Sprouty Proteins Are Negative Regulators of Interferon (IFN) Signaling and IFN-inducible Biological Responses

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Interferons (IFNs) have important antiviral and antineoplastic properties, but the precise mechanisms required for generation of these responses remain to be defined. We provide evidence that during engagement of the Type I IFN receptor (IFNR), there is up-regulation of expression of Sprouty (Spry) proteins 1, 2, and 4. Our studies demonstrate that IFN-inducible up-regulation of Spry proteins is Mnk kinase-dependent and results in suppressive effects on the IFN-activated p38 MAP kinase (MAPK), the function of which is required for transcription of interferon-stimulated genes (ISGs). Our data establish evidence that during engagement of the Type I IFN receptor (IFNR), there is up-regulation of expression of Sprouty (Spry) proteins, consistent with negative feedback regulatory roles for Spry proteins in IFN-mediated signaling. In other studies, we found that siRNA-mediated knockdown of Spry1, Spry2, or Spry4 promotes IFN-inducible antileukemic effects in vitro and results in enhanced suppressive effects on malignant hematopoietic progenitors from patients with polycythemia vera. Altogether, our findings demonstrate that Spry proteins are potent regulators of Type I IFN signaling and negatively control induction of Type I IFN-mediated biological responses.

Due to the important biological effects of interferons (IFNs) (1–3) and the clinical efficacy of these cytokines in the treatment of various diseases (4), there has been a substantial interest in defining cellular pathways activated by IFN receptors and dissecting their contributions in the generation of the biological effects of IFNs. Although IFNs were originally discovered and described as agents that block replication of different viruses (1–3), subsequent work established that they also act as modulators of innate immune responses and exhibit important growth inhibitory and antineoplastic properties (1–3). Three major IFN groups exist, each of which includes different subgroups and members. These include Type I (α, β, ω, γ, κ, ε, and δ); Type II (γ); and the more recently identified class of Type III IFNs (A1, A2, and A3) (1–6). All different IFN classes utilize Jak-Stat pathways to activate elements in the promoters of IFN-stimulated genes (ISGs)3 promoting transcriptional activation and induction of gene expression (7, 8). Common and distinct combinations of receptor-associated Jak kinases and Stat proteins are engaged by Type I, II, and III IFN receptors, and in each case, the coordinated functions of Jak-Stat pathways are essential for ultimate generation of ISG products and associated biological responses (1, 3, 7, 8). Importantly, there is also evidence that unphosphorylated Stats exhibit important functions as transcription factors, as well as modifiers of transcription factors and chromatin structure (9).

In addition to classical Jak-Stat pathways, other signaling cascades engaged by IFN receptors are essential for optimal transcriptional activation and mRNA translation of ISGs, and ultimately, the generation of IFN biological responses. The p38 MAP kinase (MAPK) pathway is activated in parallel to Jak-Stat pathways, and its function is essential for IFN-dependent gene transcription (10, 11), whereas the AKT/mTOR (mammalian target of rapamycin) signaling cascade is also engaged by IFN receptors and is required for mRNA translation of ISGs and ultimate production of ISG proteins (12–15). Other studies have shown that IFN-dependent engagement of the Erk MAP

**Background:** The potential involvement of Spry proteins in IFN signaling is unknown.

**Results:** Type I IFN treatment results in up-regulation of Spry proteins, which negatively control generation of IFN responses.

**Conclusion:** Spry proteins play important regulatory roles in IFN signaling and the generation of the biological effects of IFNs.

**Significance:** This study provides evidence for the existence of a key signaling pathway that controls IFN responses.

3 The abbreviations used are: ISG, IFN-stimulated gene; Spry, Sprouty; mSpry, mouse Spry; MEF, mouse embryonic fibroblast; BFU-E, burst-forming unit, erythroid; CFU-L, colony-forming unit, lymphocyte; Mkk, Map kinase kinase; Mnk, MAPK-interacting kinase.
kinase pathway participates in ISG mRNA translation/protein expression via regulatory effects on the activation of Mnk kinases and elf4E phosphorylation (16, 17).

