

Expression and Regulatory Effects of Murine Schlafen (*Slfn*) Genes in Malignant Melanoma and Renal Cell Carcinoma^{*[5]}

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Background: The role of *Slfn* genes in antineoplastic responses is yet to be defined.

Results: Murine *Slfn* genes are up-regulated in an IFN-dependent manner in melanoma, and RCC cells and members of this family differentially suppress proliferation and anchorage-independent malignant cell growth.

Conclusion: *Slfns* play important roles in controlling malignant melanoma and RCC tumorigenesis.

Significance: Modulation of *Slfn* expression may provide a novel approach for the treatment of malignancies.

There is emerging evidence that the IFN-inducible family of *Slfn* genes and proteins play important roles in cell cycle progression and control of cellular proliferation, but the precise functional roles of different *Slfn* members in the regulation of tumorigenesis remain unclear. In the present study, we undertook a systematic analysis on the expression and functional relevance of different mouse *Slfn* genes in malignant melanoma and renal cell carcinoma cells. Our studies demonstrate that several mouse *Slfn* genes are up-regulated in response to IFN treatment of mouse melanoma and renal cell carcinoma cells, including *Slfn1*, *Slfn2*, *Slfn4*, *Slfn5*, and *Slfn8*. Our data show that *Slfn2* and *Slfn3* play essential roles in the control of mouse malignant melanoma cell proliferation and/or anchorage-independent growth, suggesting key and non-overlapping roles for these genes in the control of malignant melanoma tumorigenesis. In renal cell carcinoma cells, in addition to *Slfn2* and *Slfn3*, *Slfn5* also exhibits important antineoplastic effects. Altogether, our findings indicate important functions for distinct mouse *Slfn* genes in the control of tumorigenesis and provide evidence for differential involvement of distinct members of this gene family in controlling tumorigenesis. They also raise the potential of future therapeutic approaches involving modulation of expression of members of this family of genes in malignant melanoma and renal cell carcinoma.

The family of Schlafen (*Slfn*) genes was first described in mouse cells, in studies that demonstrated up-regulation of members of this family during thymocyte maturation and T cell development (1). At that time, it was shown that *Slfn* expression in thymoma cells and fibroblasts suppresses cell proliferation (1). It is now well established that members of the *Slfn* family are widely expressed in mammals (2). There are currently many

known *Slfn* genes in mice and humans (2). Although the precise function of the different *Slfn* genes and proteins remains to be established, there is emerging evidence that several members of the family are involved in development, immune response, and cell proliferation (2).

In mouse cells, *Slfns* are classified in three major groups based on their molecular masses. *Slfn1* and -2 belong to group I or short *Slfns*; *Slfn3* and -4 belong to group II or intermediate *Slfns*; and *Slfn5*, -8, -9, and -14 to group III or long *Slfns* (2, 3). All *Slfns* share a specific *slfn* box domain, localized adjacent to an AAA domain (3, 4). Groups II and III *Slfns* share an additional *Slfn*-specific domain defined by the sequence Ser-Trp-Ala-Asp-Leu, called the SWADL domain (3, 4). Finally, members of group III exhibit a C-terminal extension containing motif, homologous to the superfamily I of DNA/RNA helicases (5–7). Additionally, the C-terminal extensions also harbor a nuclear localization signal (RKRRR) further supporting the notion of nuclear function for the group of long *Slfns* (7).

The mouse members of the *Slfn* family that have been most extensively studied are *Slfn1*, *Slfn2*, and *Slfn3* (4, 8–15). *Slfn2* protein appears to play a role in maintaining quiescence in cells of the immune response, as mice harboring a *Slfn2* mutation (*elektra*) are more susceptible to both bacterial and viral infections, which is a consequence of T cells undergoing apoptosis via the intrinsic pathway (10). More evidence that *Slfn2* is involved in the immune response is the observation that this member of the *Slfn* family is up-regulated by LPS, in an NF- κ B- and AP-1-dependent manner (6). There is also recent evidence that *Slfn2* is an IFN-inducible gene and suppresses cyclin D1 expression, suggesting that it plays an important role in the control of cell cycle progression (9). Additionally, *Slfn2* has been shown to be induced by RANKL, a member of the TNF family of cytokines, and is involved in osteoclastogenesis through increasing c-Jun activation and NFATc1 expression (8). Regulation of cell cycle via control of cyclin D1 expression has been also described in the case of *Slfn1* (4). *Slfn3* has been mostly implicated in differentiation as it is involved in the control of differentiation of the intestinal epithelial cells as well as in T cell differentiation (13–15), whereas it has been postu-

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[5] This article contains supplemental Figs. S1 and S2.

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lated that *Slfn3* may provide a new marker for T cell activation (15). The only evidence for regulatory effects of *Slfn3* on cell growth stems from prior work, in which ectopic expression of this *Slfn* member in human colon cancer cells inhibited cellular growth (16). The function of *Slfn4* is not well known at this time, as there is only one report showing its involvement in macrophage activation and differentiation (17). Similarly, the functions of the remaining members of the *Slfn* family are largely unknown.

As there are no consistent homology patterns between mouse and human *Slfn* genes, it has been difficult to define the evolution of this gene family and its precise role in immune surveillance against cancer. In previous studies, we demonstrated that among human SLFNs, SLFN5 is selectively up-regulated in response to IFN treatment in malignant melanoma cells (18). In the present study, we undertook a systematic analysis on the IFN-inducible expression and biological relevance of different mouse *Slfn* genes in mouse malignant melanoma and renal cell carcinoma (RCC)² cells. Our results demonstrate that *Slfns* 1, 2, 4, 5, and 8 are induced by IFN α in malignant melanoma as well as in RCC cells. Knockdown of group I and II *Slfns* (*Slfn2*, *Slfn3*) results in enhanced cell proliferation and anchorage-independent malignant melanoma cell growth, suggesting important roles for these *Slfn* subgroups in the regulation tumorigenesis and the control of neoplastic cell growth. However, in renal cell carcinoma, a member of group III *Slfns*, *Slfn5*, also appears to regulate cell proliferation and anchorage-independent growth suggesting differential involvement and requirements for distinct *Slfn* proteins in controlling malignant cell proliferation in different cell types.

MATERIALS AND METHODS

Cell Lines, Reagents, and Antibodies—Mouse melanoma cells (B16-F1) were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics. The melanocytic cell line (L10BIOBR) was cultured in Ham's F-10 medium supplemented with 50 ng/ml phorbol 12-myristate 13-acetate with 7% horse serum and antibiotics. The Cloudman S91 melanoma cells (clone M3) were cultured in high-glucose Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, and antibiotics. The renal cell carcinoma cell line (RENCA) was maintained in RPMI 1640 supplemented with 10% fetal bovine serum, antibiotics, sodium pyruvate, nonessential amino acids, and L-glutamine. The murine kidney carcinoma cell line (CLS-CD3575) was grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, and antibiotics. A polyclonal antibody against *Slfn2* has been described previously (9). Antibodies to GAPDH were obtained from Millipore.

