Molecular Medicine 5: 11-20, 1999

Molecular Medicine

Original Articles

Systemic Interferon-α (IFN-α) Treatment Leads to Stat3 Inactivation in Melanoma Precursor Lesions

John M. Kirkwood,[1] Daniel L. Farkas,[2,5] Arup Chakraborty,[1] Kevin F. Dyer,[1] David J. Tweardy,[1] John L. Abernethy,[3] Howard D. Edington,[4] Sandra S. Donnelly,[1] and Dorothea Becker[3]

Departments of [1]Medicine, [2]Bioengineering, [3]Pathology, and [4]Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania, U.S.A.
[5]Center for Light Microscope Imaging and Biotechnology, Carnegie Mellon University, Pittsburgh, Pennsylvania, U.S.A.

Accepted December 15, 1998

Abstract

Background: In the setting of familial melanoma, the presence of atypical nevi, which are the precursors of melanoma, is associated with a nearly 100% risk of developing primary melanoma by age 70. In patients with sporadic melanoma, it is estimated that 40–60% of melanomas develop in contiguous association with atypical nevi. Currently, the only way to prevent atypical nevi from progressing to melanoma is to monitor and excise them as soon as they exhibit changes in their clinical features. Activation of the transcription factor, Stat3, has been linked to abnormal cell growth and transformation as well as to interferon α (IFN-α)-mediated growth suppression in vitro.

Materials and Methods: To determine whether IFN-α, used for adjuvant therapy of high-risk, resected melanoma, induces changes in Stat3 in atypical nevi, patients with a clinical history of melanoma who have multiple atypical nevi were treated for 3 months with low-dose IFN-α. Thereupon, the new technology of microscopic spectral imaging and biochemical assays such as electrophoretic mobility shift assays (EMSAs) and immunoblot analysis were used for the study of atypical nevi, obtained before and after IFN-α treatment.

Results: The results of the investigations provided evidence that, as a result of systemic IFN-α treatment, Stat1 and Stat3, which are constitutively activated in melanoma precursor lesions, lose their ability to bind DNA, and as shown in the case of Stat3, become dephosphorylated.

Conclusions: Unlike primary and metastatic melanomas, melanoma precursor lesions cannot be established as cell cultures. Thus, the only way to explore pathways and treatment regimens that might help prevent progression to melanoma is within the context of a melanoma precursor lesion study conducted prospectively. The findings presented here suggest that down-regulation of the transcription factors Stat1 and Stat3 by systemic IFN-α treatment may represent a potential pathway to prevent the activation of gene(s) whose expression may be required for atypical nevus cells to progress to melanoma.

Address correspondence and reprint requests to: Dr. Dorothea Becker, Department of Pathology, University of Pittsburgh, BST, E1057, 211 Lothrop Street, Pittsburgh, PA 15213, U.S.A. Phone: 412-624-1047; Fax: 412-624-7737; E-mail: dbecker+@pitt.edu

Introduction

Congenital nevi and common acquired nevi, which first appear in early childhood, present in three clinical and histologic patterns—junctional,

compound, and dermal. Junctional nevi are collections of normal melanocytes along the basal layer of the epidermis appearing as single cells or junctional nests. In a compound nevus, melanocytes are present in the epidermis and dermis, and when nevus cells disappear from the epidermis, the lesion often loses its pigment and becomes a dermal nevus. Each of these types of nevi is considered benign until the following clinical and histologic features are observed: size $\geq$5 mm in diameter, macular surface component, irregular or diffuse borders, variegated pigmentation, continuous proliferation of basal melanocytes, bridging and confluence of the rete ridges, nuclear pleomorphism, and the presence of lymphocytic infiltrate. It is the combination of these features that defines atypical nevi, which are the precursors and risk markers of tumor progression (1,2).

For patients with familial melanoma and/or dysplastic nevus syndrome (3), and likewise, for patients who have a clinical history of sporadic melanoma and numerous atypical nevi, the only way to currently prevent these melanocytic lesions from progressing to melanoma is to persistently monitor and resect them at the earliest signs of changes in shape, size, and/or pigmentation. Thus, it would be of great clinical benefit for these patients if one could implement a systemic treatment that, by modulating the molecular, immunologic, histologic, and clinical features of these melanoma precursor lesions, would ultimately lead to their regression.

Interferons (IFNs) are a family of antiviral, antiproliferative, antiangiogenic, immunoregulatory, and cell surface antigen-modulating molecules that are effectively used in the therapy of several human malignancies including hairy-cell leukemia, lymphoma, and Kaposi's sarcoma. In patients with resected melanoma, either with deep primary invasion or lymph node metastases, who are at high risk for relapse, high-dose IFN-α given for 1 year has been shown to benefit relapse-free and overall survival, while patients with resected primary melanoma thicker than 1.5 mm, who had no clinically detectable lymph node metastases, have shown delayed relapse with low-dose IFN-α given for 12 or 18 months. Given the results of these clinical trials, high- and low-dose IFN-α (4–6) are currently used for adjuvant therapy of high-risk, resected melanoma.