In the present study, we provide the first evidence for engagement of Sprouty (Spry) proteins in IFN signaling. The family of Spry proteins includes four members (18–20), all of which are homologues of the *Drosophila melanogaster* Spry, which was originally identified as an inhibitor of FGF signaling (21). These proteins act as negative regulators of growth factor signaling pathways (18–20, 22), and their expression is deregulated in several malignancies (20). Our studies demonstrate that treatment of sensitive cells with IFNα or IFNβ results in stabilization/up-regulation of expression of Spry proteins in a Mnk kinase-dependent manner. IFN-activated Spry proteins act as negative feedback regulators and exhibit inhibitory effects on the p38 MAPK pathway and ISG expression. This leads to negative control of IFN-inducible antiviral effects and growth inhibitory responses. Importantly, inhibiting expression of the various Spry proteins results in enhanced IFN-dependent antileukemic effects and antiviral responses, suggesting that selective targeting of these proteins may provide an approach to enhance and optimize the therapeutic potential of IFNs.

**EXPERIMENTAL PROCEDURES**

**Cells and Reagents**—U937 cells were grown in RPMI supplemented with 10% (v/v) fetal bovine serum and antibiotics. Immortalized Sprouty1,2,4 knock-out MEFs (23) were grown in DMEM supplemented with 10% (v/v) fetal bovine serum and antibiotics. Recombinant human IFNα was obtained from Hoffmann-La Roche. Recombinant human and mouse IFNβ were from Biogen Idec. Antibodies against human Spry1 and Spry2 were purchased from Abcam (Cambridge, MA). An antibody against human Spry4 was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antibodies against mouse Spry1 and Spry2 have been previously described (23). Antibodies against the phosphorylated forms of STAT1, STAT3, ERK1/2 (Thr-202/Tyr-204), and p38 MAPK (Thr-180/Tyr-182), as well as antibodies against ERK1/2 and p38 MAPK, were obtained from Cell Signaling Technology (Danvers, MA). An antibody against GAPDH was obtained from Millipore. siRNAs targeting human Sprouty1, Sprouty2, and Sprouty4 as well nontargeting siRNAs were obtained from Santa Cruz Biotechnology.

**Cell Lysis and Immunoblotting**—Cells were treated with 5 × 10^3 or 1 × 10^4 IU/ml of IFNα and IFNβ for the indicated times. For the experiments in which ISG15 expression was assessed, the concentration of IFN used was 2.5 × 10^3 IU/ml. Cells were then collected and lysed in phosphorylation lysis buffer as in our previous studies (12). Immunoblotting was performed using an enhanced chemiluminescence (ECL) method were performed as in previous studies (12).

**mRNA Expression Assays**—Cells were treated with 5 × 10^3 units/ml of IFNo or IFNβ for 6 h, and quantitative RT-PCR was carried out as described previously (12). Real-time RT-PCR to determine expression of ISG15 mRNA was carried out by using commercially available 6-carboxyfluorescein-labeled probes and primers (Applied Biosystems). For knockdown experiments, cells were nucleofected with siRNA, and Spry1,2,4 mRNA expression was assessed using real-time RT-PCR. GAPDH was used for normalization.

**RESULTS**

In initial studies, we examined the effects of Type I IFNs (IFNα and IFNβ) on protein expression of different Spry family members. U937 cells were treated with IFNα or IFNβ for different times, and Spry protein levels were assessed by immunoblotting. Type I IFN treatment resulted in the up-regulation of expression of Spry2 that was detectable within 60 min of treatment and persisted for 4–5 h (Fig. 1, A and B). Similarly, IFN treatment resulted in sustained up-regulation of Spry4 (Fig. 1, C and D) and Spry1 (Fig. 1, E and F).

Evidence from previous work shows that phosphorylation of Spry2 on serines 112 and 121 by Mnk1 stabilizes the protein and antagonizes c-Cbl binding and polyubiquitination that would otherwise lead to its degradation (24). As we have shown that Mnk1 is engaged in Type I IFN signaling and plays critical roles in the generation of IFN responses (16), we examined whether Mnk kinase activity is required for stabilization/up-regulation of Spry protein expression by the Type I IFN receptor. Immortalized MEFs from mice with targeted disruption of both Mnk1 and Mnk2 (25) or control wild-type (WT) MEFs were treated with mouse IFNβ for increasing times, and protein levels of Spry1 and Spry2 were analyzed in parallel. Treatment of WT MEFs with mouse IFNβ resulted in rapid up-regulation of Spry1 levels, consistent with stabilization of protein expression, but this up-regulation was not seen in Mnk1/2 double knock-out MEFs (Fig. 2A). Similarly, up-regulation of expression of Spry2 was Mnk kinase-dependent (Fig. 2B), suggesting that IFN-dependent, Mnk-mediated phosphorylation of Spry proteins stabilizes them and promotes their expression (24).