Cell Proliferation Assays—Cell counting was performed using a Scepter Handheld Automatic Cell Counter (version 2.0, EMD Millipore). Assays using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetra-zolium bromide method were performed after treating cells with IFN α for 5 to 7 days, as in our previous studies (9, 18).

RNAi-mediated Knockdown of Target Genes—Transient knockdown of *Slfn2*, *Slfn3*, and *Slfn5* was performed using a pool of three target-specific siRNAs for each *Slfn* as well as non-targeting control pool siRNA. Validated, inventoried siRNA pools were purchased from Santa Cruz Biotechnology and used according to the manufacturer's instructions. The siRNA used were as follows: *Slfn2*, sc-40925; *Slfn3*, sc-40926; *Slfn5*, sc-153590; and control siRNA, sc-44230. siRNA was introduced to the cells using Lipofectamine RNAiMAX according to the manufacturer's instructions (Invitrogen).

RNA Isolation and Real Time PCR—Cells were treated with 1×10^3 IU/ml IFN α , as indicated. Isolation of RNA and conversion into cDNA was performed using the respective kits from Qiagen, according to the manufacturer's instructions. Validated, inventoried probes and primers for real time PCR and Taqman PCR master mix were purchased from Applied Biosystems and used according to the manufacturer's instructions. The probes and primers used were as follows: GAPDH, Mm99999915_g1; *Slfn1*, Mm00488306_m1; *Slfn2*, Mm00488307_m1; *Slfn3*, Mm00488309_g1; *Slfn4*, Mm01298330_m1; *Slfn5*, Mm00806095_m1; *Slfn8*, Mm00824405_m1. GAPDH was used as an internal control.

Soft Agar Assays—Anchorage-independent cell growth was analyzed in soft agar assays as described previously (9, 18, 19). Equal number of cells were plated in soft agar and allowed to form colonies for 14 days, and colony formation was assessed.

Cell Lysis and Immunoblotting—Cell lysis in phosphorylation lysis buffer and immunoblotting using an ECL kit for protein detection were performed as described previously (20, 21).

Statistical Analysis—Results were expressed as means \pm S.E., with the number of experiments indicated in each figure. Statistical significance was assessed using paired *t* test analysis.

RESULTS

To determine the inducible patterns of expression of *Slfns* in response to type I IFN treatment of murine melanoma cells, we assessed the mRNA expression profile of different *Slfns* in B16-F1 cells treated with IFN α for different times. *Slfn1*, *Slfn2*, *Slfn4*, *Slfn5*, and *Slfn8* were all strongly IFN-inducible, whereas there was no significant induction of *Slfn3* (Fig. 1, A–F). The peak of induction for all inducible *Slfn* genes was seen at 6 h of treatment (Fig. 1). Notably, in the case of *Slfn2*, a biphasic pattern of induction was seen with a second peak noticeable at 24 h (Fig. 1A). The intensity of induction was similar for *Slfn2*, *Slfn4*, *Slfn8*, and *Slfn1* (Fig. 1, A, C, E, and F), whereas the induction of *Slfn5* was the highest (Fig. 1D). When the induction of different mouse *Slfns* in another murine melanoma cell line, clone M3, was studied, we found very high levels of expression of *Slfn5* (Fig. 2A). *Slfn1*, 2, and 4 transcripts were undetectable at base line, but *Slfn2* and 4 increased after IFN treatment (Fig. 2B). Similarly, when the immortalized murine melanocyte cell line L10BIOBR was studied, we found IFN-dependent up-regulation of *Slfn2*, 3, 4, and 8 (Fig. 2C). *Slfn1* and 5 transcripts were undetectable at base line in these cells, but the expression of both of them increased and was detectable after IFN treatment (Fig. 2D).

² The abbreviations used are: RCC, renal cell carcinoma; RENCA, renal cell carcinoma cell line.