Stat proteins, which were first identified by studying early signaling events in IFN-treated cells (7–9), are activated in response to a variety of growth factors and cytokines. Binding of these growth factors/cytokines to cell surface receptors leads to receptor autophosphorylation at a tyrosine residue. Latent cytoplasmic Stat proteins, which contain an SH2 domain that recognizes the receptor phosphotyrosine, are then recruited to the receptor. Upon association with the receptor, Stats become phosphorylated, either by the activated receptor or by a receptor-associated Janus kinase (Jak). Thereupon, the phosphorylated Stats form homo- or heterodimers and translocate to the nucleus where they function as transcription factors for a number of different genes (10,11). Of the seven currently known Stat genes, there are three (Stat1, Stat2, Stat3), whose proteins are activated by the cytokine IFN-α.

In the adjuvant setting of high-risk melanoma, one cannot determine whether IFN-α-induced Stat activation is one of the pathways that leads to the observed clinical benefits because the melanoma is resected prior to IFN-α treatment. Likewise, since atypical nevi cannot be established as cell cultures, it is not possible to analyze in vitro the impact of IFN-α on Stats in melanoma precursor lesions. Thus, the only way to explore pathways of IFN-α action that might help prevent melanoma at its inception is within the context of a melanoma precursor lesion study conducted prospectively.

The data presented here provide first-time evidence that systemic IFN-α treatment leads to dephosphorylation and abrogation of the DNA-binding activity of Stat3 protein in melanoma precursor lesions.

Materials and Methods

IFN-α Treatment and Histologic Analysis of Nevus Specimens

In compliance with an approved clinical protocol [University of Pittsburgh Cancer Institute (UPCI) protocol 96-043, Institutional Review Board (IRB) protocol 960915], patients entering the 3-month clinical trial with low-dose IFN-α2a had to fulfill the following criteria of eligibility. They had to have at least four nevi exhibiting the established features of clinical atypia, which are: size $\geq$5 mm, a macular surface component, irregular or diffuse borders, and variegated pigmentation, and they had to have a clinical history of prior melanoma of stage IA or greater. Ineligible for the study were patients with active

metastatic melanoma or a history of disease that precluded or included treatment with IFN-α. Likewise, individuals who were treated with known immunosuppressive drugs and/or steroids that can counteract or modulate the effects of IFN-α were not eligible to enter the study.

Upon identification and photographic recording of four atypical nevi, two of them were selected at random by members of the UPCI Biostatistics Center. The two nevi were then excised, and parts of them were snap frozen or formalin fixed and paraffin embedded. Following removal of the two nevi, the patients received subcutaneous injections of low-dose IFN-α2a (Roche Laboratories, Nutley, NJ) at a dosage of 3×10^6 units/m^2 three times a week for 3 months. In each patient, the other two atypical nevi were removed at the end of the 3-month treatment period, and parts of the specimens were snap frozen or formalin fixed. The data reported here are based upon the analysis of 18 nevi obtained from five patients.

The degree of architectural and cytologic atypia of each nevus specimen was determined according to established histopathologic criteria (2,12). All specimens were diagnosed as nevi exhibiting a moderate or severe degree of histologic atypia.

Immunohistochemistry

Snap-frozen nevus sections were analyzed by immunohistochemistry as previously described (12,13). Polyclonal antibodies to Stat1, Stat2, and Stat3 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). A polyclonal antibody, recognizing Stat3 protein only when activated by phosphorylation at Tyr705, was obtained from New England Biolabs (Beverly, MA). A mouse IgG1 antibody (Tago, Burlingame, CA) was used as an isotype control.

To minimize the possibility that experimental conditions would lead to differences in Stat expression, adjacent nevus sections were cut and probed at the same time with similar concentrations of the respective antibodies.

Microscopic Spectral Imaging Analysis

Nevus sections probed with antibodies to Stat1, Stat2, and Stat3 and counterstained with hematoxylin were imaged at 20× using a Nikon Eclipse E800 microscope coupled to a Fourier-transform imaging spectrometer (14) (SpectraCube, Applied Spectral Imaging, Carlsbad, CA). The spectral cubes were transferred to a computer where the images were converted from transmission to optical density. Spectra corresponding to the Stat-specific hybridization signals and the hematoxylin-stained nuclei were selected manually. A linear unmixing algorithm, implemented in custom-designed spectral imaging analysis software (15), was applied to each image to detect the presence and intensity of the immunohistochemical and hematoxylin stains. The output of this algorithm was used to generate pseudocolored images for maximum visibility.

Electrophoretic Mobility Shift Assay

Protein extracts (20 μg/sample), prepared from snap-frozen sections of atypical nevi and normal skin, were analyzed by gel shifts and supershifts as previously described (16–19).

Immunoblot Analysis

Total cell lysates (20 μg/sample) of snap-frozen nevus and adjacent normal skin sections were separated on 8% SDS-polyacrylamide gels. Upon electrotransfer onto nylon membrane, the protein samples were probed with the Stat3 polyclonal antibody, which recognized only phosphorylated Stat3 protein. Following incubation with this primary and a secondary antibody, proteins were visualized by enhanced chemiluminescence (ECL) (Amersham Life Sciences, Arlington Heights, IL).

