Decitabine Up-regulates S100A2 Expression and Synergizes with IFN-γ to Kill Uveal Melanoma Cells

Jared A. Gollob1,2 and Catherine J. Sciambi1,2

Abstract

Purpose: Metastatic uveal melanoma is resistant to conventional chemotherapy and immunotherapy. In this study, we investigated the responsiveness of uveal melanoma cell lines to IFNs and the hypomethylating agent decitabine.

Experimental Design: The uveal melanoma cell lines 92-1, UW-1, OCM-1, and MKT-BR were exposed to varying concentrations of IFN-α, IFN-γ, and decitabine, alone and in combination. The effects of decitabine on gene expression were examined using DNA microarray analysis.

Results: We found that IFN-γ and decitabine induced cell death in uveal melanoma. Whereas a high concentration of IFN-γ (1,000 units/mL) was required to induce cell death, we observed a dose-related increase in cell death when decitabine was used at a range of 0.1 to 10 μmol/L. Strikingly, 1 μmol/L decitabine synergized with 10 to 1,000 units/mL IFN-γ to induce massive cell death. In contrast, decitabine had no effect on three cutaneous melanoma cell lines and exhibited no synergy with either IFN. In uveal melanoma, decitabine up-regulated the expression of genes involved in growth control and apoptosis and down-regulated genes that have been implicated in the malignant phenotype of cutaneous melanoma. The gene up-regulated to the greatest degree by decitabine and whose expression showed a dose-effect across the three concentrations of decitabine was S100A2, a putative tumor suppressor. The genes modulated by decitabine in uveal melanoma were largely unaffected in cutaneous melanoma.

Conclusions: These findings form a basis for testing the decitabine/IFN-γ combination in metastatic uveal melanoma and for exploring the role of S100A2 in the susceptibility of uveal melanoma to IFN-mediated cell death.

Metastatic uveal melanoma is an aggressive malignancy with a median survival of <6 months (1). Patients with locally advanced primary lesions also have a poor prognosis because up to 50% will subsequently develop disseminated metastases within 10 years (2). Although hepatic metastases can respond partially to intra-arterial chemotherapy or chemoembolization, metastatic uveal melanoma is largely resistant to systemic chemotherapy (3). Immunomodulatory drugs used to treat advanced cutaneous melanoma, including interleukin 2 (IL-2) and IFN-α, have also had very limited activity in uveal melanoma (4).

The mechanism underlying the antitumor effect of IFN-α or IL-2 in select patients with advanced cutaneous melanoma remains poorly defined. The association of good outcome with the development of autoimmune breakthrough events (5, 6) suggests that overcoming tolerance to self-antigens plays a role in cytokine-mediated antitumor immunity. However, IFN-α and IFN-γ can also exert direct antiproliferative and/or pro-apoptotic effects on cutaneous melanoma cell lines (7, 8), illustrating how certain cytokines can have antitumor effects that are independent of the induction of antigen-specific cellular or humoral immunity.

The epigenetic suppression of gene expression through DNA methylation has been linked to the relative resistance of a cutaneous melanoma cell line to the direct proapoptotic effect of type I IFNs (9). In that model, the hypomethylating agent decitabine (5-aza-2'-deoxycytidine) induced the re-expression of the apoptosis-associated IFN response gene XAF1. Decitabine has also been shown to augment the expression of other proapoptotic genes in cutaneous melanoma, including Apaf-1 (10) and RASSF1A (11).

There is very little known about the direct effects of IFNs on uveal melanoma. Several papers have shown that type I and type II IFNs can have variable effects on cell proliferation and integrin/adhesion molecule expression in uveal melanoma (12, 13). Like cutaneous melanoma, the biology of uveal melanoma can also be affected through decitabine-induced gene re-expression. For example, p16INK4a repression through promoter methylation has been associated with worse outcome in uveal melanoma, and re-expression of p16INK4a by decitabine resulted in growth inhibition (14).

In this report, we have further evaluated the effect of IFNs and decitabine on uveal melanoma cell lines and show for the
first time that IFN-γ and decitabine synergize to induce cell death in uveal melanoma. Through DNA microarray analysis, we further show that decitabine has a striking effect on the expression of genes implicated in apoptosis and cell growth, including the tumor suppressor gene S100A2, whose up-regulation may underlie the synergy between decitabine and IFN-γ.