Antineoplastic Effects of Mouse *Slfn* Genes

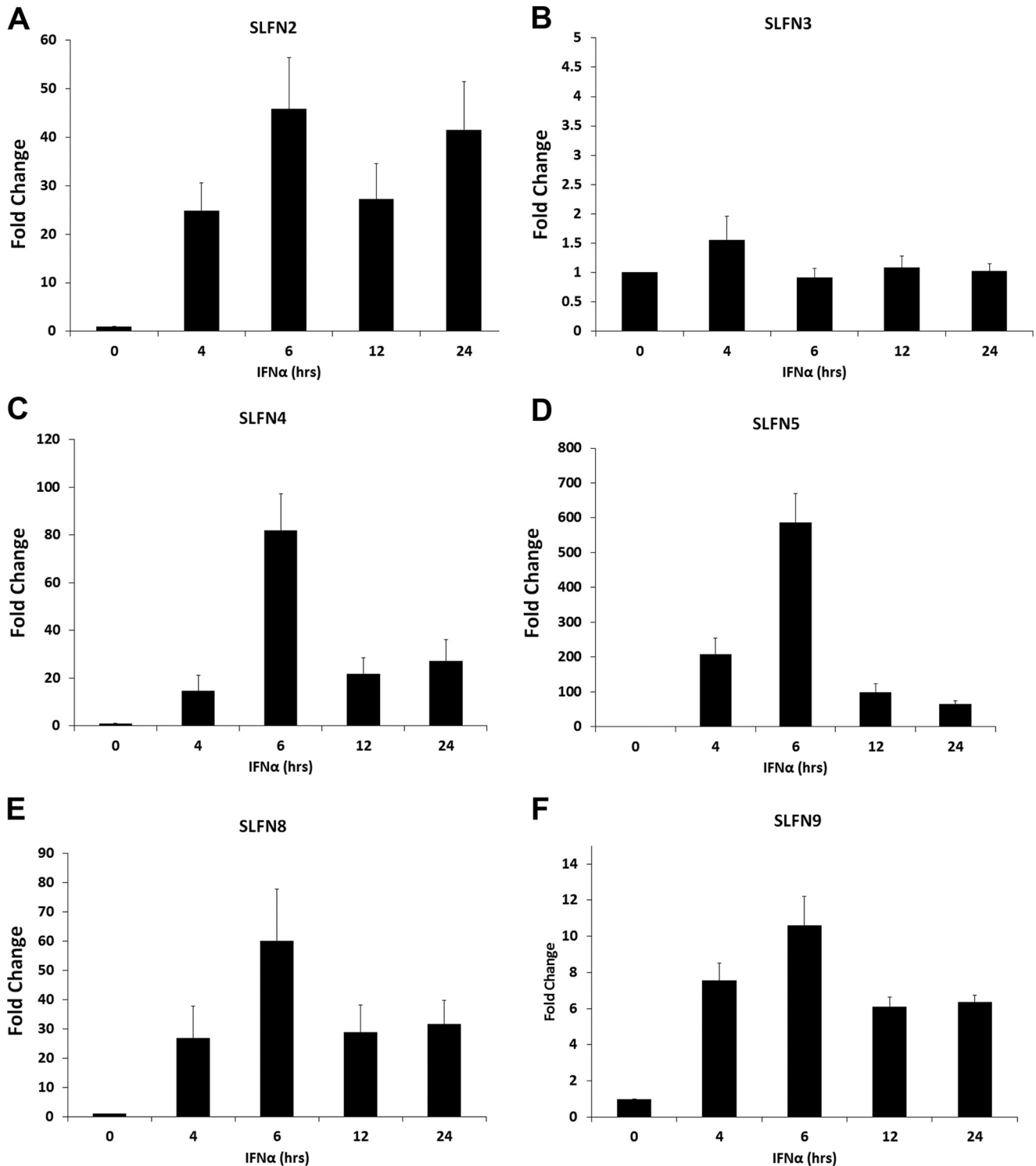


FIGURE 1. **Type I IFN-dependent expression of mouse *Slfn* genes in malignant melanoma cells.** B16-F1 cells were treated with IFN α for the indicated times. Expression of *Slfn2* (A), *Slfn3* (B), *Slfn4* (C), *Slfn5* (D), *Slfn8* (E), and *Slfn1* (F) was measured by quantitative real-time RT-PCR using specific primers and was normalized to GAPDH. Data are expressed as fold increase compared with untreated samples and represent means \pm S.E. of the indicated number of experiments: *Slfn2* ($n = 4$), *Slfn3* ($n = 4$), *Slfn4* ($n = 4$), *Slfn5* ($n = 4$), *Slfn8* ($n = 4$), and *Slfn1* ($n = 2$).

To determine the potential regulatory effects of different Slfns on proliferation and survival of malignant melanoma cells, we used specific siRNAs to knock down the expression of selected *Slfn* genes (supplemental Fig. S1) and assess responses in their presence or absence. Using siRNAs against specific sequences in distinct Slfns, selective targeting and specificity

was achievable to a large extent (supplemental Fig. S1), allowing evaluation of distinct Slfns in the generation of growth inhibitory responses in B16-F1 malignant melanoma cells. As shown in Fig. 3A, mouse melanoma cells exhibited higher proliferation rates when *Slfn2* and *Slfn3* were individually knocked down, whereas mouse *Slfn5* knockdown did not significantly affect

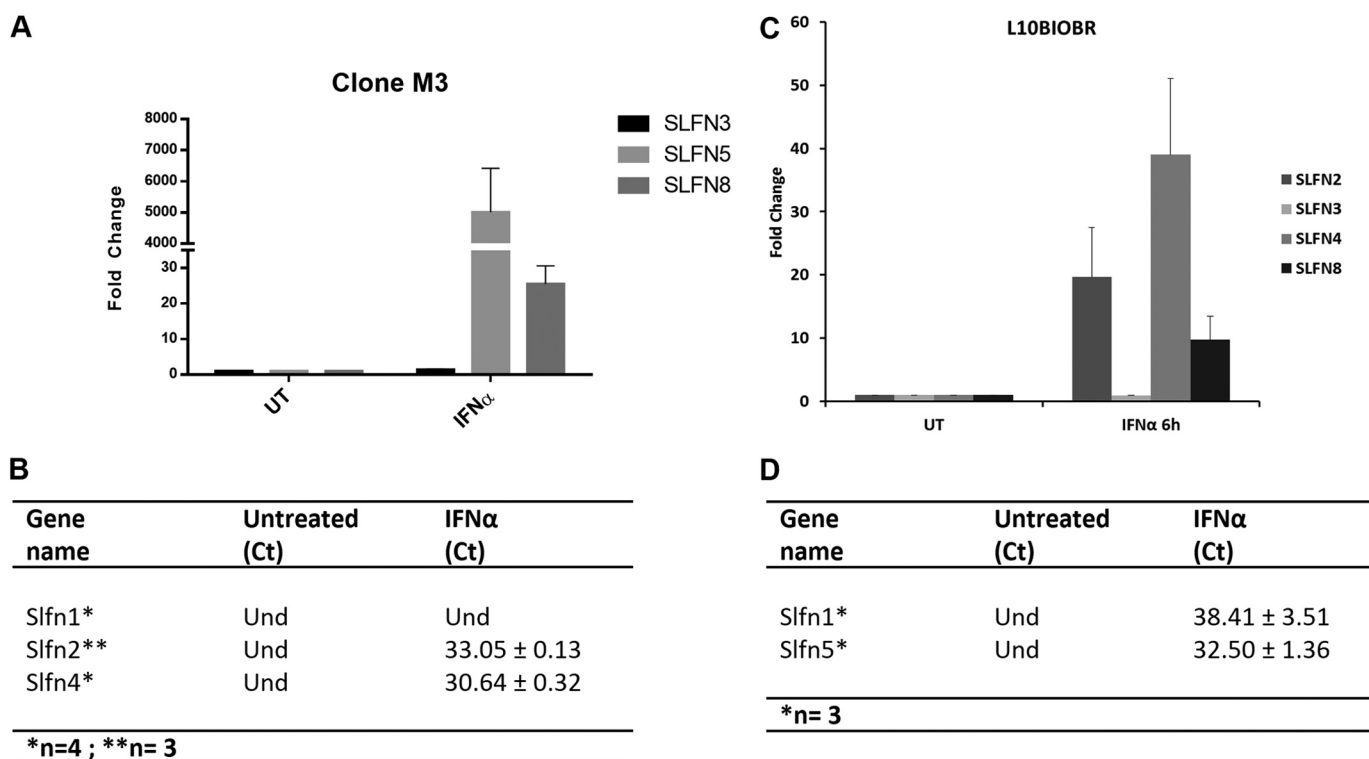


FIGURE 2. **Type I IFN-dependent expression of mouse *Slfn* genes in malignant melanoma and immortalized murine melanocyte cells.** Clone M3 and L10BIOBR cells were treated with IFN α for the indicated time. Expression of *Slfn3*, *Slfn5*, and *Slfn8* (A); *Slfn1*, *Slfn2*, and *Slfn4* (B) in clone M3; and the expression of *Slfn2*, *Slfn3*, *Slfn4*, and *Slfn8* (C); and *Slfn1* and *Slfn5* (D) in L10BIOBR were measured by quantitative real time RT-PCR using specific primers and were normalized to GAPDH. Data are expressed as fold increase compared with untreated (UT) samples and represent means \pm S.E. of the indicated number of experiments: *Slfn3*, *Slfn5*, *Slfn8*, *Slfn1*, and *Slfn4* (n = 4) and *Slfn2* (n = 3). Und, undetected.