Results

Systemic IFN-α Treatment and Histologic Analysis of Atypical Nevi

Patients with a clinical history of resected primary melanoma who had at least four atypical nevi and who, in compliance with an Institutional Review Board (IRB)-approved clinical protocol, were found eligible to undergo 3 months of systemic IFN-α treatment, had their four most atypical nevi marked and photographed. Using randomization, two of the nevi were surgically removed, and parts of the sectioned nevi were snap frozen for subsequent molecular analyses or formalin fixed and paraffin embedded for histologic evaluation. Following removal of the two nevi, the patients received subcutaneous injec-

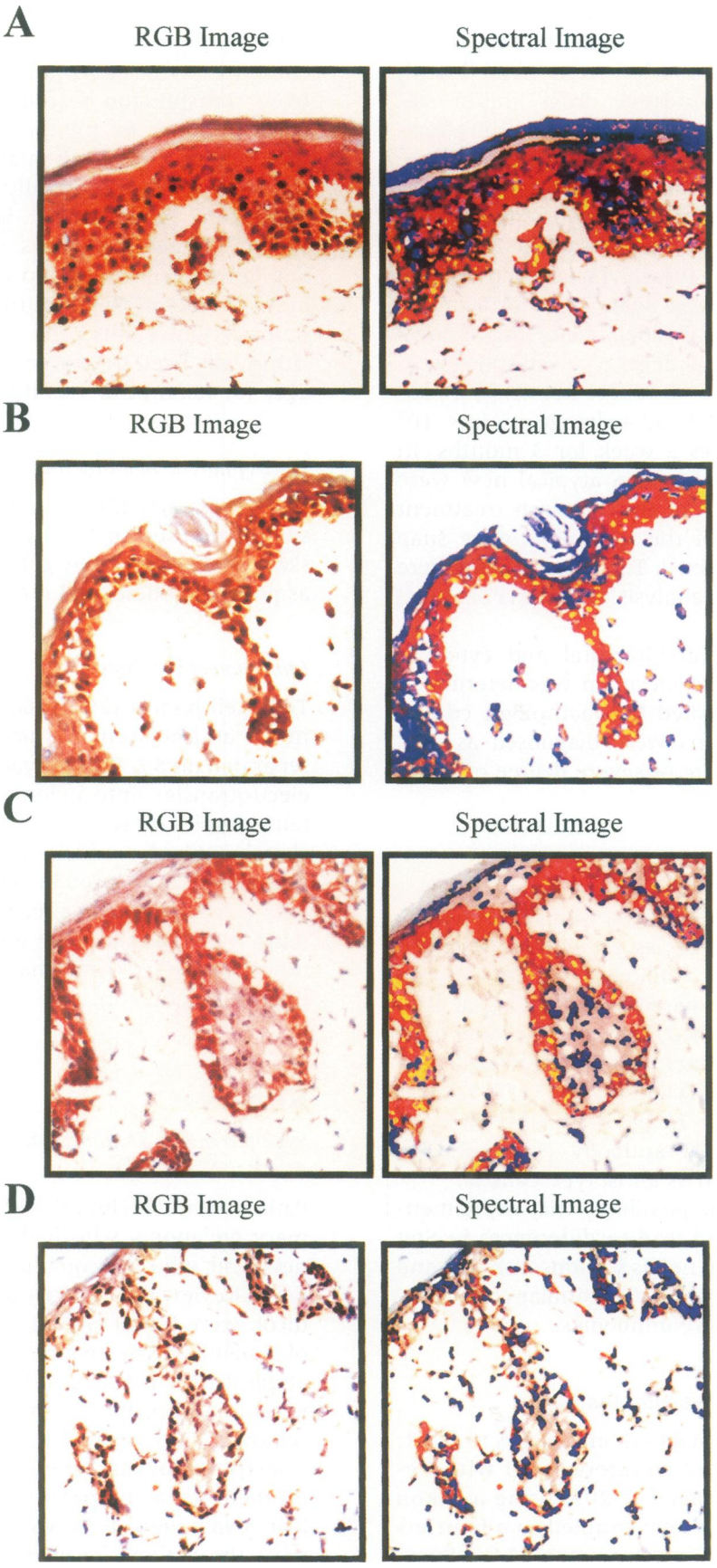
A
RGB Image
Spectral Image
B
RGB Image
Spectral Image
C
RGB Image
Spectral Image
D
RGB Image
Spectral Image

Fig. 1. Cellular location of Stat proteins in melanoma precursor lesions before and after systemic IFN-α treatment. Following immunohistochemistry with polyclonal antibodies to Stat1, 2, and 3, the stained nevus sections were subjected to spectral imaging. (A) Section of a nevus obtained before IFN-α treatment, probed with a Stat3-specific antibody. (B) Section of a nevus excised after treatment, stained with the same Stat3 antibody. (C) Section of an atypical nevus resected after treatment, probed with an antibody to Stat1. (D) Section of a nevus removed after treatment, stained with a Stat2-specific antibody. The tissue sections presented in (A) and (B) were prepared from nevi of the same patient as were those shown in (C) and (D). Depicted on the left of each panel is a Red-Green-Blue (RGB) image of the nevus section, and on the right, a corresponding spectrally segmented image. In each of the spectral images, the assigned pseudocolor, red, represents Stat protein located in the cytoplasm; blue, the hematoxylin (nuclear) counterstain; and yellow, Stat protein residing in the nucleus.

tions of low-dose IFN-α for a period of 3 months, at which point the remaining two nevi were excised and processed for research analysis and histologic examination.

