UV-induced DNA damage in melanocytes is regulated hormonally. What biological advantage is conferred by maintaining intermediate levels of repair that can be upregulated and downregulated by hormonal factors produced locally and centrally? Is it possible that DNA repair is playing a role in the balance between cell survival, senescence, and immortalization of cancerous melanocytes? Could reduction of repair mechanisms through antagonism of MC1R function make melanocytes or melanoma cells more vulnerable to immunologic attack (e.g., in vitiligo or immunotherapy for melanoma) or sensitize these cells to traditional DNA damaging chemotherapeutics? These questions and ideas are certainly not the only ones that could be raised, but they will hopefully increase readers' appreciation of the clinical relevance of this work as well as the implications that extend from the fields of pigmentation and melanoma to inflammation, immunology, and, even, infectious disease.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Melanocyte Regeneration in Vitiligo Requires WNT beneath their Wings

John E. Harris[1]

Melanocytes in patients with vitiligo possess intrinsic abnormalities that contribute to its pathogenesis. Regazzetti *et al.* report that CXCL10 expression reflects subtle inflammation in normal-appearing skin but not in stable depigmented lesions, supporting the hypothesis that melanocytes themselves initiate autoimmune inflammation prior to clinically evident disease. In addition, they find that oxidative stress in melanocytes impairs WNT signaling and that targeting this pathway induces melanoblast differentiation. Thus, activating the WNT pathway may serve as an adjunctive strategy to support repigmentation in patients with vitiligo.

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Regazzetti *et al.* (2015) report that WNT signaling is impaired in lesional and nonlesional skin of patients with vitiligo. Because WNT signaling promotes the differentiation of melanocyte precursors in skin (Yamada *et al.*, 2013), impaired signaling may affect the ability of melanocytes to proliferate and differentiate into functional melanocytes during therapeutic repigmentation. Current treatments for vitiligo include topical anti-inflammatory compounds, such as corticosteroids and calcineurin inhibitors, which inhibit autoimmune responses, as well as narrow-band ultraviolet radiation B (nbUVB) (Ezzedine *et al.*, 2015), which likely inhibits autoimmunity and promotes melanocyte regeneration. Repigmentation in vitiligo begins within 6–12 weeks after initiating treatment, and it frequently occurs in a perifollicular pattern (Figure 1). A recent study has reported that nbUVB treatment of vitiligo is associated with proliferation, migration, and differentiation of melanocytes in the hair follicles and epidermis

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Clinical Implications

- Oxidative stress, which is elevated in melanocytes from patients with vitiligo, may directly inhibit WNT activation. Because WNT signaling is important for melanoblast differentiation, this impaired signaling may inhibit melanocyte regeneration during therapy.
- Therapeutic WNT activation might serve as an adjunctive therapeutic strategy employing anti-inflammatory agents to support melanocyte regeneration.
- CXCL10 is expressed in normally pigmented skin in patients with vitiligo but not in completely depigmented skin, from which melanocytes are absent. This supports the hypothesis that melanocytes themselves initiate autoimmune inflammation in vitiligo.

of lesional skin (Goldstein *et al.*, 2015). The current study suggests that impaired WNT signaling in vitiligo slows down this process and that promoting this pathway may improve repigmentation by enhancing melanocyte differentiation.

It is now well established that melanocytes in vitiligo have increased oxidative stress, reflected by elevated levels of reactive oxidative species in cultured cells *in vitro* and within the epidermis *in vivo* (Glassman, 2011; Ezzedine *et al.*, 2015). This may have an important role in its pathogenesis, possibly by initiating inflammation and autoimmunity in the skin (Richmond *et al.*, 2013). However, the current study now indicates that oxidative stress may also be partly responsible for impaired WNT signaling, as melanocytes exposed to oxidative stress *in vitro* decreased the expression of WNT family members. Thus, oxidative stress may both promote autoimmune inflammation and impair melanocyte regeneration during treatment. The authors reasoned that, if WNT signaling were important for melanocyte differentiation but impaired in patients with vitiligo, then pharmacological activators of this pathway might help promote melanocyte differentiation in the skin. Indeed, the authors demonstrated that members of the WNT signaling pathway are induced in the skin by a chemical WNT agonist and two antagonists of GSK-β (a negative regulator of the pathway) and that this treatment promotes the differentiation of melanoblast precursors in skin affected by vitiligo. They suggest that stimulating WNT signaling may serve as an adjunct to current therapies. It is possible that WNT activators could synergize with anti-inflammatory treatments to simultaneously shut down autoimmunity and promote melanocyte regeneration.

We previously reported transcriptional differences in the lesional skin of vitiligo patients compared with healthy control skin, but focused on lesions with active inflammation to reveal the cytokine and chemokine patterns responsible for driving autoimmunity. This approach revealed an IFN-γ-specific signature, and CXCL10, an IFN-γ-induced chemokine, was the most highly expressed gene in lesional skin. We further determined that CXCL10 was elevated in a mouse model of vitiligo that we developed, and that it was required functionally for both the progression of vitiligo as well as for the maintenance of disease, as neutralizing CXCL10 both prevented depigmentation as well as reversed established disease (Rashighi *et al.*, 2014). The current study by Regazzetti *et al.* also found that CXCL10 is elevated in perilesional skin in vitiligo, although the magnitude of expression was lower than in our study. This is likely due to the fact that we profiled only lesional skin with a significant mononuclear infiltrate by histology, whereas the present study profiled perilesional skin indiscriminately, which would usually include sites with very mild inflammation.