Materials and Methods

**Cell culture.** Human uveal melanoma cell lines 92-1, UW-1, OCM-1, and MKT-BR were provided by Dr. Prithvi Nuthuynjaya (Duke University Eye Center, Durham, NC). Human cutaneous melanoma cell lines DM6 and DM93 were provided by Drs. James Grichnik and Hilliard Seigler (Duke University Medical Center, Durham, NC) and 501mel by Dr. David Fisher (Dana-Farber Cancer Institute, Boston, MA). Uveal melanoma cell lines were grown in RPMI supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin. 501mel cells were grown in F-10 medium supplemented with 10% heat-inactivated FBS and penicillin/streptomycin. The other cell lines were grown in MEM supplemented with 5% FBS and penicillin/streptomycin. Cultures were maintained at 37°C with 5% CO₂, and adherent cells were harvested from plastic plates using trypsin/EDTA (Invitrogen). When cells were cultured with decitabine (5-aza-2'-deoxycytidine; Sigma), fresh decitabine in DMSO (Sigma) was added to cultures every day, and cells were split every 48 h. When cells were exposed to decitabine and either IFN-γ or IFN-α2a (R&D Systems), the IFN was added to the culture on the day the cells were split after a prior 48-h incubation with decitabine. For cells exposed to IFN-γ or IFN-α alone, the IFN was added to the culture on the day the cells were split after a prior 48-h incubation with DMSO.

**Cell cycle analysis.** Adherent and suspension cells were harvested and combined, and cells were fixed with 3 mL absolute ethanol for 1 h at 4°C. The cells were then stained with a 50 μg/mL propidium iodide (BD Biosciences) solution containing 10 μg/mL RNase A (Roche Applied Science) for 3 h at 4°C. The cells were analyzed by flow cytometry using the CellQuest program.

**Arrays.** Arrays were printed at the Duke Microarray Facility using the Genomics Solutions OmniGrid 100 Arrayer. The arrays contain the Operon Human Genome Oligo Set version 4.0 (Operon) that possess 35,354 optimized 70-mers, representing 35,354 genes.

**Probe preparation and microarray hybridization.** Total RNA (10 μg) from each cell line and the reference (Universal Human Reference RNA, Stratagene) was hybridized to oligo-dT primers at 65°C and then incubated at 42°C for 2 h in the presence of reverse transcriptase, Cy5 or Cy3-dUTP and Cy5 or Cy3-dCTP, and a deoxynucleotide mix. NaOH was used to destroy residual RNA. Cell line and reference cDNA were pooled and mixed with 1× hybridization buffer (50% formamide, 5× SSC, and 0.1% SDS), COT-1 DNA, and poly-dA to limit non-specific binding and heated to 95°C for 2 min. This mixture was pipetted onto a microarray slide, coverslipped, and hybridized overnight at 42°C. The array was then washed at increasing stringencies and scanned on a GenePix 4400B microarray scanner (Axon Instruments). 3

**Data processing and statistical analysis.** GeneSpring 6.1 program (Agilent Technologies, Silicon Genetics) was used to perform data analysis. Intensity-dependent (Lowess) normalization was done on the entire dataset. Based on triplicates of each condition, a threshold of 2-fold increase or decrease in expression relative to the control and a two-way ANOVA with a P value cutoff of 0.05 were done. The expression of each gene was reported as the ratio of the value obtained for each condition relative to control conditions after normalization of the data. 4

**Western blotting.** Following indicated treatment, whole cell extracts were prepared in lysis buffer [20 mmol/L Tris-HCl (pH, 8.0), 1% Igepal/NP40, 10 mmol/L EDTA, 137 mmol/L NaCl, 100 mmol/L NaF, complete protease inhibitor mixture (Roche Molecular Biochemicals),

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4 All raw data files and gene lists are found at http://data.cgt.duke.edu/Gollob3.php.
and complete phosphatase inhibitor cocktail (Sigma)]. Protein in the lysates was quantitated, and equal amounts were subjected to SDS-PAGE and immunoblotting. Blots were incubated with the indicated primary antibody and then incubated with horseradish peroxidase-conjugated goat anti-mouse, goat anti-rabbit, or rabbit anti-goat IgG antibody for 1 h at room temperature. Blots were then developed using the enhanced chemiluminescence system (Pierce). Primary antibodies used included antibodies to S100A2 (the kind gift of Professor C.W. Heizmann, Zurich, Switzerland), Tubulin (NeoMarkers), IL-24/mda-7 (R&D Systems), p15 INK4B, and phosphoinositide-3-kinase (PI3K) p110 γ (Cell Signaling, Technology).