Microscopic Spectral Imaging Reveals Cytoplasmic and Nuclear Location of Stat1 and Stat3 in Melanoma Precursor Lesions Before and After Systemic IFN-α Treatment

To assess the status of expression and cellular location of Stat1, Stat2, and Stat3 in atypical nevi before and after systemic low-dose IFN-α treatment, we probed sections of atypical nevi, obtained before and after treatment, with antibodies specific for each of the three proteins.

In the context of immunohistochemical studies, it is often difficult to assess on a cell-by-cell basis whether a protein is located in the cytoplasm or the nucleus. This task becomes even more complicated in the case of pigmented lesions, where the melanin of junctional and dermal melanocytes frequently masks the hybridization signal(s). To circumvent these potential problems, we subjected the stained nevus sections to microscopic spectral imaging analysis, which made it possible to visualize the location of the Stats in individual cells.

Spectral imaging, defined as the application of spatially resolved spectroscopic analyses to macroscopic and microscopic samples (15,20), allows a high-resolution spectrum—intensity as a function of wavelength—to be acquired at each pixel in an image. The result is an image cube that contains both spectral and spatial information. Unlike conventional transmittance and fluorescence microscopy, spectral imaging has the ability to generate qualitative and quantitative multiparameter high-resolution images at the single cell level.

As depicted in Figure 1A and B, upon staining with a Stat3-specific antibody, tissue sections prepared from atypical nevi obtained before (Fig. 1A) and after (Fig. 1B) IFN-α treatment demonstrated cytoplasmic and, to a lesser extent, nuclear location of Stat3 protein in epidermal and basal layer keratinocytes, junctional melanocytes, and fibroblasts residing in the dermis. Stat1 was also detected in the cytoplasm and in some nuclei of atypical nevi resected before (data not shown) and after IFN-α treatment (Fig. 1C). However, unlike Stat3, Stat1 was not expressed in the upper epidermis. In fact, strongest expression of Stat1 was observed in basal layer keratinocytes and junctional melanocytes (Fig. 1C). In contrast to Stat1 and Stat3, Stat2 was expressed in significantly fewer keratinocytes and junctional melanocytes both before (data not shown) and after IFN-α treatment (Fig. 1D). Furthermore, microscopic spectral imaging analysis of the Stat-2-stained nevus sections documented an almost exclusively cytoplasmic location of Stat2 protein (Fig. 1D).

Systemic IFN-α Treatment Extinguishes the DNA-Binding Activity of Constitutively Expressed Stat1 and Stat3 in Melanoma Precursor Lesions

To investigate whether systemic IFN-α treatment changed the activity of Stat1, 2, and/or 3 in atypical nevi, we carried out gel-shift analyses with protein extracts prepared from adjacent sections of the nevi that were analyzed by immunohistochemistry and subsequent microscopic spectral imaging. In addition, we conducted supershift assays with protein extracts of atypical nevi and adjacent normal skin obtained before IFN-α treatment.

Regarding the first part of the analyses, electrophoretic mobility shift assays (EMSAs) of nevus extracts were performed with the high-affinity form of the serum-inducible element (hSIE),

