERK (34), we have widely used this approach in our studies. Cells infected with VSV indeed displayed phosphorylation of eIF2α paralleled by activation of p38 kinase and subsequent down-regulation of IFNAR1 (Fig. 5A). Intriguingly, we found that the VSV load is decreased in human or mouse cells where p38α was either knocked out or knocked down (Fig. 7). Thus, the interpretation of inefficient IFNAR1 down-regulation, degradation, and signaling seen in p38-defective cells should include both the abrogation of p38-mediated effects on IFNAR1 itself (also evident from experiments that used p38 pharmacologic inhibitors added after VSV infection has been completed) and resistance of these cells to viral replication. Nevertheless, all presented data are supportive of our conclusions that p38 kinase plays an important role in regulating type I IFN signaling and antiviral defenses. Furthermore, experiments that use TG as a non-viral UPR stimulus clearly demonstrate that UPR-induced IFNAR1 phosphorylation (Figs. 1 and 2), IFNAR1 ubiquitination (Fig. 3), down-regulation of IFNAR1 (Fig. 5, D and F) and attenuation of IFNα/β signaling (Fig. 6, D and E) require the activity of p38α.

Although present data establish the role of p38 kinase in priming phosphorylation of IFNAR1, it remains to be seen whether this regulation is direct. Our pilot biochemical data suggest that immunopurified p38 kinase is capable of phosphorylating the priming site (Ser-532) within IFNAR1 in an in vitro kinase reaction.

Given that p38 kinase mediates priming phosphorylation-degron phosphorylation, ubiquitination, and degradation (14). As VSV infection presents a convenient and robust yet inexpensive tool for inducing UPR and PERK (34), we have widely used this approach in our studies. Cells infected with VSV indeed displayed phosphorylation of eIF2α paralleled by activation of p38 kinase and subsequent down-regulation of IFNAR1 (Fig. 5A). Intriguingly, we found that the VSV load is decreased in human or mouse cells where p38α was either knocked out or knocked down (Fig. 7). Thus, the interpretation of inefficient IFNAR1 down-regulation, degradation, and signaling seen in p38-defective cells should include both the abrogation of p38-mediated effects on IFNAR1 itself (also evident from experiments that used p38 pharmacologic inhibitors added after VSV infection has been completed) and resistance of these cells to viral replication. Nevertheless, all presented data are supportive of our conclusions that p38 kinase plays an important role in regulating type I IFN signaling and antiviral defenses. Furthermore, experiments that use TG as a non-viral UPR stimulus clearly demonstrate that UPR-induced IFNAR1 phosphorylation (Figs. 1 and 2), IFNAR1 ubiquitination (Fig. 3), down-regulation of IFNAR1 (Fig. 5, D and F) and attenuation of IFNα/β signaling (Fig. 6, D and E) require the activity of p38α. Whereas genetic data obtained in fibroblasts and fibrosarcoma cells highlight the importance of p38α form, the importance of other forms (β, γ, and δ) in other cell types cannot be ruled out.