**Results**

Decitabine synergizes with IFN-γ and IFN-α to induce cell death in uveal melanoma cell lines. We have previously shown that cell lines derived from human cutaneous melanoma vary in their ability to undergo apoptosis in response to relatively high concentrations (1,000 units/mL) of IFN-γ or IFN-α (8). To determine the effect of IFNs on uveal melanoma, we exposed four different uveal melanoma cell lines (92-1, UW-1, OCM-1, and MMT-BR) to IFN-γ and IFN-α. After 48 h, none of the cell lines exhibited any morphologic changes or alteration in viability in response to 1,000 units/mL IFN-α. Although concentrations of IFN-γ of 100 units/mL or less also had no effect, 1,000 units/mL IFN-γ induced morphologic changes (characterized by enlargement of cells, rounding, and loss of dendrites) in all four cell lines as well as variable degrees of cell death within 48 h of exposure. Figure 1 illustrates the variable effect of 1,000 units/mL IFN-γ on cell viability, showing that it induced a greater than 3-fold increase in cell death over control in 92-1 compared with only a slight increase in cell death in UW-1.

We next examined the effect of decitabine on the uveal melanoma cell lines. Testing decitabine concentrations of 0.1, 1, and 10 μmol/L, we observed that all three concentrations induced the same morphologic effects on all cell lines that were observed with 1,000 units/mL IFN-γ. In addition, after 96 h of exposure, decitabine induced cell death in all cell lines (Fig. 2). The degree of cell death depended on the decitabine concentration (Fig. 3): 1.5- to 2-fold increase in cell death with 0.1 μmol/L, 3- to 4-fold increase with 1 μmol/L, and 5- to 6-fold increase with 10 μmol/L. To determine whether decitabine-induced cell death could be augmented through the addition of IFNs, 92-1 and UW-1 were incubated with 1 μmol/L decitabine together with concentrations of IFN-α and IFN-γ (1,000 and 100 units/mL, respectively), which, by themselves, did not induce cell death. Although IFN-α did augment decitabine-induced cell death in both cell lines, the strongest synergy was seen between decitabine and IFN-γ (Fig. 2). Following the addition of IFN-γ to cells preincubated with decitabine for 48 h, more than a third of cells were dead within 48 h (Fig. 2), and the vast majority of cells were dead by 96 h.

Combining 1,000 units/mL IFN-γ with 1 μmol/L decitabine had no greater effect on cell death than 100 units/mL IFN-γ. Strikingly, as little as 10 units/mL IFN-γ synergized with 1 μmol/L decitabine to induce cell death (Fig. 3A), and synergy was still observed when 0.1 μmol/L decitabine was combined with 10 units/mL IFN-γ (Fig. 3B).

![Fig. 2. IFN-γ and IFN-α synergize with decitabine to augment cell death in uveal melanoma. The 92-1 (A) and UW-1 (B) cell lines were incubated for 48 h in medium with either DMSO or 1 μmol/L decitabine (DAC), following which cells were split and incubated for an additional 48 h with either DMSO, decitabine, or decitabine in combination with the indicated concentrations of IFN-γ or IFN-α. The number embedded in each panel above the sub-G1 region of the histogram shows the percentage of dead cells.](image-url)
In contrast to this effect of decitabine on uveal melanoma cells, decitabine had no effect whatsoever on the morphology, growth, or viability of three cutaneous melanoma cell lines (DM6, DM93, and 501mel). In addition, decitabine did not render any of these cell lines more susceptible to killing by IFN-α or IFN-γ (data not shown).