Spry expression is stimulated by growth factors and plays key regulatory roles in growth factor signaling (18–22, 24). By contrast to growth factors, IFNs are cytokines that generally inhibit cell proliferation, and our finding that Spry protein levels are regulated by IFNs raised the possibility that Spry proteins might...
modulate IFN-mediated growth inhibition. To examine the effects of Spry proteins on Type I IFN-induced signaling, we utilized MEFs created by Cre-mediated excision of lox P-linked alleles deficient for all three Spry proteins up-regulated in response to Type I IFNs (Spry1,2,4/H11002/H11002/H11002) (23). Serum-starved parental Spry1,2,4flox/flox or Spry1,2,4/H11002/H11002/H11002 MEFs were treated with mouse IFNα or IFNβ for the indicated times. Proteins in lysates were resolved by SDS-PAGE and immunoblotted with an antibody against Spry2. The signals for Spry2 and tubulin from the experiments shown in A and B were quantitated by densitometry, and the intensities of Spry2 relative to tubulin expression were calculated. C and D, U937 cells were treated with human IFNα or IFNβ for the indicated times. Proteins in lysates were resolved by SDS-PAGE and immunoblotted with an antibody against Spry4. The signals for Spry4 and GAPDH from the experiment shown in C and D were quantitated by densitometry, and the intensities of Spry4 relative to GAPDH expression were calculated. E and F, U937 cells were treated with human IFNα or IFNβ for the indicated times. Proteins in lysates were resolved by SDS-PAGE and immunoblotted with an antibody against Spry1. The signals for Spry1 and tubulin (E) or GAPDH (F) were quantitated by densitometry, and the intensities of Spry1 relative to tubulin or GAPDH expression were calculated.
negative feedback regulators of the MEK/Erk pathway. Importantly, targeted disruption of the Spry1, Spry2, and Spry4 genes also resulted in enhancement of phosphorylation/activation of p38 MAPK (Fig. 3F), suggesting unique regulatory effects of Spry proteins in IFN signaling.

Previous work established that the p38 MAPK pathway complements the function of Stat pathways and is required for optimal transcription of ISGs with interferon-sensitive response elements in their promoters (26, 27). As our data demonstrated regulatory effects of Spry proteins on p38 MAPK activity, we determined the effects of the targeted disruption of Spry genes on mRNA expression of the ISG15 gene, the protein product of which accounts for IFN-dependent ISGylation and plays an important role in the control of IFN responses (28).

Spry1,2,4flox/flox and Spry1,2,4flox/flox MEFS were treated with murine IFNα or IFNβ, and ISG15 mRNA expression was determined by quantitative real-time RT-PCR. As shown in Fig. 4A, there was strong induction of ISG15 mRNA expression in response to either IFNα or IFNβ in Spry1,2,4flox/flox MEFS, but this expression increased further in Spry1,2,4flox/flox cells (Fig. 4A). Consistent with this, IFN-dependent ISG15 protein expression was strongly enhanced in Spry1,2,4flox/flox cells (Fig. 4, B–C). To

**FIGURE 2.** Type I IFN-dependent up-regulation of Spry proteins is Mnk kinase dependent. A, Mnk1/2+/+ and Mnk1/2−/− MEFS were treated with mouse IFNβ for the indicated times, and proteins in cell lysates were separated by SDS-PAGE and immunoblotted with an antibody against Spry1. The same blot was probed with an antibody against GAPDH. The signals for Spry1 and GAPDH were quantitated by densitometry, and the intensity of Spry1 relative to GAPDH expression was calculated. B, Mnk1/2+/+ and Mnk1/2−/− MEFS were treated with mouse IFNβ for the indicated times, and proteins in cell lysates were separated by SDS-PAGE and immunoblotted with an antibody against Spry2. The same blot was probed with an antibody against GAPDH. The signals for Spry2 and GAPDH were quantitated by densitometry, and the intensity of Spry2 relative to GAPDH expression was calculated.
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To evaluate the functional relevance of Spry proteins in the biological effects of IFNs, we compared the antiviral properties of mouse IFNβ with those of IFNα. We found that Spry proteins exhibit a suppressive effect on the generation of the suppressive effects of IFNα on leukemic progenitor colony formation (Fig. 5A). Ectopic expression of Spry1 or Spry2 substantially decreases the levels of IFN-dependent expression of ISG15 protein (Fig. 4F).