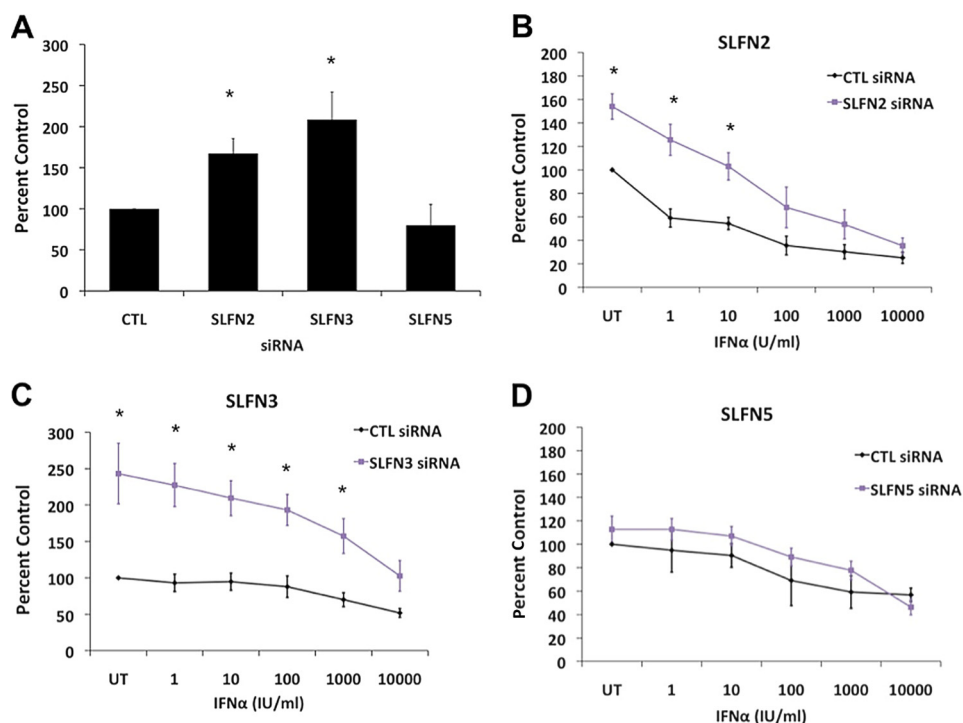


FIGURE 3. **Effect of knockdown of different mouse *Slfns* on cell proliferation of malignant melanoma cells.** A, B16-F1 cells were transfected with control siRNAs or siRNAs specific for *Slfn2*, *Slfn3*, or *Slfn5* as indicated. 48 h post-transfection, an equal number of cells was plated and allowed to proliferate for 72 h, at which time point the cells were counted. Data are expressed as % control siRNA samples and represent means \pm S.E. of four experiments. *, $p < 0.05$ versus control siRNA cells. B–D, B16-F1 cells transfected with siRNAs against the indicated *Slfn* genes were treated with indicated doses of IFN α , and proliferation was measured by MTT assays. Data are expressed as % untreated (UT) control (CTL) siRNA samples and represent means \pm S.E. of the several experiments. *, $p < 0.05$, *Slfn2* (n = 5), *Slfn3* (n = 5), and *Slfn5* (n = 3). Und, undetected.

Antineoplastic Effects of Mouse *Slfn* Genes

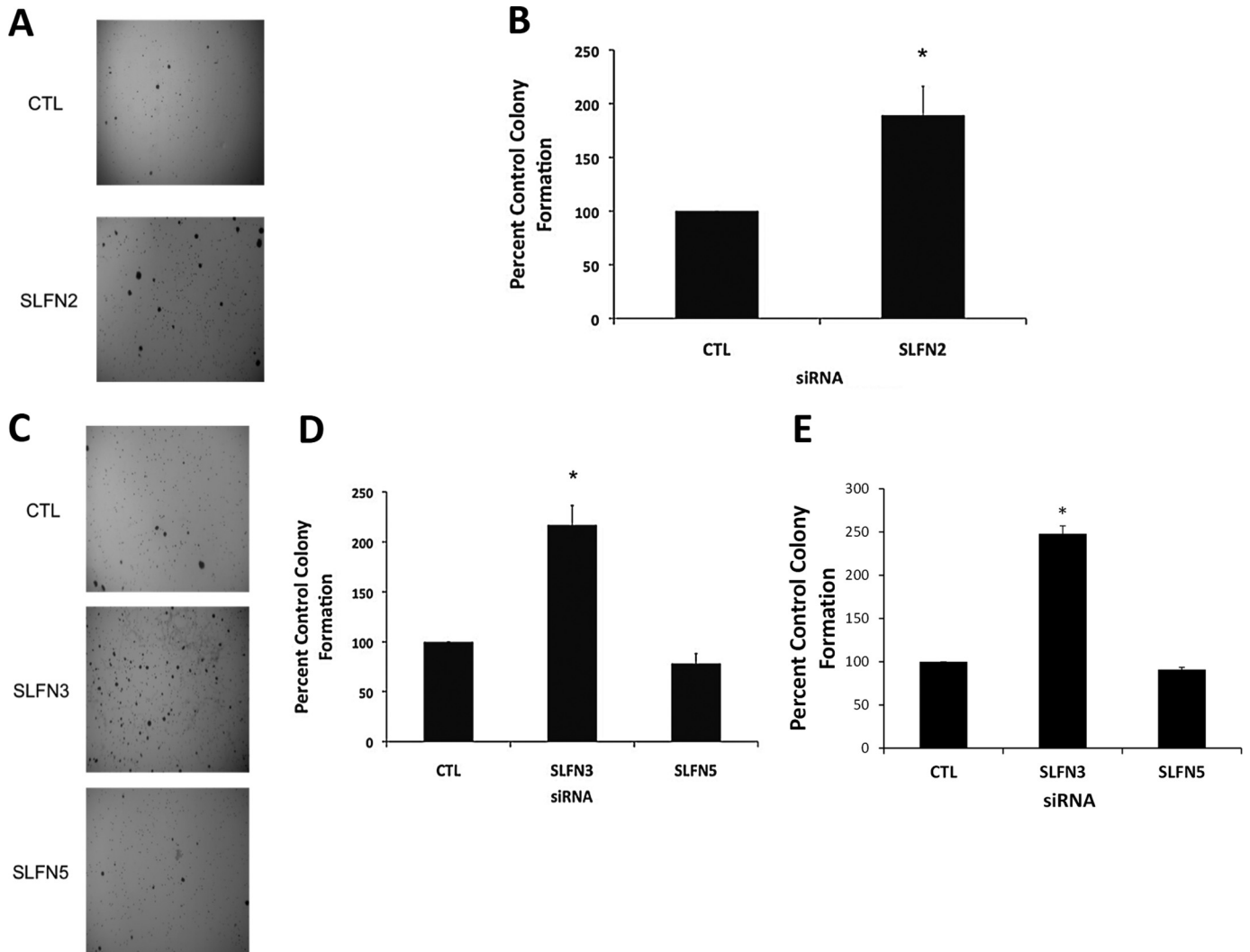


FIGURE 4. Effects of knockdown of different *Slfn* genes on anchorage-independent malignant melanoma cell growth. *A*, B16-F1 cells were transfected with control siRNA or *Slfn2* siRNA. Equal number of cells were plated in soft agar and allowed to form colonies for 14 days. Representative images of the soft agar wells are shown. *B*, quantitation of colonies from four independent experiments, including the one shown in *A*. Data are expressed as % control siRNA transfected cells and represent means \pm S.E. of four experiments. *, $p < 0.05$. *C*, B16-F1 cells were transfected with control siRNA or *Slfn3* or *Slfn5* siRNAs. An equal number of cells was plated in soft agar and allowed to form colonies for 14 days. Representative images of the soft agar wells are shown. *D*, quantitation of colonies from four independent experiments, including the one shown in *C*. Data are expressed as % control siRNA transfected cells and represent means \pm S.E. of four experiments. *, $p < 0.05$. *E*, clone M3 cells were transfected with control (CTL) siRNA or *Slfn3* or *Slfn5* siRNAs. An equal number of cells was plated in soft agar, and colonies from four independent experiments were quantified after 14 days. Data are expressed as % control siRNA-transfected cells and represent means \pm S.E. of four experiments. *, $p < 0.05$.