Decitabine up-regulates the expression of multiple genes involved in apoptosis and growth inhibition in uveal melanoma but not in cutaneous melanoma. To better understand the mechanism by which decitabine was augmenting the sensitivity of uveal melanoma cells to killing by IFNs, we used DNA microarray analysis to assay changes in gene expression in 92-1 induced by decitabine concentrations ranging from 0.1 to 10 μmol/L. Setting the threshold at genes up-regulated or down-regulated 2-fold or greater, we discovered that 599 genes were affected by 0.1 μmol/L decitabine, 1,414 genes were affected by 1 μmol/L, and 2,155 genes were affected by 10 μmol/L. A total of 396 genes were affected by all three decitabine concentrations, 741 genes were common to just 1 and 10 μmol/L, and 66 genes were common to just 0.1 and 1 μmol/L.

Among these genes affected by decitabine, we further focused our search to genes involved in apoptosis, inhibition of cell growth, IFN response, or other aspects of melanoma biology. Through this approach, we discovered 18 genes of interest up-regulated by decitabine (Table 1) and 6 genes down-regulated by decitabine (Table 2). Among the 18 up-regulated genes, the two that showed the strongest up-regulation and have been previously implicated in melanoma growth inhibition and apoptosis were S100A2 (15) and mda-7 (IL-24; ref. 16). S100A2 showed the greatest dose-dependent change in response to decitabine because its expression increased 27-, 79-, and 128-fold over the baseline with decitabine concentrations of 0.1, 1, and 10 μmol/L, respectively (Table 1). Two other S100 family members (S100D and S100E) whose functions are unknown were also up-regulated to a lesser extent by decitabine, but did not show the same dose-dependent change as S100A2 within the 1 to 10 μmol/L range. Mda-7 was also significantly up-regulated by decitabine at 1 μmol/L (although not at 0.1 μmol/L), but this up-regulation did not further increase at 10 μmol/L. The expression of four genes with the potential to modulate the IFN response [IRF7A, IRF-1, mitogen-activated protein/extracellular signal-regulated kinase (ERK) kinase (MEK) 1, and PI3K] was only modestly increased by decitabine, predominantly at higher concentrations, as was the expression of two genes involved in cell cycle control (p15 INK4B and p19 INK4D; Table 1). Among the six genes down-regulated by decitabine, three of these genes (CAPN3-p94, SLUG, and MITF) have been implicated in the malignant phenotype of cutaneous melanoma (17–19).

Because decitabine had no effect on the cutaneous melanoma cell lines examined, either alone or in conjunction with the IFNs, we examined whether any of the genes of interest whose expression was altered by decitabine in 92-1 were also similarly modulated in DM6 and 501mel. In DM6, the expression of only 112 genes changed 2-fold or more in response to 1 μmol/L decitabine (data not shown), and these did not include any of the genes in Tables 1 and 2. In 501mel, the expression of 1,757 genes was affected by 1 μmol/L decitabine, including six genes from Table 1 (S100A2, S100E,
Decitabine Synergizes with IFN-γ to Kill Uveal Melanoma

This is the first report to show that decitabine induces cell death in uveal melanoma and sensitizes uveal melanoma to killing by IFN-γ and, to a lesser extent, IFN-α. The morphologic changes induced in uveal melanoma by IFN-γ and decitabine, the increase in the sub-G1 peak seen by flow cytometry following propidium iodide staining, and prior data showing that the IFN-γ–induced increase in the sub-G1 peak in a cutaneous melanoma cell line is associated with an increase in cell staining by terminal nucleotidyl transferase–mediated nick end labeling assay (8) suggest that the cell death induced by decitabine and IFN-γ in uveal melanoma may be due to apoptosis rather than necrosis. Our finding that synergy occurred with as little as 10 to 100 units/mL IFN-γ is important because these concentrations are achievable in patients with acceptable toxicity. For example, in healthy male subjects, a 100 µg/m² dose of IFN-γ-1b (Actimmune) resulted in peak plasma concentrations of ~20 units/mL (0.6 ng/mL) and 50 units/mL (1.5 ng/mL) when administered by s.c. and i.m. injection, respectively (data reported in Actimmune package insert, put out by InterMune, Inc.). Because low doses of decitabine have been shown to induce DNA hypomethylation and changes in gene expression in vivo with minimal toxicity aside from reversible neutropenia (20), it seems that combination therapy with decitabine and IFN-γ could be a viable strategy for patients with uveal melanoma. It is interesting to note that a patient with metastatic uveal melanoma treated on a phase I clinical trial with low-dose decitabine plus high-dose IL-2 had a minor response (29% tumor regression of multiple lung masses) associated with vitiligo (20), raising the possibility that IL-2–induced IFN-γ might have played a role in the response in conjunction with the decitabine.