To test the functional relevance of Spry proteins in the biological effects of IFNs, we compared the antiviral properties of mouse IFNα with those of Spry knockdowns on the generation of the suppressive effects of IFNα or IFNβ on leukemic progenitor (CFU-L) colony formation. Treatment with either IFNα or IFNβ resulted in partial inhibition of CFU-L colony formation (Fig. 5F, B and C), which was significantly enhanced in cells depleted of Spry1 or Spry2 expression (Fig. 5B). A similar trend was seen in cells in which Spry4 was knocked down (Fig. 5C). Thus, Spry proteins oppose both the antiviral and the antiproliferative/antileukemic effects of Type I IFNs.

To ascertain the clinical importance of this latter effect, we determined the function of Spry proteins on the action of IFNα on primary malignant hematopoietic progenitors from patients with polycythemia vera. As shown in Fig. 5D, selective targeting of Spry1, Spry2, or Spry4 resulted in enhanced IFN-inducible suppressive effects on malignant early erythroid progenitor (BFU-E) colony formation (Fig. 5D), further implicating these proteins as negative regulators of IFN responses, specifically in primary malignant progenitors.

**DISCUSSION**

The first member of the Sprouty family (dSpry) was originally discovered in *Drosophila* as a novel cysteine-rich protein functioning as an FGF antagonist (21). In that original study, which ultimately defined the existence of the Spry family of proteins, it was demonstrated that the FGF pathway is overactive in *Sprouty* mutant embryos and is associated with ectopic branches, as compared with wild-type embryos (21). Subsequent work identified four mammalian homologs, Spry1, Spry2, Spry3, and Spry4 (29). Expression of Spry1, Spry2, and Spry4
proteins is widespread in embryonic and adult tissues, whereas expression of Spry3 is restricted to brain and testes in adult tissues (30, 31). Spry proteins play critical roles in normal cells, and there is accumulating evidence for important regulatory effects during embryogenesis and organ development, including expansion of the organ of Corti (auditory sensory epithelium) (32), diastema tooth development (33), and morphogenesis of the ureteric epithelium in kidney development (34).

These important functions of Spry proteins during development are to a large extent reflections of their properties as modifiers of intracellular pathways and growth factor responses during development.

It is now well established that Spry proteins inhibit signaling from various growth factor receptors, including epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth fac-

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**FIGURE 4. Regulatory effects of Spry proteins on ISG expression.**