malignant melanoma cell proliferation (Fig. 3*A*). Similar results were obtained in studies in which cell viability/cell proliferation was assessed using MTT assays. Knockdown of *Slfn2* (Fig. 3*B*) or *Slfn3* (Fig. 3*C*) melanoma cells resulted in enhanced cellular proliferation. Also, cells in which either *Slfn2* or *Slfn3* were knocked down were less sensitive to IFN-induced anti-proliferative responses (Fig. 3, *B* and *C*). However, selective knockdown of mouse *Slfn5* (Fig. 3*D*) did not augment malignant melanoma cell growth and did not reverse the inhibitory effects of mouse IFN α (Fig. 3*D*).

To assess the potential regulatory effects of different mouse *Slfn* genes on anchorage-independent malignant melanoma cell growth, different *Slfn* genes were knocked down using specific siRNAs, and colony formation in soft agar was determined (9, 20). In cells in which either *Slfn2* or *Slfn3* were knocked down, there was marked increase in colony numbers, reflecting

anchorage-independent cell growth (Fig. 4, *A–D*), whereas *Slfn5* knockdown did not have significant effects (Fig. 4, *C* and *D*). Similarly, knockdown of *Slfn3* increased colony formation from clone M3 cells, whereas as in the case of B16-F1 cells, *Slfn5* knockdown had no significant effects on colony formation (Fig. 4*E*). Thus, members of the mouse *Slfn* family appear to play regulatory roles on anchorage-independent malignant melanoma cell growth, underscoring their relevance in malignant melanoma tumorigenesis.

Beyond malignant melanoma, one of the most IFN-responsive malignant tumors is renal cell carcinoma (22). To examine the patterns of expression of different mouse *Slfn*s in response to type I IFN treatment, RENCA cells were treated for different times, and *Slfn* mRNA expression was assessed by real-time RT-PCR. As shown in Fig. 5, all different mouse *Slfn* genes examined (*Slfn* 2, 3, 4, 5, 8, 1) were induced at variable levels in

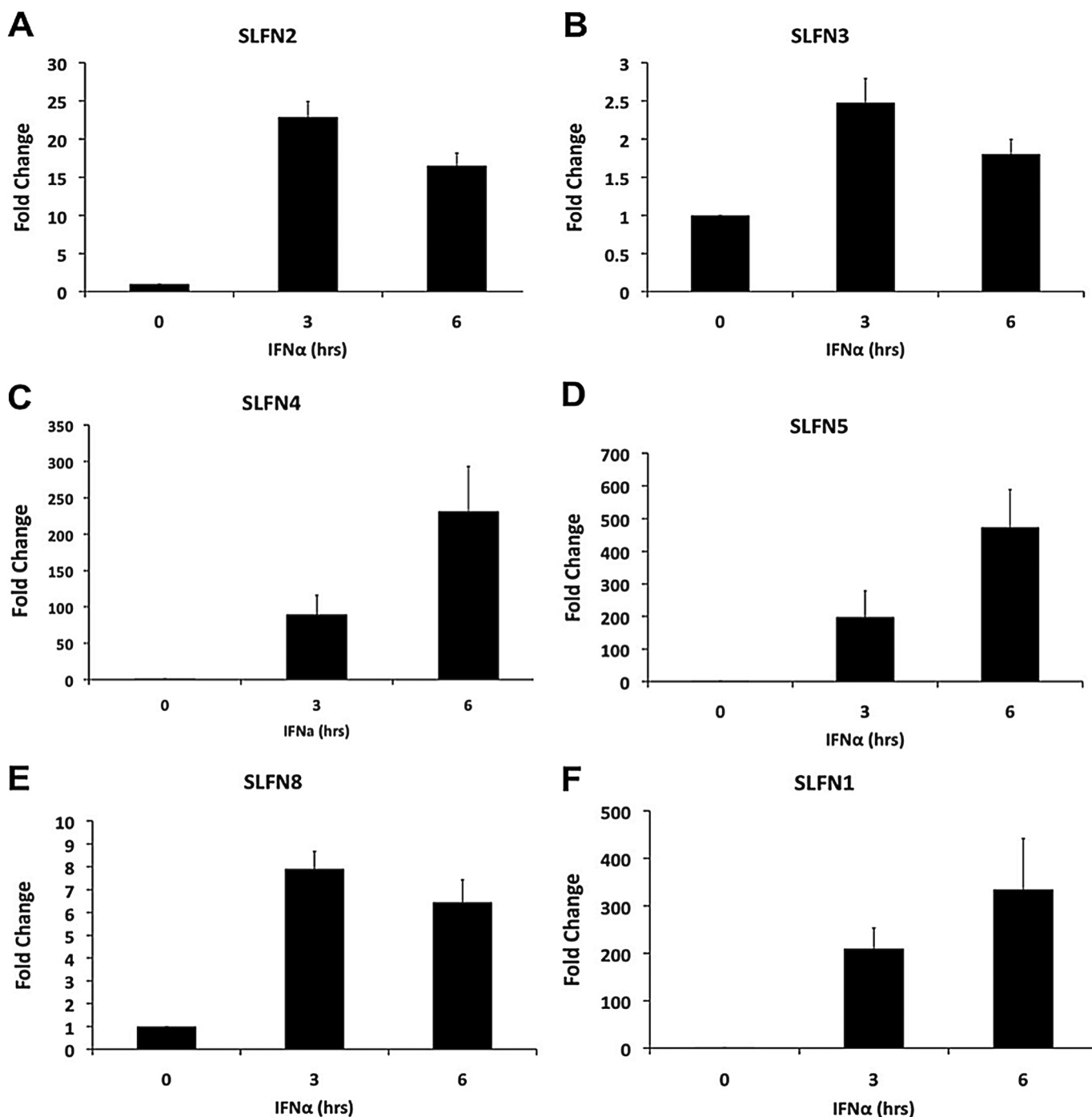


FIGURE 5. **Type I IFN-dependent expression of mouse *Slfn* genes in renal cell carcinoma.** A–E, RENCA cells were treated with IFN α for the indicated times. mRNA expression for *Slfn2* (A), *Slfn3* (B), *Slfn4* (C), *Slfn5* (D), *Slfn8* (E), and *Slfn1* (F) was measured by quantitative real-time RT-PCR using specific primers and was normalized to GAPDH. Data are expressed as fold increase over untreated samples. Data represent means \pm S.E. of several experiments for each *Slfn* gene as follows: *Slfn2* (n = 4), *Slfn3* (n = 4), *Slfn4* (n = 4), *Slfn5* (n = 3), *Slfn8* (n = 4), and *Slfn1* (n = 3).