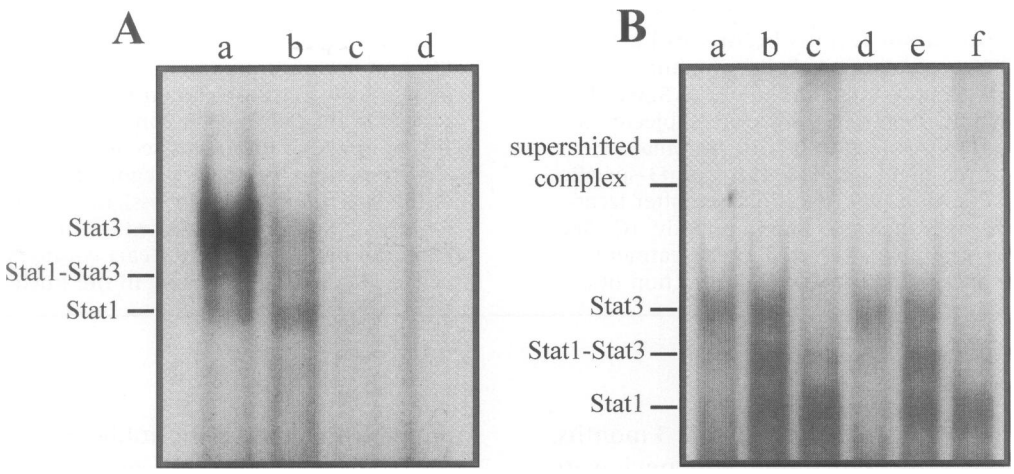

Fig. 2. IFN-α treatment extinguishes the DNA-binding activity of constitutively activated Stat1 and Stat3 in melanoma precursor lesions. (A) Protein extracts (20 μg/sample) of sections of atypical nevi, obtained from a patient before (a and b) and after treatment (c and d), were incubated with radiolabeled hSIE probe and subjected to EMSA. (B) Protein extracts (20 μg/sample), prepared from sections of an atypical nevus (a–c) and normal skin surrounding the nevus (d–f), obtained from a patient before IFN-α treatment, were incubated with a Stat1 (a and d), Stat2 (b and e), and Stat3 (c and f) antibody, followed by incubation with the radiolabeled hSIE duplex oligonucleotide and EMSA.

contained within the c-fos promoter (21). Following incubation of the protein extracts with a ^{32}P-labeled hSIE probe, the resultant DNA-binding protein complexes were analyzed by gel shifts (18,22). As shown in Figure 2A, two atypical nevi, obtained from a patient before IFN-α treatment (Fig. 2A, a and b), demonstrated the presence of three protein complexes, Stat3/Stat3 homodimer, Stat1/Stat3 heterodimer, and Stat1/Stat1 homodimer (16), that bound the radiolabeled hSIE duplex oligonucleotide. In contrast, these complexes were not detected in two atypical nevi obtained from the same patient following IFN-α treatment (Fig. 2A, c and d). These findings suggested that Stat1 and Stat3 are constitutively activated in atypical nevi and that systemic IFN-α treatment extinguishes this activity.

To confirm the presence of Stat1 and Stat3 in the hSIE-protein complexes, we performed supershift assays with nevus and normal skin extracts that had been preincubated with antibodies to Stat1 and Stat3 and serving as a control, an antibody to Stat2. The results, presented in Figure 2B, document that the Stat3 antibody supershifted major portions of the Stat3/Stat3 homodimer and Stat1/Stat3 heterodimer but not the Stat1/Stat1 homodimer in an atypical nevus (Fig. 2B, c) and its surrounding normal skin (Fig. 2B, f), both of which were excised before IFN-α treatment. Similarly, preincubation of protein extracts of the same nevus (Fig. 2B, a) and adjacent normal skin (Fig. 2B, d) with a Stat1-specific antibody diminished formation of the Stat1/Stat3 heterodimer and the Stat1/Stat1 homodimer. Although in each case, antibody to Stat1 and Stat3 was added in an amount sufficient to supershift its own Stat protein, the Stat1 antibody failed to supershift. To confirm that only Stat1 and Stat3 were present in the hSIE DNA-protein complexes, equal portions of the two protein extracts were preincubated with a Stat2-specific antibody. No changes in DNA binding to the three different protein complexes were observed, either in the nevus (Fig. 2B, b) or in its surrounding normal skin (Fig. 2B, e).

Systemic IFN-α Treatment Results in Dephosphorylation of Stat3 Protein in Melanoma Precursor Lesions

Since tyrosine phosphorylation of Stats is essential for their DNA-binding activity and subsequent translocation from the cytoplasm to the nucleus, we analyzed the Stat3 phosphorylation status in atypical nevi, resected before and after IFN-α treatment. Using an antibody that detects Stat3 protein only when activated by phosphorylation at tyrosine 705 (Tyr705), we performed immunochemistry, followed by spectral imaging analysis of the stained nevus sections. As de-