The primary effect of decitabine and the combination of decitabine and IFN in uveal melanoma was the induction of cell death. Although two genes with the potential to control cell cycling (p15 and p19) were up-regulated by decitabine, p15 protein expression could not be detected by Western blot. Although the inability to detect up-regulation of p15, mda-7, or PI3K p110 γ does not rule out low-level protein up-regulation that could not be detected with the available antibodies, it nonetheless brings into question the functional significance of some of the gene expression changes detected by DNA microarray in this study. It is of interest that six genes with known proapoptotic/growth inhibitory function were up-regulated by decitabine; however, it was S100A2 that exhibited

### Discussion

This is the first report to show that decitabine induces cell death in uveal melanoma and sensitizes uveal melanoma to killing by IFN-γ and, to a lesser extent, IFN-α. The morphologic changes induced in uveal melanoma by IFN-γ and decitabine, the increase in the sub-G1 peak seen by flow cytometry following propidium iodide staining, and prior data showing that the IFN-γ–induced increase in the sub-G1 peak in a cutaneous melanoma cell line is associated with an increase in cell staining by terminal nucleotidyl transferase–mediated nick end labeling assay (8) suggest that the cell death induced by decitabine and IFN-γ in uveal melanoma may be due to apoptosis rather than necrosis. Our finding that synergy occurred with as little as 10 to 100 units/mL IFN-γ is important because these concentrations are achievable in patients with acceptable toxicity. For example, in healthy male subjects, a 100 µg/m² dose of IFN-γ-1b (Actimmune) resulted in peak plasma concentrations of ~20 units/mL (0.6 ng/mL) and 50 units/mL (1.5 ng/mL) when administered by s.c. and i.m. injection, respectively (data reported in Actimmune package insert, put out by InterMune, Inc.). Because low doses of decitabine have been shown to induce DNA hypomethylation and changes in gene expression in vivo with minimal toxicity aside from reversible neutropenia (20), it seems that combination therapy with decitabine and IFN-γ could be a viable strategy for patients with uveal melanoma. It is interesting to note that a patient with metastatic uveal melanoma treated on a phase I clinical trial with low-dose decitabine plus high-dose IL-2 had a minor response (29% tumor regression of multiple lung masses) associated with vitiligo (20), raising the possibility that IL-2–induced IFN-γ might have played a role in the response in conjunction with the decitabine.

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### Table 1. Genes up-regulated ≥2-fold by decitabine in 92-1 cells

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*<2-fold change.

<sup>[1]</sup> Reduced by 2-fold.
the most dramatic up-regulation. Although the proapoptotic mda-7 (IL-24) gene was also up-regulated, albeit to a lesser extent than S100A2, we were unable to detect the up-regulation of protein expression in the uveal melanoma cell lines. Furthermore, mda-7 gene expression was also up-regulated to a fairly significant degree in 501mel despite the fact that neither decitabine nor IFN-\(\gamma\) affected 501mel growth or viability. It therefore seems somewhat less likely that mda-7 would have been responsible for the synergy between decitabine and the IFNs. On the other hand, S100A2 protein was detected, albeit weakly, following decitabine treatment, and both gene and protein expression correlated with decitabine concentration throughout the 0.1 to 10 \(\mu\)mol/L range, just as the degree of cell death correlated with decitabine concentration. Although this does not rule out a possible role for the other up-regulated proapoptotic genes, including CMT1A, IKCA1, CDF, or p53 (which was up-regulated but remained below the level of expression in the reference sample), this seems less likely given their relatively low peak levels of gene expression and the absence of further up-regulation when the decitabine was increased from 1 to 10 \(\mu\)mol/L.