mouse RENCA renal cell carcinoma cells (Fig. 5). The highest levels of IFN-induced gene expression were seen in the case of *Slfn5* (Fig. 5D), whereas *Slfn3* was the least inducible member of the family in these cells (Fig. 5B). Different degrees of IFN-dependent up-regulation of different *Slfns* were also seen in the mouse CD3575 kidney cell carcinoma cell line (Fig. 6). To examine whether different *Slfns* play regulatory roles on cell proliferation of RCC cells, we knocked down *Slfn2*, *Slfn3*, and *Slfn5* using specific siRNAs (supplemental Fig. S2). As in the

case of melanoma cells, knockdown of either *Slfn2* or *Slfn3* resulted in enhanced RCC cell proliferation (Fig. 7A). However, in contrast to what was observed in melanoma cells, knockdown of *Slfn5* increased the proliferation of RENCA cells (Fig. 7B). In addition, when the regulatory effects of distinct mouse *Slfns* on anchorage-independent growth of RCC cells were assessed, we found that knockdown of *Slfn2*, *Slfn3*, or *Slfn5* resulted in substantial enhancement of malignant cell colony formation in RENCA cells (Fig. 8, A and B). Similarly, knock-

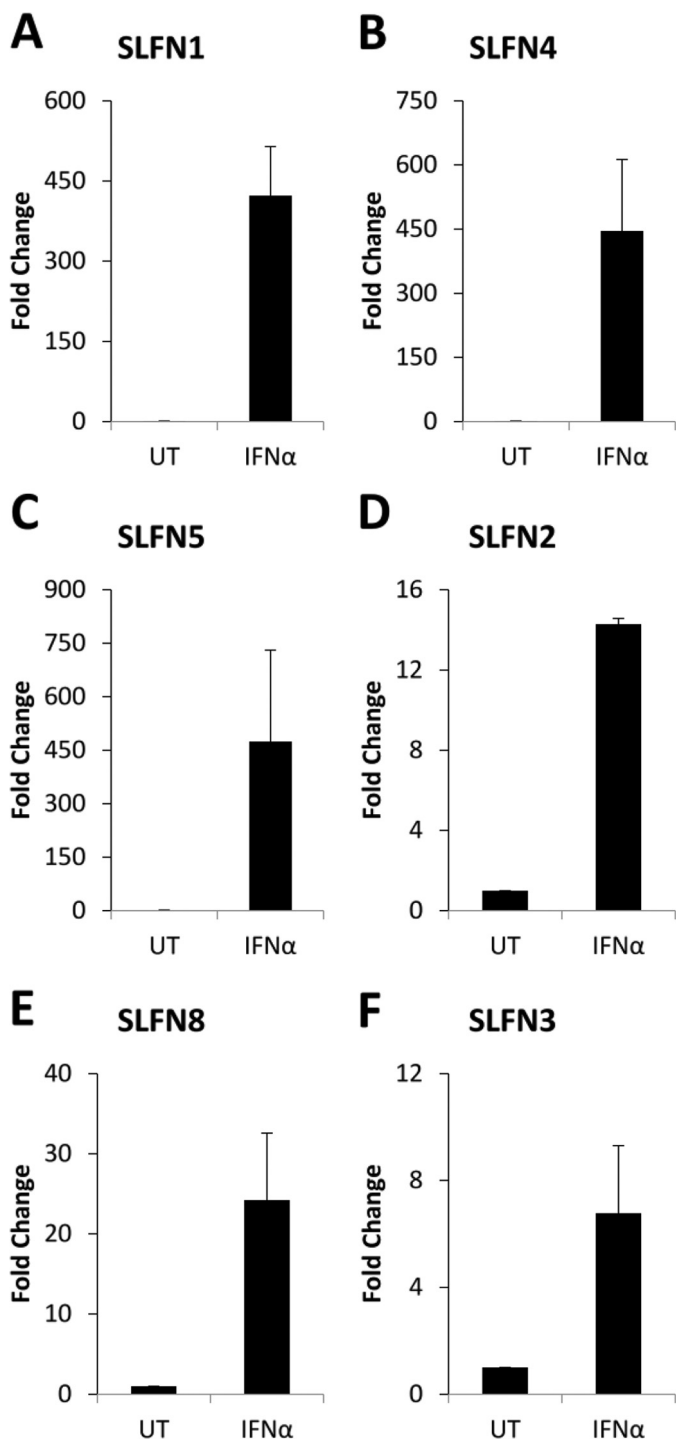


FIGURE 6. Type I IFN-dependent expression of mouse *Slfn* genes in kidney carcinoma cells. CD3575 cells were treated with mouse IFN α for the indicated time. Expression of *Slfn1* (A), *Slfn4* (B), *Slfn5* (C), *Slfn2* (D), *Slfn8* (E), and *Slfn3* (F) in CD3575 was measured by quantitative real-time PCR using specific primers and was normalized to GAPDH. Data are expressed as fold increase compared with untreated (UT) samples and represent means \pm S.E. of three independent experiments.

down of *Slfn3* and *Slfn5* resulted in significantly enhanced colony formation in CD3575 cells (Fig. 8C). We also performed studies in which we examined the effects of overexpression of *Slfn2* (Fig. 9, A and B) on anchorage-independent CD3575 malignant cell growth. *Slfn2* overexpression suppressed malignant cell colony formation (Fig. 9, C and D), further