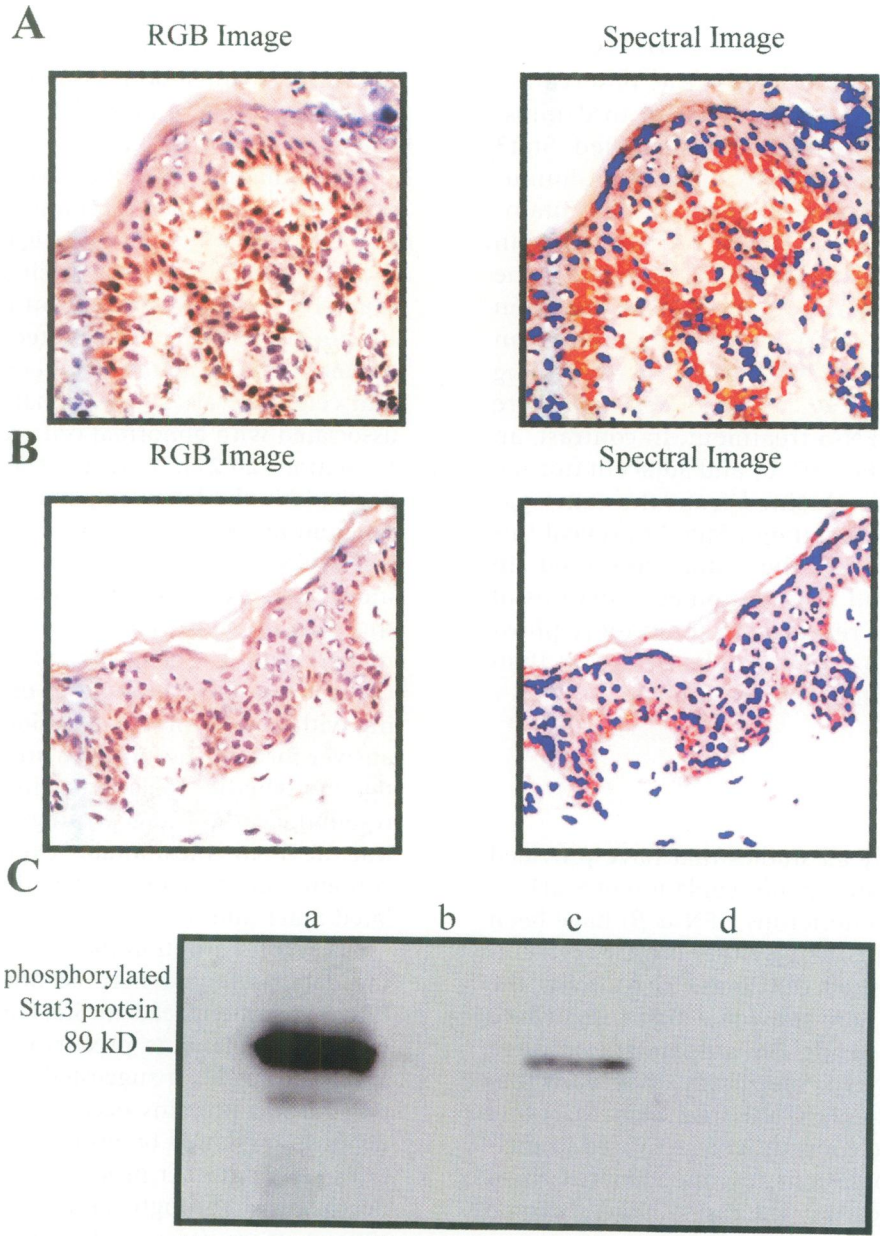

Fig. 3. Stat3 protein, constitutively phosphorylated in atypical nevi and adjacent normal skin, is dephosphorylated following systemic IFN-α treatment. Sections of atypical nevi obtained from a patient before (A) and after treatment (B) were stained with a Stat3 antibody that recognized Stat3 protein only when phosphorylated at Tyr705. Depicted on the left is the RGB image of the nevus section, and on the right, the corresponding spectrally segregated image. In the spectral image, the assigned pseudocolor, red, represents Stat protein located in the cytoplasm; blue, the hematoxylin (nuclear) counterstain; and yellow, Stat protein residing in the nucleus. (C) Total cell lysates (20 μg/sample), prepared from snap-frozen sections of an atypical nevus (a and b) and normal skin surrounding the nevus (c and d), obtained from a same patient before (a and c) and after treatment (b and d), were analyzed by immunoblotting with the phospho-Stat3 (Tyr705) antibody.

picted in Figure 3A, a section of an atypical nevus, excised before IFN-α treatment, demonstrated both cytoplasmic and nuclear location of phosphorylated Stat3 protein in the basal layer. In contrast, in a nevus obtained from the same patient after IFN-α treatment (Fig. 3B), some of the cells residing in the basal layer exhibited a weak cytoplasmic hybridization signal but no

phosphorylated Stat3 protein was detected in nuclei.

To independently verify the observation that systemic IFN-α treatment led to dephosphorylation of constitutively activated Stat3 protein in atypical nevi, we carried out immunoblot analysis. The probing of a blot, containing equal amounts of nevus and normal skin lysates, with the antibody that recognized the phosphorylated form of Stat3, led to detection of an 89 kD phosphorylated Stat3 protein in an atypical nevus (Fig. 3C, a) and its surrounding normal skin (Fig. 3C, c), both of which were excised before IFN-α treatment. In contrast, an atypical nevus (Fig. 3C, b) and adjacent normal skin (Fig. 3C, d) obtained from the same patient after IFN-α treatment failed to reveal this protein. Regarding the data presented in Figure 3C, (a and c), densitometry analysis of the immunoblot revealed 15 times more phosphorylated Stat3 protein in the nevus (a) than in the surrounding normal skin (c).

Discussion

To date, virtually all studies that have provided information regarding the regulation of Stat1, 2, and 3 by type I interferons (IFN-α/β) have been conducted in vitro, using either primary cell cultures or established cell lines. In contrast, the data presented here provide a first insight into the effects of systemic IFN-α treatment on these Stat proteins in vivo. Specifically, they show that as a result of systemic IFN-α treatment, Stat1 and Stat3, which are constitutively activated in melanoma precursor lesions, lose their ability to bind DNA, and as shown in the case of Stat3, become dephosphorylated.