S100A2 is a putative tumor suppressor gene whose expression relative to normal epithelium is strongly down-regulated in breast (21), lung (22), and prostate cancer (23). Importantly, S100A2 is strongly expressed in benign nevi but expressed at only very low levels in primary melanomas and is absent from melanoma metastases (24), suggesting that the loss of S100A2 may contribute to the malignant phenotype of cutaneous melanoma. Furthermore, in cutaneous melanoma, there is evidence that S100A2 up-regulation by transforming growth factor-\(\beta\) (TGF-\(\beta\)) contributes to synergistic growth inhibition together with IFN-\(\alpha\) (15). In that report, overexpression of S100A2 led to modest growth inhibition, which was then greatly augmented by IFN-\(\alpha\). In uveal melanoma, we have found that decitabine induces S100A2 expression, and it is possible that IFNs synergize with decitabine by stimulating calcium influx (15), which would then augment S100A2 activation. Although growth inhibition was observed with TGF-\(\beta\) and IFN-\(\alpha\) in cutaneous melanoma, cell death ensued when decitabine and either IFN-\(\gamma\) or IFN-\(\alpha\) were combined in uveal melanoma. If S100A2 is responsible for that cell death, that would be in line with the finding that p53 and p53 homologues stimulate S100A2 transcription (25, 26) as well as the finding that S100A2 interacts with p53 and modulates its transcriptional activity (27). In addition, S100A2 may play a role in the ability of chemoradiation to induce cell death in esophageal cancer because S100A2 expression was associated with a higher pathologic complete response (CR) rate (28).

Although the up-regulation of gene expression is the expected outcome with decitabine, it is clear that decitabine can also down-regulate gene expression (20), raising the possibility that DNA hypomethylation may not be the only mechanism by which decitabine can influence gene expression. In uveal melanoma, it is of interest that decitabine downregulated CAPN3, MITF, and SLUG because these same genes were among multiple genes whose down-regulation in cutaneous melanoma was associated with the direct antimelanoma effect of IFN-\(\gamma\) (8). Although we did not assess the effect of IFN-\(\gamma\) on the expression of these genes in uveal melanoma, it is possible that some of the synergy between decitabine and

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\(<2\text{-fold change.}

Fig. 4. S100A2 protein expression is induced by decitabine in uveal melanoma. A, Western blot showing S100A2 protein expression in 92-1 and UW-1 cells following a 96-h incubation with either 1 or 10 \(\mu\)mol/L decitabine (DAC) or with DMSO (control). An antibody to \(\alpha\)-tubulin was used as a loading control. B, Western blot showing S100A2 protein expression in 92-1 after a 96-h incubation with 1 \(\mu\)mol/L decitabine (D0) and then at 3, 5, and 7 d following the removal of decitabine from the culture medium. Bold arrow, location of the S100A2 band.
IFN-γ with respect to cell death could have involved the more effective down-regulation of CAPN3, MITF, and SLUG.

It is notable that the functional effect of decitabine on uveal melanoma cell lines was strikingly different from its effect on cutaneous melanoma cell lines. This was associated with a stark difference in the gene expression changes induced by decitabine in the two types of melanoma, lending further support to the possible central role of changes in expression of genes like S100A2 in the impact of decitabine on uveal melanoma viability and sensitivity to IFNs. Although the explanation for the differential effect of decitabine on gene expression in uveal versus cutaneous melanoma is not currently known, it is possible that the three cutaneous melanoma cell lines examined did not express those permissive transcription factors required for the expression of S100A2 and the other genes listed in Table 1 following removal of methyl groups from promoter regions by decitabine. This does not necessarily imply that all uveal melanoma cell lines and all cutaneous melanoma cell lines will respond the same way to decitabine because decitabine has been shown to augment the pro-apoptotic effect of type I IFNs in a cutaneous melanoma cell line different from those tested here (9). Therefore, just as uveal and cutaneous melanoma cell lines can differ in their direct response to IFNs, they may also vary in the transcriptional program activated by decitabine. Based on our results and the prior data pertaining to the synergy between TGF-β and IFN-α (15), we hypothesize that melanoma cells capable of responding to IFN and up-regulating S100A2 in response to decitabine will be efficiently killed by the decitabine/IFN combination.

In summary, we have shown that decitabine and IFN-γ synergize to kill uveal melanoma cells and have provided indirect evidence that S100A2 may be a potential mediator of that synergy. Further studies examining the effects of S100A2 overexpression, the overexpression of other genes listed in Table 1, and the silencing of genes like CAPN3, MITF, and SLUG on IFN responsiveness will be necessary to better elucidate how decitabine is altering the sensitivity of uveal melanoma to IFNs. Given the absence of effective therapies for advanced uveal melanoma, the findings presented here provide a basis for testing the combination of decitabine plus IFN-γ in this devastating disease.

References

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