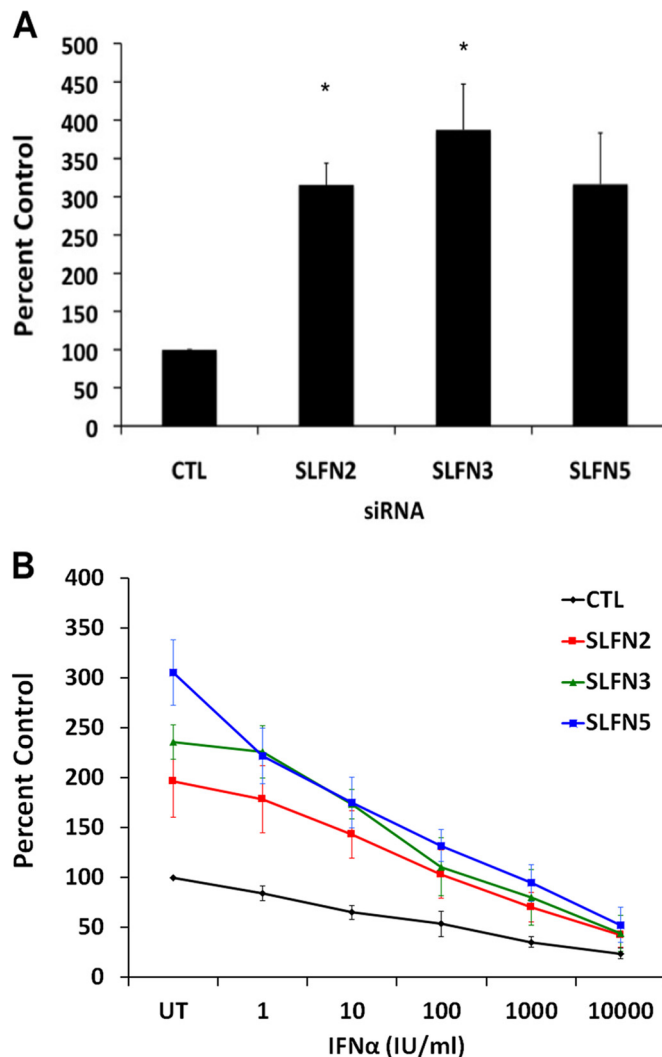


FIGURE 7. Effects of knockdown of different mouse *Slfn*s on cell proliferation of mouse renal cell carcinoma cells. A, RENCA cells were transfected with control siRNAs or siRNAs specific for *Slfn2*, *Slfn3*, or *Slfn5* as indicated. 48 h post-transfection, an equal number of cells was plated and allowed to proliferate for 72 h, at which time the cells were counted. Data are expressed as % of control (CTL) siRNA-transfected samples and represent means \pm S.E. of three experiments. *, $p < 0.05$ versus control siRNA cells. B, transfected RENCA cells were treated with indicated doses of IFN α , and viability was assessed by MTT assays. Data are expressed as % untreated control (CTL) siRNA samples and represent means \pm S.E. of five experiments. *, $p < 0.05$ control versus *Slfn2*, control versus *Slfn5*, **, $p < 0.05$ control versus *Slfn2*, control versus *Slfn3*, control versus *Slfn5*.

establishing a role for *Slfn*s on anchorage-independent malignant cell growth.

DISCUSSION

The *Slfn* family includes a large and diverse group of genes and proteins, with substantial variability and functional diversity among species. There are several murine *Slfn*s and human *Slfn*s, whereas there is only one *Slfn* in pigs (2). Virtually all mouse *Slfn*s do not have actual human homologues and *vice versa* (2), raising the question of whether a functional analogy between mouse and human *Slfn*s exists and/or whether the mouse and human *SLFN* systems play distinct and species-specific roles. Several mouse *Slfn* genes, including *Slfn1* (4), *Slfn2* (9), and *Slfn3* (13, 14, 16) have been shown to play key regula-

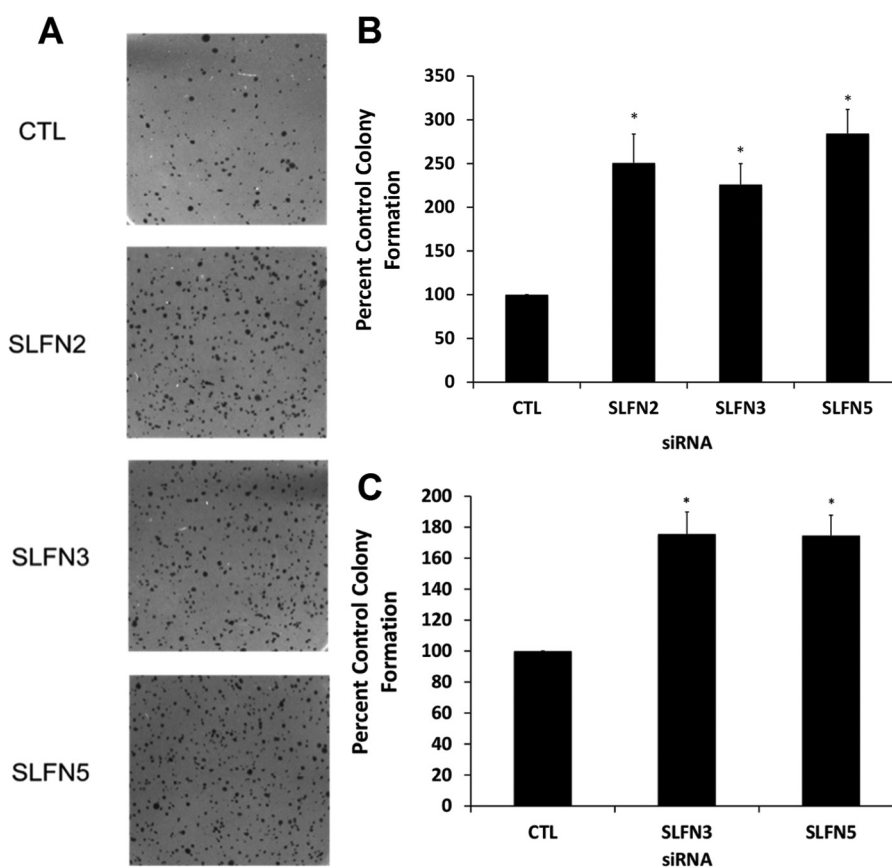


FIGURE 8. **Effects of knockdown of *Slfn2*, *Slfn3*, and *Slfn5* on anchorage-independent RCC cell growth.** A, RENCA cells were transfected with control siRNA or *Slfn2*, *Slfn3*, or *Slfn5* siRNAs. Equal number of cells were plated in soft agar and allowed to form colonies for 14 days. Representative images of the soft agar wells are shown. B, quantitation of colonies from four independent experiments, including the one shown in A. Data are expressed as % control siRNA-transfected cells and represent means \pm S.E. of four experiments. * $p < 0.05$. C, CD3575 cells were transfected with control siRNA or *Slfn3* or *Slfn5* siRNAs. Equal number of cells were plated in soft agar and colonies were quantified after 14 days. Data are expressed as % control (CTL) siRNA-transfected cells and represent means \pm S.E. of four experiments.

tory roles in control of cell proliferation and/or differentiation. Mouse *Slfn2* has been implicated in immune cell quiescence (10), whereas *Slfn4* has been implicated in the control of myelopoiesis (17).

Although there continues to be emerging evidence that different mouse *Slfn* genes play important regulatory functions during normal development, as regulators of the immune, hematopoietic, and other systems, very little is known on their potential involvement in tumorigenesis and whether abnormalities in their expression contribute to the pathophysiology of various malignancies. This may be of particular interest, as several *Slfn* genes appear to mediate growth inhibitory responses, and, therefore, deregulation of *Slfn* gene expression or other abnormalities in their function may contribute to the malignant phenotype. As our previous studies have demonstrated that *Slfn* genes are IFN-inducible (9, 18), we have examined the patterns of induction of different mouse *Slfn* genes and their functions, in malignant melanoma and renal cell carcinoma cells, diseases that exhibit relatively high sensitivity to IFN treatment as compared with several other malignancies (22–24).

