Seeing the light in melanoma

By Chris Cain, Staff Writer

UV light exposure is strongly linked to the development of melanoma, but the molecular mechanisms underlying the connection have been poorly understood. Now, an American research team has elucidated an important link by showing that UV light induces the expression of interferon- γ , which in turn can drive the formation and growth of melanoma.¹ The findings could open up a new therapeutic area for companies developing anti-interferon- γ antibodies.

A major hurdle to studying melanoma formation is that the tumors develop from melanocytes—pigment-producing cells that represent only a small fraction of the skin and thus are difficult to isolate or study *in vivo*.

To overcome this obstacle, a team led by Glenn Merlino and M. Raza Zaidi at the **National Cancer Institute** and Edward De Fabo at **The George Washington University Medical Center** developed a transgenic mouse with fluorescently tagged melanocytes that can be tracked *in vivo* and isolated for detailed gene expression analysis.

Merlino is chief of the laboratory of cancer biology and genetics at NCI, and Zaidi is a research fellow at NCI. De Fabo is a research professor in the Department of Immunology at GWU.

The researchers started by exposing one-day-old, postnatal, transgenic mice to UV light. Six days later, the group selectively induced GFP expression in melanocytes and used fluorescence-activated cell sorting (FACS) to isolate these cells for microarray gene expression analysis. The team separately tested both major types of UV radiation—UVA and UVB. The former had little effect on gene expression, but the latter induced a gene signature that resembled the cellular response to interferon- γ (IFNG; IFN- γ).

IFN- γ is a cytokine produced by immune cells that is typically induced in response to bacterial or viral infection. Previously, its production had not been associated with melanoma.

“We were completely stunned by the IFN- γ signature,” said Merlino. “We wanted to take an unbiased approach to identify UV-responsive genes in melanocytes, and this just popped out of the analysis.”

To identify the source of IFN- γ , the researchers performed immunohistochemistry on UV-irradiated skin samples and found robust infiltration of macrophages. Although a handful of previous reports have suggested that macrophages can express IFN- γ , the cytokine is more commonly associated with T cells.

Gene expression profiling confirmed that the infiltrating macrophages expressed IFN- γ . In addition, IFN- γ -expressing macrophages were found

in tissue samples from 19 of 27 melanoma patients.

Finally, the researchers evaluated the tumor-promoting role of IFN- γ in a mouse model of melanoma. Macrophages were isolated from UV-irradiated, neonatal mouse pups and then co-injected with a melanoma cell line into mice. The co-injection increased melanoma tumor volume compared with injection of melanoma cells alone—an effect that IFN- γ antibodies blocked.

Results were published in *Nature*.

Pursuing progression

An unanswered question is whether IFN- γ blockade can prevent melanoma formation and progression in a model of established disease. Indeed, the model in the paper only tested the effect of IFN- γ blockade when macrophages and melanoma cells were co-injected into otherwise normal mice.

Merlino and De Fabo think they have the right tool for the job. Ten years ago they published a transgenic mouse model of melanoma that develops progressive disease comparably to humans with melanoma.² In this model, neonatal exposure to UV light induces the formation of lesions that can be followed over time.

“What we would like to do now is to block IFN- γ signaling at different stages of melanoma development and see if we can prevent the initiation or progression of cancer,” Merlino told SciBX. “This mouse provides a relevant preclinical model, where we can block the pathway at different time points using antibodies or small molecules, systemically and topically, and then measure the effect.”

Although the researchers used antibodies in the *Nature* paper, their next step is to test small molecule inhibitors of the IFN- γ receptor (IFNGR) in the model. IFN- γ exerts its effects by binding IFNGR, and the researchers believe small molecule inhibitors could be applied topically and be more cost effective than antibodies.

Merlino and De Fabo are developing the small molecules in collaboration with Howard Young, an author on the paper who is deputy laboratory chief of the laboratory of experimental immunology at NCI.

Two companies are developing IFN- γ -blocking antibodies to treat immune diseases: **Amgen Inc.** and **NovImmune S.A.** Amgen’s AMG 811 is in Phase I testing to treat systemic and discoid lupus erythematosus. Amgen declined to comment.

This year, NovImmune plans to start clinical trials of its anti-IFN- γ antibody, NI-0501, in an undisclosed inflammatory indication. CSO Marie Kosco-Vilbois said the paper “presented an intriguing story with fantastic early stage data, but additional patient samples and functional follow-up assays are necessary to bridge this work to clinical relevance.”

Like the authors, she suggested that a key question is whether inhibiting IFN- γ can halt disease progression once premalignant lesions are detected.

Jin Mo Park, assistant professor of dermatology at **Massachusetts General Hospital** and **Harvard Medical School**, said macrophages are probably not solely responsible for IFN- γ production.

“In UV-exposed skin, multiple cell types work together to create a pro-tumorigenic tissue microenvironment,” he said. “What remains to be fully clarified is the relative contributions of macrophages versus other types

of cells as sources of IFN- γ . Some immune cells—such as plasmacytoid dendritic cells and NK cells—are much harder to detect and isolate from skin tissues yet may be playing a substantial role in supplying melanocytes with IFN- γ .”

Park’s lab is focused on identifying cytokines and other inflammatory mediators produced by distinct skin cell types and understanding their roles in melanoma formation.

In addition to testing therapeutic strategies, Merlino and De Fabo also plan to use their model to better understand how IFN- γ signaling promotes melanoma growth.

Merlino said the team’s findings are unpatented and that the GFP melanocyte mouse model in the paper is available for licensing.

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