With respect to the biological implications of the findings described here, several important aspects warrant discussion. In the context of a previously conducted melanoma precursor lesion study (12), we reported a correlation between increasing degrees of histological atypia and expression of epidermal growth factor receptor (EGFR) in the epidermal keratinocytes of atypical nevi. This finding, combined with the facts that EGFR is one of the receptor tyrosine kinases that catalyzes Stat phosphorylation and EGFR-activated Stat complexes typically consist of Stat1 and Stat3 homo- and heterodimers, points to a likely association between expression of EGFR and activation of Stat1 and 3 in epidermal and basal layer keratinocytes of atypical nevi and to a possible explanation as to why melanoma precursor lesions contain 15 times more constitutively phosphorylated Stat3 than their surrounding normal skin.

In addition to the extensively studied and by now well-understood mechanisms of Stat activation, the other focus of investigations pertaining to Stats is to understand their biological functions. For example, analyses of normal and malignant cell lines have suggested that expression of Stat3 is required for cell differentiation (23,24) and cell survival (25), and that its activation is associated with abnormal cell growth and transformation (26,27). In light of these observations, particularly the latter, one may propose a model wherein EGFR activates Stat1 and Stat3 in atypical nevi which, in turn, leads to Stat1 and Stat3-induced transcription of genes that play a role in the development of melanoma. Assuming this model is correct, the question then arises: what is the status of these proteins in melanomas evolving within these precursor lesions? To obtain an answer for this question, we are presently investigating whether melanomas in situ, which represent the earliest stage of progression from atypical nevi to melanoma, express EGFR and contain constitutively activated and phosphorylated Stat1 and Stat3.

A second question that arises with respect to the data presented here is: how does systemic IFN-α treatment lead to Stat1 and Stat3 inactivation in melanoma precursor lesions? For example, it has been suggested that down-regulation of Stat proteins occurs as a result of their dephosphorylation by protein tyrosine phosphatases (28). Another proposed mechanism is Stat degradation through ubiquitination pathways (29). A more recently described third pathway involves blocking of the DNA binding activity of Stats by protein inhibitors of activated Stat (PIAS) (30). Among the members of the family of PIAS, PIAS3 was found to be a specific inhibitor of Stat3 signaling (30), while PIAS1 blocked the DNA-binding activity of Stat1 (31). In addition, the latter report documented that in vitro, treatment of human Daudi B-cells with IFN-α led to association of PIAS1 with Stat1. This raises the possibility that similarly, systemic IFN-α treatment may induce PIAS1 to associate with Stat1 and perhaps, PIAS3 with Stat3. Consequently, these Stats may no longer be able to activate genes whose expression may be required for nevic cells to progress to melanoma.

Although we cannot deduce from the data presented here whether suppressing Stat1 and Stat3 activity in melanoma precursor lesions might serve as a potential strategy for melanoma prevention, it should be mentioned that immunoblot analysis of atypical nevi excised from a patient a month instead of directly after completion of low-dose IFN-α treatment also revealed dephosphorylation of Stat3. Furthermore, atypical nevi obtained from a patient with a clinical history of melanoma who received high-dose IFN-α for a period of 3 months also demonstrated loss of Stat1 and Stat3 DNA-binding activity upon EMSA analysis. These preliminary findings suggest that IFN-α-induced inactivation of Stat1 and Stat3 in atypical nevi is not rapidly reversed upon completion of IFN-α treatment, and that both low- and high-dose systemic IFN-α treatment lead to Stat1 and Stat3 inactivation in melanoma precursor lesions.

Finally, these investigations establish that it is possible to study gene regulation in atypical nevi despite their small size, and that the nevic cells from these lesions cannot be cultured and propagated in vitro.

Acknowledgments

This work was supported by grants from the National Institutes of Health (D.B. and D.J.T.), the National Science Foundation (D.L.F.), and the Cancer Treatment Research Foundation (D.B.).