A, Spry1,2,4<sup>flox/flox</sup> MEFs and Spry1,2,4<sup>−/−</sup> MEFs were treated with mouse IFNα or IFNβ for 6 h, as indicated. The expression of ISG15 mRNA was assessed by quantitative RT-PCR, normalized for GAPDH expression. Data are expressed as -fold induction over corresponding untreated samples and represent means ± S.E. of three independent experiments. B, Spry1,2,4<sup>flox/flox</sup> MEFs and Spry1,2,4<sup>−/−</sup> MEFs were treated with mouse IFNβ for 24 and 48 h, as indicated. Equal amounts of protein were resolved by SDS-PAGE and immunoblotted with antibody against mouse ISG15. The same blot was probed with an antibody against GAPDH. C, Spry1,2,4<sup>flox/flox</sup> MEFs and Spry1,2,4<sup>−/−</sup> MEFs were treated with mouse IFNα or IFNβ for 48 h, as indicated. Equal amounts of protein were resolved by SDS-PAGE and immunoblotted with antibody against mouse ISG15. The same blot was probed with an antibody against GAPDH. D, Spry null MEFs complemented with empty vector or vectors containing cDNA for mSpry1 or mSpry2 were lysed, and equal amounts of protein were resolved by SDS-PAGE and immunoblotted with antibody against mouse ISG15. The same blot was probed with an antibody against GAPDH. E, Spry null MEFs complemented with empty vector or cDNA for mSpry1 or mSpry2 vector were lysed, and equal amounts of protein were resolved by SDS-PAGE and immunoblotted with an antibody against mSpry1. The same blot was probed with an antibody against tubulin. F, Spry1,2,4<sup>flox/flox</sup> MEFs and Spry1,2,4<sup>−/−</sup> MEFs in which mSpry1 or mSpry2 were ectopically re-expressed, as indicated, were treated with mouse IFNβ 24 h, as indicated. Equal amounts of protein were resolved by SDS-PAGE and immunoblotted with antibody against mouse ISG15. The same blot was probed with an antibody against GAPDH.
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FIGURE 5. Negative regulatory effects of Spry proteins in induction of IFN-dependent antiviral and antileukemic effects. A, Spry1,2,4+/floxed MEFs and Spry1,2,4−/− MEFs were incubated with the indicated doses of mouse IFN-α. The cells were subsequently challenged with encephalomyocarditis virus (EMCV), and the viral cytopathic effects (CPE) were quantified 4 days later. Data are expressed as the percentage of control colony formation of the control untreated siRNA-transfected cell-derived colony formation and represent means ± S.E. of five independent experiments. For panel B, paired t test analysis showed p = 0.0009 for the combination of control siRNA and IFN-α versus the combination of Spry1 siRNA and IFN-α; p = 0.0001 for the combination of Spry2 siRNA and IFN-α; p = 0.019 for the combination of control siRNA and IFN-β versus the combination of Spry1 siRNA and IFN-β for CFU-L; and p = 0.035 for the combination of control siRNA and IFN-β versus the combination of Spry2 siRNA and IFN-β for CFU-L colonies. For panel C, paired t test analysis showed p = 0.02 for the combination of control siRNA and IFN-α versus the combination of Spry4 siRNA and IFN-α and p = 0.008 for the combination of control siRNA and IFN-β versus the combination of Spry4 siRNA and IFN-β for CFU-L. D, mononuclear cells derived from peripheral blood of patients with polycythemia vera were transfected with the indicated siRNAs and were then plated in a methylcellulose assay system, in the absence or presence of human IFN-α. BFU-E progenitor colonies were scored after 14 days in culture. Data are expressed as the percentage of control colony formation of the control untreated siRNA-transfected cell-derived colony formation and represent means ± S.E. of five independent experiments. For panel D, paired t test analysis showed p = 0.0008 for the combination of control siRNA and IFN-α versus the combination of Spry1 siRNA and IFN-α; p = 0.0017 for the combination of control siRNA and IFN-α versus the combination of Spry2 siRNA and IFN-α; and p = 0.0074 for the combination of control siRNA and IFN-α versus the combination of Spry4 siRNA and IFN-α.

A key mechanism by which Spry proteins modulate cell proliferation and survival is by their ability to inhibit the Ras/MEK/Erk pathway (35). Several mechanisms for these responses have been delineated and involve direct interactions with known regulators or effector molecules of this signaling cascade, such as Grb2 (36), SHP2 (37), Raf1 (38), and Gap1 (39). There is also recent evidence that Spry proteins modulate other cellular signals and pathways, such as the phosphatidylinositol-specific phospholipase C-γ, which accounts for regulatory effects on calcium-mediated signaling and T cell proliferation (23). Notably, the effects of Spry proteins on growth factor-activated MAP kinase pathways appear to be limited to the MEK/Erk pathway (20–22), and there have been no reports on effects on other MAP kinase cascades.