Our studies demonstrate that there is IFN-dependent induction of expression of several murine *Slfn* genes in malignant melanoma and renal carcinoma cells, including *Slfn1*, *Slfn2*, *Slfn4*, *Slfn5*, and *Slfn8*. Importantly, selective knockdown of

group I and II *Slfns* (*Slfn2*, *Slfn3*) results in enhanced cell proliferation and anchorage-independent cell growth, raising the possibility that group I *Slfns* are regulators of growth of malignant melanoma cells. However, in renal cell carcinoma cells, *Slfns* from all three groups appear to regulate cell proliferation and anchorage-independent malignant cell growth, including *Slfn5*. Notably, our previous studies have shown that in the human system, only *SLFN5* is up-regulated by type I IFNs in malignant melanoma cells and that its inducible expression correlates with IFN responses (18). Human *SLFN5* contains an RNA/DNA helicase motif, as well as a nuclear localization signal, which is not seen in group I *Slfns* but only in group III *Slfns* in the mouse system (3, 25). Although mouse *Slfn5* is not a functional homologue of human *SLFN5*, the fact that murine *Slfn5* does not appear to play a key role in the control of proliferation of mouse malignant melanoma cells, further underscores the functional diversity and differences of function between murine *versus* human *SLFN* isoforms. It is of particular interest that selective knockdown of either *Slfn2* or *Slfn3* was sufficient to promote malignant melanoma cell proliferation, indicating that these mouse *Slfns* exhibit essential but distinct non-overlapping functions. Moreover, in the case of RCC cells, in addition to *Slfn2* and *Slfn3*, there was no compensatory mechanism for the suppressive effects of mouse *Slfn5*, indicat-

Antineoplastic Effects of Mouse *Slfn* Genes

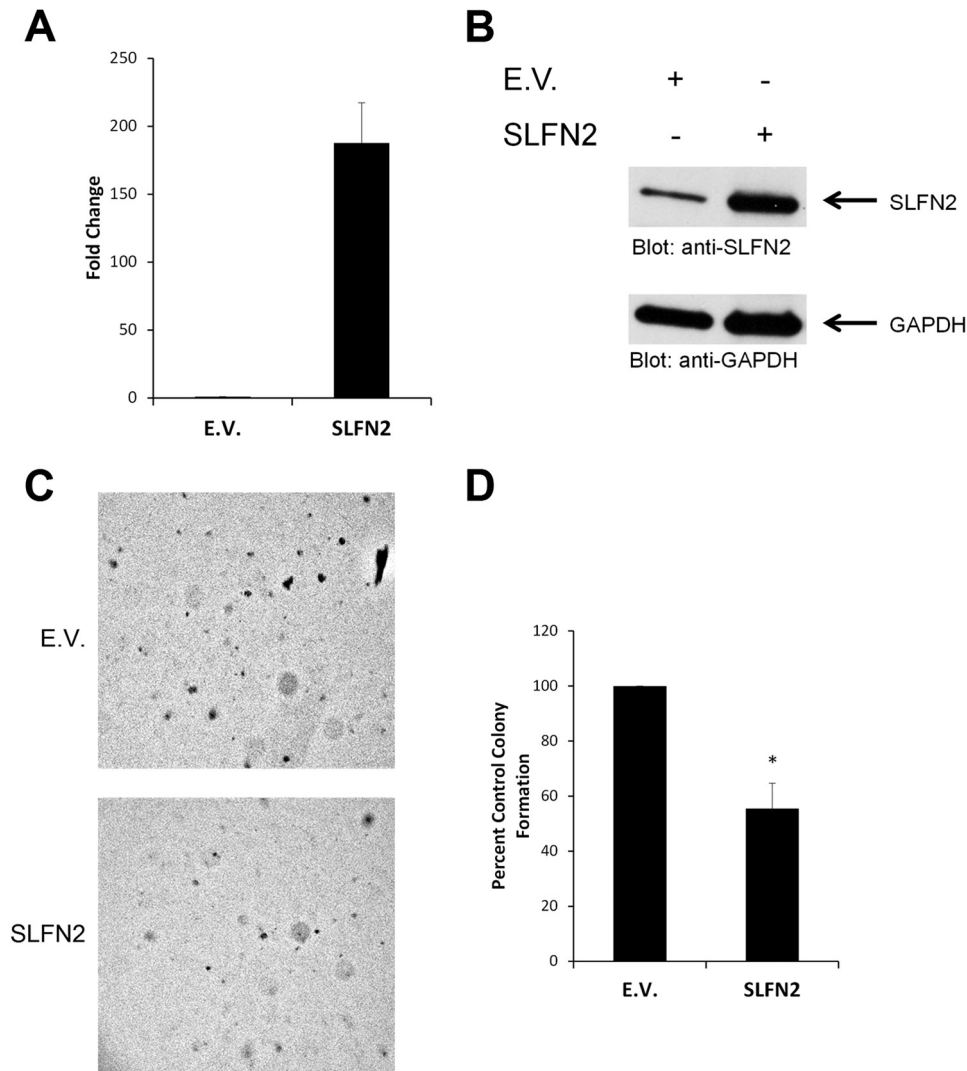


FIGURE 9. Effects of overexpression of mouse *Slfn2* on cell proliferation and anchorage-independent growth in kidney carcinoma cells. *A* and *B*, CD3575 cells were transiently transfected with either empty vector (*E.V.*) or *Slfn2* cDNA. Levels of *Slfn2* were quantified at 48 h post-transfection by quantitative RT-PCR (*A*) or examined by immunoblotting (*B*). The quantitative RT-PCR data are expressed as fold increase in the *Slfn2*-transfected samples over empty vector transfected samples normalized to GAPDH and represent means \pm S.E. from two independent experiments performed in triplicate. *C*, CD3575 cells were transiently transfected with either empty vector (*E.V.*) or *Slfn2* cDNA. An equal number of cells was plated in soft agar and allowed to form colonies for 8 days. Representative images of the soft agar wells are shown. *D*, quantitation of colonies from three independent experiments, including the one shown in *A*. Data are expressed as % control empty vector transfected cells and represent means \pm S.E. of three experiments performed in triplicate. *, $p < 0.05$.

ing an essential role for this member of the family of proteins in the suppression of RCC tumorigenesis.

The precise mechanisms accounting for such selective requirements for distinct Slfns in the control of tumorigenesis remain to be defined in future studies. It is possible that some of their common activities reflect effects on elements of cell cycle progression in malignant melanoma or renal cell carcinoma cells. It is also possible that, beyond their suppressive properties on anchorage-independent malignant cell growth, additional, yet unknown, functions for various murine Slfns may exist, which define their unique biological specificities. Beyond delineating such putative functions in future studies, it would be important to expand this work in other types of tumors, beyond malignant melanoma and RCC. For instance, it would be interesting to examine the role of Slfn family in the pathophysiology of various other type I IFN-sensitive solid tumors, such as carcinoids, as well as hematological malignancies such as chronic

myeloproliferative neoplasms and cutaneous T cell lymphoma (26–28). Nevertheless, independently of the precise mechanisms accounting for tissue specificity for different Slfns, identification, or design of pharmaceutical agents that may promote expression of distinct Slfns may ultimately provide a novel therapeutic approach for the treatment of such malignancies.

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