References

1. Clark WH Jr. (1991) Tumor progression and the nature of cancer. *Br. J. Cancer* **64:** 101–112.
2. Elder DE, Clark WH Jr, Glenitsas R, Guerry D, IV, Halpern AC. (1993) The early and intermediate precursor lesions of tumor progression in the melanocytic system: common acquired nevi and atypical (dysplastic) nevi. *Semin. Diagn. Pathol.* **10:** 18–35.
3. Greene MH, Clark WH Jr, Tucker MA, et al. (1985) Acquired precursors of cutaneous malignant melanoma. The familial dysplastic nevus syndrome. *New Engl. J. Med.* **312:** 91–96.
4. Kirkwood JM, Strawderman MH, Ernstoff MS, et al. (1996) Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: The Eastern Cooperative Oncology Group Trial EST 1684. *J. Clin. Oncol.* **14:** 7–17.
5. Grob JJ, Dreno B, de la Salmoniere P, et al. (1998) Randomized trial of interferon alpha-2a as adjuvant therapy in resected primary melanoma thicker than 1.5 mm without clinically detectable node metastases. *Lancet* **351:** 1905–1910.
6. Pehamberger H, Soyer HP, Steiner A, et al. (1998) Adjuvant interferon alfa-2a treatment in resected primary stage II cutaneous melanoma. *J. Clin. Oncol.* **16:** 1425–1429.
7. Darnell JE, Kerr IM, Stark GR. (1994) Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* **264:** 1415–1421.
8. Schindler C, Darnell JE Jr. (1995) Transcriptional responses to polypeptide ligands: the Jak-STAT pathway. *Annu. Rev. Biochem.* **64:** 621–651.
9. Leaman DW, Leung S, Stark GR. (1996). Regulation of STAT-dependent pathways by growth factors and cytokines. *FASEB J.* **10:** 1578–1588.
10. Ihle JN. (1996) STATs: signal transducers and activators of transcription. *Cell* **84:** 331–334.
11. Darnell JE. (1997) Stats and gene regulation. *Science* **277:** 1630–1635.
12. Wang Y, Rao U, Mascari R, et al. (1996) Molecular analysis of melanoma precursor lesions. *Cell Growth Differ.* **7:** 1733–1740.
13. Wang Y, Becker D. (1997) Antisense targeting of bFGF/FGFR-1 in human melanomas blocks intratumoral angiogenesis and tumor growth. *Nat. Med.* **3:** 887–893.
14. Levenson RL, Farkas DL. (1997) Digital spectral imaging for histopathology and cytopathology. *Proc. SPIE* **2983:** 123–135.
15. Farkas DL, Du C, Fisher GW, et al. (1998) Noninvasive image acquisition and advanced processing in optical bioimaging. *Comput. Med. Imaging Graph.* **22:** 89–102.
16. Sadowski HB, Shuai K, Darnell JE Jr, Gilman MZ. (1993) A common nuclear signal transduction pathway activated by growth factor and cytokine receptors. *Science* **261:** 1739–17434.
17. Wong P, Severns CW, Gayer NB, Wright TM. (1994) A unique palindromic element mediates gamma interferon induction of mig gene expression. *Mol. Cell. Biol.* **14:** 914–922.
18. Tweardy DJ, Wright TM, Ziegler SF, et al. (1995) Granulocyte colony-stimulating factor rapidly activates a distinct STAT-like protein in normal myeloid cells. *Blood* **86:** 4408–4416.
19. Chakraborty A, White SM, Schaefer TS, et al. (1996) Granulocyte colony-stimulating factor activation of Stat3α and Stat3β in immature normal and leukemic human myeloid cells. *Blood* **88:** 2442–2448.
20. Farkas DL, Ballou BT, Fisher GW, Taylor DL. (1995) From in vitro to in vivo by dynamic multi-wavelength imaging. *Proc. SPIE* **2386:** 138–149.
21. Wagner BJ, Hayes TE, Hoban CJ, Cochran BH. (1990) The SIF binding element confers sis/PDGF

inducibility onto the c-fos promoter. *EMBO J.* **9:** 4477–4484.
22. Hou J, Schindler U, Henzel WJ, et al. (1994) An interleukin-4-induced transcription factor: IL-4 Stat. *Science* **265:** 1701–1706.
23. Minami M, et al. (1996) Stat3 activation is a critical step in gp130-mediated terminal differentiation and growth arrest of a myeloid cell line. *Proc. Natl. Acad. Sci. U.S.A.* **93:** 3963–3966.
24. Ihara S, Nakajima K, Fukada T, et al. (1997) Dual control of neurite outgrowth by STAT3 and MAP kinase in PC12 cells stimulated with interleukin-6. *EMBO J.* **16:** 5345–5352.
25. Fukada T, Hibi M, Yamanaka Y, et al. (1996) Two signals are necessary for cell proliferation induced by a cytokine receptor gp130: involvement of STAT3 in anti-apoptosis. *Immunity* **5:** 449–460.
26. Migone TS, Lin JX, Cereseto A, Mulloy JC, et al. (1995) Constitutively activated Jak-STAT pathway in T cells transformed with HTLV-1. *Science* **269:** 79–81.
27. Yu CL, Meyer DJ, Campbell GS, et al. (1995) Enhanced DNA binding activity of a Stat3-related protein in cells transformed by the Src oncoprotein. *Science* **269:** 81–83.
28. David M, Grimley PM, Finbloom DS, Larner AC. (1993) A nuclear tyrosine phosphatase downregulates interferon-induced gene expression. *Mol. Cell. Biol.* **13:** 7515–7521.
29. Haspel RL, Salditt-Georgieff M, Darnell JE Jr. (1996) The rapid inactivation of nuclear tyrosine phosphorylated Stat1 depends upon a protein tyrosine phosphatase. *EMBO J.* **15:** 6262–6268.
30. Chung CD, Liao J, Liu B, et al. (1997) Specific inhibition of Stat3 signal transduction by PIAS3. *Science* **278:** 1803–1805.
31. Liu B, Liao J, Rao X, et al. (1998) Inhibition of Stat1-mediated gene activation by PIAS1. *Proc. Natl. Acad. Sci. U.S.A.* **95:** 10626–10631.