However, the current study went further, analyzing also stably depigmented and normally pigmented skin from vitiligo, and it compared these results with normal, control skin as well. This revealed that the expression of CXCL10 is also mildly elevated in nonlesional, normally pigmented skin in vitiligo, when compared with healthy control skin, which suggests that subtle inflammation is present even before the appearance of clinically evident lesions. This may indicate a predisposition in all of the skin of patients to develop vitiligo, and this may reflect the ability of tolerance mechanisms to keep low-level inflammation "in check", thereby preventing new lesions from appearing. Previous studies have reported an important role for T-regulatory cells in controlling the spread of depigmentation in mouse models of vitiligo (Gregg *et al.*, 2010; Chatterjee *et al.*, 2014), which may be a key mechanism that controls further depigmentation. Importantly, in contrast to normally pigmented skin, completely depigmented skin from patients with vitiligo (old, inactive lesions) did not have elevated CXCL10, suggesting that the presence of melanocytes was required to fuel the low-level inflammation. Melanocytes in vitiligo are not simply targets of autoimmunity; rather, they contain intrinsic defects that help initiate the autoimmune destruction that is observed in vitiligo (Passeron

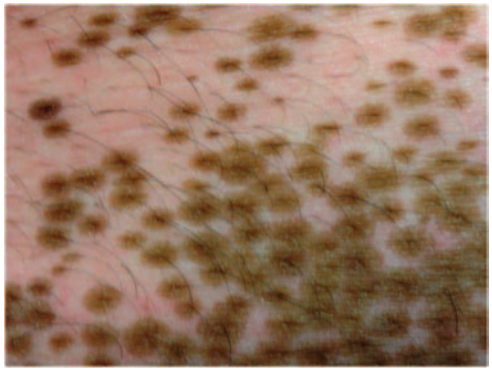

Figure 1. Perifollicular repigmentation in a patient with vitiligo during nbUVB phototherapy.

and Ortonne, 2012; Richmond *et al.*, 2013). Future studies may reveal how "abnormal" melanocytes promote autoimmunity in vitiligo, and specifically how CXCL10 is initially induced in disease.

> Targeted therapy may promote repigmentation in vitiligo by serving as the 'WNT beneath the wings' of regenerating melanocytes.

The study by Regazzetti *et al.* (2015) confirms our findings that CXCL10 is expressed within active lesional skin in vitiligo (Rashighi *et al.*, 2014), and, further, it suggests that subtle inflammation is ongoing in even normally pigmented skin. In addition, this study connects oxidative stress in melanocytes to the WNT signaling pathway, revealing that intrinsic defects in melanocytes from such patients likely include impaired WNT signaling. Finally, the authors hypothesize that pharmacological activation of the pathway could help promote melanocyte differentiation and repigmentation during treatment, serving as the "WNT beneath the wings" of regenerating melanocytes, which must go through a series of steps, beginning with differentiation of melanocyte precursors in the hair follicles or other niches of the skin and then progressing through proliferation, migration, and further differentiation into functional melanocytes. The study used an innovative new *ex vivo* vitiligo skin culture model to demonstrate that WNT activators begin this process by differentiating melanoblasts but, because of the limited time that the cultures could be maintained *ex vivo*, the authors were unable to determine whether this would progress to promote fully functional melanocytes. The role of WNT signaling in events downstream of melanocyte differentiation is not clear, and future studies will hopefully to assess this in order to translate these results into new treatments.

CONFLICT OF INTEREST
The author states no conflict of interest.

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Microenvironment-Driven Resistance to BRAF Inhibition Comes of Age

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Increasingly comprehensive observations indicate that the tumor microenvironment contributes to drug resistance toward small molecule inhibitors. Fedorenko *et al.* describe a role for fibroblasts in creating a favorable niche for melanoma cell survival if treated with the BRAF inhibitor vemurafenib. TGF-β released by vemurafenib-treated melanoma cells stimulated fibroblasts for increased α-smooth muscle actin, neuregulin (NRG), and fibronectin expression. Off-target effects of vemurafenib led to paradoxical secretion of hepatocyte growth factor (HGF) by fibroblasts. Combined inhibition of BRAF/MET/HER kinases was insufficient to reverse the protective effect of the fibroblasts, whereas reversal was achieved by combined BRAF/PI3K inhibition. A thorough understanding of the complex spatiotemporal interactions in tumor microenvironments holds promise for improved targeting using combination therapies in patients with melanoma.

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Small molecule inhibitors have brought about a strategic shift in the way melanomas are treated. Melanoma patients harboring BRAF mutations in particular have reaped significant benefit from small molecule BRAF–MEK

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