In contrast to growth factors, IFNs are cytokines with important antiproliferative properties and tumor suppressive effects on malignant cells (1–3). In addition to the classical Jak-Stat pathways that regulate transcription of IFN-stimulated genes and their products (1, 3–8), IFNs activate several other cellular cascades, the functions of which complement the activities of Jak-Stat pathways in the generation of IFN responses. In recent studies, we demonstrated that activation of the Mnk/elf4E pathway plays important roles in the generation of IFN-induced biological effects (16, 17). As there is evidence in other systems that phosphorylation of Spry2 on Ser-112 and Ser-121 by Mnk kinase activity stabilizes the protein and antagonizes c-Cbl binding and polyubiquitination (24), we examined the effects of Type I IFN treatment on expression of different members of the Spry family of proteins. Our data established that IFN treatment leads to increased levels of Spry proteins, whereas such expression is defective in Mnk1/2 double knock-out MEFs, establishing that Mnk activity is required for the process. In experiments using Spry1/Spry2/Spry4 triple knock-out MEFs, we found that IFN-dependent phosphorylation/activation of the MEK/Erk pathway is augmented, in a manner similar to what others have reported previously for growth factor signaling (35). However, the p38 MAPK was also strongly enhanced in the absence of expression of these Spry proteins, establishing regulatory effects of Spry proteins on the p38 MAPK pathway. In parallel studies, we found that induction of ISG15 gene transcription and ISG15 protein expression are Spry-regulated and that targeted disruption of all three widely expressed Spry genes (Spry1, Spry2, and Spry4) results in enhanced ISG15 expression. Such effects do not reflect effects of Spry proteins on IFN-activated Jak-Stat pathways as IFN-dependent phosphorylation/activation of the MEK/Erk pathway is augmented, in a manner similar to what others have reported previously for growth factor signaling (35). However, the p38 MAPK was also strongly enhanced in the absence of expression of these Spry proteins, establishing regulatory effects of Spry proteins on the p38 MAPK pathway. In parallel studies, we found that induction of ISG15 gene transcription and ISG15 protein expression are Spry-regulated and that targeted disruption of all three widely expressed Spry genes (Spry1, Spry2, and Spry4) results in enhanced ISG15 expression. Such effects do not reflect effects of Spry proteins on IFN-activated Jak-Stat pathways as IFN-dependent phosphorylation/activation of the MEK/Erk pathway is augmented, in a manner similar to what others have reported previously for growth factor signaling (35). However, the p38 MAPK was also strongly enhanced in the absence of expression of these Spry proteins, establishing regulatory effects of Spry proteins on the p38 MAPK pathway. In parallel studies, we found that induction of ISG15 gene transcription and ISG15 protein expression are Spry-regulated and that targeted disruption of all three widely expressed Spry genes (Spry1, Spry2, and Spry4) results in enhanced ISG15 expression. Such effects do not reflect effects of Spry proteins on IFN-activated Jak-Stat pathways as IFN-dependent phosphorylation/activation of the MEK/Erk pathway is augmented, in a manner similar to what others have reported previously for growth factor signaling (35). However, the p38 MAPK was also strongly enhanced in the absence of expression of these Spry proteins, establishing regulatory effects of Spry proteins on the p38 MAPK pathway.
with enhanced p38 MAP kinase activity and are in agreement with previous work that has established that the p38 MAPK pathway plays an essential role in ISG transcription without modulating tyrosine or serine phosphorylation of Stat proteins (26, 27). Such Spry-dependent regulatory effects on the IFN-activated p38 MAPK pathway have important functional consequences as our data demonstrate that knockdown of Spry1, Spry2, or Spry4 potentiates the inhibitory effects of IFNα or IFNβ on U937-derived leukemic precursor or primary malignant erythroid hematopoietic progenitors from patients with polycythemia vera. It is of interest that although selective knock-out of distinct Spry proteins only partially enhances the suppressive effects of Type I IFNs on malignant hematopoiesis, different Spry proteins do not appear to compensate for each other in that context. It should also be noted that previous work demonstrated that the p38 MAP kinase pathway is essential for the generation of the suppressive effects of IFNα on normal (40) and leukemic hematopoietic progenitors (41), and recent work has shown that p38 MAPK is required for the inhibitory effects of IFNα on malignant erythroid progenitors from patients with polycythemia vera (42). Taken together with the findings of the current study, the data suggest that the enhancing effects of Spry knockdown on IFN-mediated suppression of malignant hematopoiesis may result from regulatory effects on the p38 MAPK pathway.

The precise protein target(s) of Spry proteins among elements of the IFN-activated p38 MAPK pathway remain to be determined. Putative candidates include various kinases in the p38 MAPK cascade, such as MAP kinase kinase (Mkk) 3, Mkk6, or Mkk4 (10, 11), or various upstream MAP kinase kinase kinases (MAPKKK) that are known to control Mkk/p38 MAPK cascades (10, 11). Alternatively, effects on early upstream G-proteins that regulate engagement of the IFN-activated pathway, such as Rac1 (27), may be involved, but this remains to be directly addressed in future studies. Independent of the precise mechanisms involved, the results of this study may prove to have important translational implications in the future use of IFNs in clinical medicine. IFNα/β is used in various clinical settings, such as the treatment of various viral infections, multiple sclerosis, and certain malignancies (4). Importantly, it has major clinical activity, and it is one of the most effective agents in the treatment of Philadelphia negative (Ph−) myeloproliferative neoplasms. In recent years, there has been a dramatic emergence of IFNα as an agent with major activity in the treatment of polycythemia vera and essential thrombocyto­sis (43–45). Nevertheless, not all patients respond or achieve long-term remissions, and efforts to further improve the clinical activity of IFNα should have a substantial impact in the management of patients with myeloproliferative neoplasms. Our studies suggest that Spry proteins are important negative feedback regulators of IFN responses and raise the potential of approaches to block their expression or inhibit their function to enhance and promote the antineoplastic effects of IFNs in vitro and in vivo.

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