4 S. Bhattacharya and S. Y. Fuchs, unpublished data.
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REFERENCES

1. Huangfu, W. C., and Fuchs, S. Y. (2010) Genes Cancer 1, 725–734
2. Pfeffer, L. M., and Dinarello, C. A. (1995) Pharmacol. Ther. 52, 149–157
3. Pfeffer, L. M., Dinarello, C. A., Herberman, R. B., Williams, B. R., Borden, E. C., Bordens, R., Walter, M. R., Nagabhushan, T. L., Trotta, P. P., and Pestka, S. (1998) Cancer Res. 58, 2489–2499
4. Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H., and Schreiber, R. D. (1998) Annu. Rev. Biochem. 67, 227–264
5. Platanias, L. C. (2005) Nat. Rev. Immunol. 5, 375–386
6. Aaronson, D. S., and Horvath, C. M. (2002) Science 296, 1653–1655
7. Du, Z., Wei, L., Murti, A., Pfeffer, S. R., Fan, M., Yang, C. H., and Pfeffer, L. M. (2007) J. Cell. Biol. 102, 1087–1094
8. Uezu, G., Schreiber, G., Pielcher, J., and Pellegrini, S. (2007) Curr. Top Microbiol. Immunol. 316, 71–95
9. Liu, J., Plotnikov, A., Banerjee, A., Suresh Kumar, K. G., Ragimbeau, J., Marijanovic, Z., Baker, D. P., Pellegrini, S., and Fuchs, S. Y. (2008) Biochem. Biophys. Res. Commun. 367, 388–393
10. Marijanovic, Z., Ragimbeau, J., Kumar, K. G., Fuchs, S. Y., and Pellegrini, S. (2006) Biochem. J. 397, 31–38
11. Kumar, K. G., Barriere, H., Carbone, C. J., Liu, J., Swaminathan, G., Xu, P., Li, Y., Baker, D. P., Peng, J., Lukacs, G. L., and Fuchs, S. Y. (2007) J. Cell Biol. 179, 935–950
12. Kumar, K. G., Tang, W., Ravindranath, A. K., Clark, W. A., Croze, E., and Fuchs, S. Y. (2003) EMBO J. 22, 5480–5490
13. Kumar, K. G., Krolewski, J. J., and Fuchs, S. Y. (2004) J. Biol. Chem. 279, 46614–46620
14. Zheng, H., Qian, J., Varghese, B., Baker, D. P., and Fuchs, S. (2011) Mol. Cell Biol. 31, 710–720
15. Liu, J., Carvalho, L. P., Bhattacharya, S., Carbone, C. J., Kumar, K. G., Leu, N. A., Yau, P. M., Donald, R. G., Weiss, M. J., Baker, D. P., McLaughlin, K. J., Scott, P., and Fuchs, S. Y. (2009) Mol. Cell Biol. 29, 6401–6412
16. Knippschild, U., Gocht, A., Wolf, S., Huber, N., Löhler, J., and Stöter, M. (2005) Cell Signal 17, 675–689
17. Bhattacharya, S., Huangfu, W. C., Liu, J., Veeranki, S., Baker, D. P., Koumenis, C., Diehl, J. A., and Fuchs, S. Y. (2010) J. Biol. Chem. 285, 2318–2325
18. Liu, J., Huangfu, W. C., Kumar, K. G., Qian, J., Casey, J. P., Hama, R. B., Grigoriadou, C., Aldabe, R., Diehl, J. A., and Fuchs, S. Y. (2009) Cell Host Microbe 5, 72–83
19. Wek, R. C., and Cavender, D. R. (2007) Antioxid Redox Signal 9, 2357–2371
20. Goldman, L. A., Zafari, M., Cutrone, E. C., Dang, A., Brickelmeier, M., Runkel, L., Benjamin, C. D., Ling, E. L., and Langer, J. A. (1999) J. Interferon Cytokine Res. 19, 15–26
21. Kumar, K. G., Varghese, B., Banerjee, A., Baker, D. P., Constantinescu, S. N., Pellegrini, S., and Fuchs, S. Y. (2008) J. Biol. Chem. 283, 18566–18570
22. Liang, S. H., Zhang, W., McGrath, B. C., Zhang, P., and Cavender, D. R. (2006) Biochem. J. 393, 201–209
23. Hama, R. B., Bobrovnikova-Marjon, E., Ji, X., Liebhaber, S. A., and Diehl, J. A. (2009) Oncogene 28, 910–920
24. Gauzzi, M. C., Barbieri, G., Richter, M. F., Uzé, G., Ling, L., Fellous, M., and Pellegrini, S. (1997) Proc. Natl. Acad. Sci. USA 94, 11839–11844
25. Rani, M., Leaman, D. W., Han, Y., Leung, S., Croze, E., Fish, E. N., Wolfman, A., and Ransohoff, R. M. (1999) J. Biol. Chem. 274, 32507–32511
26. Bennett, B. L., Sasaki, D. T., Murray, W. O., Leary, E. C., Sakata, S. T., Xu, W., Leisten, J. C., Motiwala, A., Pierce, S., Satoh, Y., Bhagwat, S. S., Manning, A. M., and Anderson, D. W. (2001) Proc. Natl. Acad. Sci. USA 98, 13681–13686
27. Cohen, P. (1997) Trends Cell Biol. 7, 353–361
28. Hale, K. E., Trolldinger, D., Rihanek, M., and Manthey, C. L. (1999) J. Immunol. 162, 4246–4252
29. Hwang, S. Y., Hertzog, P. J., Holland, K. A., Sumarsono, S. H., Tymms, M. J., Hamilton, J. A., Whitty, G., Bertoncello, I., and Kola, I. (1995) Proc. Natl. Acad. Sci. USA 92, 11284–11288
30. Su, H. L., Liao, C. L., and Lin, Y. L. (2002) J. Virol. 76, 4162–4171
31. Kyriakis, J. M. (2000) Sci STKE 2000, pe1
32. Kyriakis, J. M., and Avruch, J. (1996) Bioessays 18, 567–577
33. Urano, F., Wang, X., Bertotti, A., Zhang, Y., Chung, P., Harding, H. P., and Ron, D. (2000) Science 287, 664–666
34. Baltzis, D., Qu, L. K., Papadopoulou, S., Blais, J. D., Bell, J. C., Sonenberg, N., and Koromilas, A. E. (2004) J. Virol. 78, 12747–12761
35. Roux, P. P., and Blenis, J. (2004) Microbiol. Mol. Biol. Rev. 68, 320–344
36. Shi, Y., and Gaestel, M. (2002) Biol. Chem. 383, 1519–1536
37. Joshi, S., Kaur, S., Redig, A. J., Goldsborough, K., David, K., Ueda, T.,
Watanabe-Fukunaga, R., Baker, D. P., Fish, E. N., Fukunaga, R., and Platanias, L. C. (2009) *Proc. Natl. Acad. Sci. U.S.A.* **106**, 12097–12102

38. Kroczynska, B., Kaur, S., Katsoulidis, E., Majchrzak-Kita, B., Sassano, A., Kozma, S. C., Fish, E. N., and Platanias, L. C. (2009) *Mol. Cell. Biol.* **29**, 2865–2875

39. Katsoulidis, E., Li, Y., Mears, H., and Platanias, L. C. (2005) *J. Interferon Cytokine Res* **25**, 749–756

40. Parmar, S., Katsoulidis, E., Verma, A., Li, Y., Sassano, A., Lal, L., Majchrzak, B., Ravandi, F., Tallman, M. S., Fish, E. N., and Platanias, L. C. (2004) *J. Biol. Chem.* **279**, 25345–25352

41. Platanias, L. C. (2003) *Pharmacol. Ther.* **98**, 129–142

42. Uddin, S., Lekmine, F., Sharma, N., Majchrzak, B., Mayer, I., Young, P. R., Bokoch, G. M., Fish, E. N., and Platanias, L. C. (2000) *J. Biol. Chem.* **275**, 27634–27640

43. Uddin, S., Majchrzak, B., Woodson, J., Arunkumar, P., Alsayed, Y., Pine, R., Young, P. R., Fish, E. N., and Platanias, L. C. (1999) *J. Biol. Chem.* **274**, 30127–30131

44. Goh, K. C., Haque, S. J., and Williams, B. R. (1999) *EMBO J.* **18**, 5601–5608

45. Johnson, R. A., Huong, S. M., and Huang, E. S. (1999) *Antiviral Res.* **41**, 101–111

46. Karaca, G., Hargett, D., McLean, T. L., Aguilar, J. S., Ghazal, P., Wagner, E. K., and Bachenheimer, S. L. (2004) *Virology* **329**, 142–156

47. Cohen, P. S., Schmidtmayerova, H., Dennis, J., Dubrovska, L., Sherry, B., Wang, H., Bukrinsky, M., and Tracey, K. I. (1997) *Mol. Med.* **3**, 339–346

48. Chen, C. J., Raung, S. L., Kuo, M. D., and Wang, Y. M. (2002) *J. Gen Virol.* **83**, 1897–1905

49. Si, X., Luo, H., Morgan, A., Zhang, J., Wong, J., Yuan, J., Esfandiairei, M., Gao, G., Cheung, C., and McManus, B. M. (2005) *J. Virol.* **79**, 13875–13881

50. Chang, W. W., Su, I. J., Chang, W. T., Huang, W., and Lei, H. Y. (2008) *J. Viral Hepat.* **15**, 490–497

51. Kono, M., Tatsumi, K., Imai, A. M., Saito, K., Kuriyama, T., and Shirasawa, H. (2008) *Antiviral Res.* **77**, 150–152

52. Mayer, I. A., Verma, A., Grumbach, I. M., Uddin, S., Lekmine, F., Ravandi, F., Majchrzak, B., Fujita, S., Fish, E. N., and Platanias, L. C. (2001) *J. Biol. Chem.* **276**, 28570–28577

---

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---

Johnson, R. A., Huong, S. M., and Huang, E. S. (1999) *Antiviral Res.* **41**, 101–111

Karaca, G., Hargett, D., McLean, T. L., Aguilar, J. S., Ghazal, P., Wagner, E. K., and Bachenheimer, S. L. (2004) *Virology* **329**, 142–156

Cohen, P. S., Schmidtmayerova, H., Dennis, J., Dubrovska, L., Sherry, B., Wang, H., Bukrinsky, M., and Tracey, K. I. (1997) *Mol. Med.* **3**, 339–346

Chen, C. J., Raung, S. L., Kuo, M. D., and Wang, Y. M. (2002) *J. Gen Virol.* **83**, 1897–1905

Si, X., Luo, H., Morgan, A., Zhang, J., Wong, J., Yuan, J., Esfandiairei, M., Gao, G., Cheung, C., and McManus, B. M. (2005) *J. Virol.* **79**, 13875–13881

Chang, W. W., Su, I. J., Chang, W. T., Huang, W., and Lei, H. Y. (2008) *J. Viral Hepat.* **15**, 490–497

Kono, M., Tatsumi, K., Imai, A. M., Saito, K., Kuriyama, T., and Shirasawa, H. (2008) *Antiviral Res.* **77**, 150–152

Mayer, I. A., Verma, A., Grumbach, I. M., Uddin, S., Lekmine, F., Ravandi, F., Majchrzak, B., Fujita, S., Fish, E. N., and Platanias, L. C. (2001) *J. Biol. Chem.* **276**, 28570–28577