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Viewpoint 4

The intriguing current debate feature brings us back to reconsidering an old hypothesis on the pathogenesis of vitiligo that we had ventilated almost two decades ago in light of recent insights into cutaneous neuroendocrinology.

Dysregulation of melanogenesis is at the centre of vitiligo pathogenesis

There is still no consensus on the aetiology of vitiligo. However, two main theories on the origin of this disease can be distinguished ((1–3) and this *Controversies* feature). One proposes that vitiligo results from an autoimmune response to melanocyte-associated autoantigens (4). The second identifies a chain reaction of ultimately cytotoxic events that is triggered by noxious endogenous or exogenous factors as the starting point of the disease, with autoimmune responses representing only secondary events (5–7).

In an attempt to develop a unifying concept on the origin of vitiligo that would promise to help developing more satisfactory therapeutic regimens for this psychosocially often devastating disease, we have proposed that vitiligo originates from a cascade of reactions that is initiated by a deregulation of melanogenesis, a process that entails the abundant generation of reactive oxygen species (ROS) and necessitates their highly controlled scavenging by several interlocking scavenging systems, including the production of ROS-scavenging melanin itself (8,9). This dysregulation would result in the massive and uncontrolled production of ROS and toxic quinone/semiquinone intermediates of melanogenesis, leading to sequential damage and ultimately destruction of both melanocytes and keratinocytes; this hypothesis also envisioned that, eventually, a secondary autoimmune response to melanocyte-associated autoantigens would be provoked that would lead to further massive and irreversible loss of melanocytes (8). Over the last decade, this – widely ignored – concept of vitiligo pathogenesis that had linked oxidative stress with endogenous production of toxic metabolites through a self-amplifying process resulting in melanocyte destruction has gained some experimental credence (5,6,8,10), and thus deserves to be re-emphasized.

Is vitiligo triggered by a malfunctioning melatonin receptor?

Eighteen years ago, we had proposed that deregulation of melanogenesis leading to melanocyte autodestruction could

be initiated by pathological activation of melatonin receptor(s) (8), even though intracutaneous melatonin receptors had not been identified yet. Since then, however, membrane-bound (MT1 and MT2) and nuclear (ROR- α) melatonin receptors have been cloned and characterized (11,12) and their expression and activity were demonstrated in a variety of skin cells (13–15). Furthermore, pathways for melatonin synthesis and metabolism in skin cells were uncovered (15–18), indicating not only the existence of an intracutaneous melatonergic system, but also the involvement of melatonin receptors in the regulation of the activities of melanocytes and keratinocytes through auto-, intra- and paracrine modes of action (17). The expression and possibly function of melatonin receptors might be modified by ultraviolet radiation (UV radiation) or skin pathology, for example, through alternative splicing (13,14). Thus, pathological activity of melatonin receptors as one of the factors initiating vitiligo through dysregulation of melanogenesis (8), still remains a viable option.

However, non-receptor-mediated actions of melatonin had not yet been identified at that time, and ROS-protective effects of melatonin through its anti-oxidative actions, which would attenuate vitiligo development, were not considered in the original hypothesis. These more recent developments invite modifications of the original hypothesis, whose basic tenants (as we argue) nevertheless remain valid.

Recently, melatonin functions have become much more complex, especially in the skin

Recent advances in melatonin research have clearly demonstrated that melatonin and its metabolites (such as N1-acetyl-N2-formyl-5-methoxykynuramine, AFMK) exert powerful direct, non-receptor-mediated bioregulatory actions (19–21). Thus, they can function as endogenous free-radical scavengers/antioxidants, pleiotropic inducers of anti-oxidative/cytoprotective responses, mitochondrial stabilizers/regulators and have anti-genotoxic and anti-mutagenic actions (19–23). Furthermore, both the cytosolic flavoprotein, quinone reductase II (NQO2) [involved in cellular resistance to oxidative stress and detoxification (24) and ubiquitously expressed in skin cells (14)] and calmodulin have been identified as important integrators of cellular melatonin protective activities (19–21). Accordingly, new roles for melatonin as a powerful protector of cellular

and organ integrity acting against a wide variety of exogenous and endogenous insults, including ROS and DNA damage, has emerged (19) with wide implications for skin pathophysiology (14,17). Interestingly, these melatonin receptor-independent protective actions of melatonin against oxidative stress in skin cells require comparatively high concentrations of melatonin (14,17,25–28) that could only be achieved by its sufficient endogenous production (14,19). Thus, low levels of intracutaneous melatonin generation and/or overly rapid melatonin degradation may impair the melatonin-dependent epidermal response system against oxidative and/or genotoxic stress, thus predisposing it to damage induced cell death.

Finally, it has now surfaced that both mammalian skin and hair follicles are potent sources of (inducible) extrapineal melatonin synthesis *in situ* (15–17), as integral components of a complex intracutaneous melatonergic system that includes the synthesis of various melatonin metabolites (14,17,18).

How melatonin could play a key role in the pathogenesis of vitiligo?

Against this background, we are revising our old theory on the role of overactive melatonin receptor as a key player in vitiligo pathogenesis (8) and propose that, in addition or predominantly, a dysregulation of the cutaneous melatonergic system contributes to the development and progression of vitiligo, along the lines summarized in Fig. 1. Insufficient local production of melatonin and/or of its (even more powerful) antioxidant/cytoprotective intermediates, such as AFMK (14,18) is envisioned to impair the epidermal buffering capacity against oxidative and/or genotoxic stress, induced by a variety of exo- and/or endogenous noxious factors, such as UVR, pro-oxidative chemicals and biomolecules deregulating melanogenesis, which consequently leads to unbalanced ROS, H₂O₂, quinones and semiquinones accumulation or DNA damage (Fig. 1). According to the extended hypothesis, this melatonin shortage will result in the melanocyte and keratinocyte (depending on the context) damages due to ROS, toxic quinone compounds and DNA damage, which would eventually induce the apoptosis/death pathways in targeted epidermal cells. However, generation of toxic reactive precursors or metabolites of melatonin as cellular intoxicators cannot entirely be excluded depending on the subcellular localization or environmental factors (e.g. solar radiation) (Fig. 1).

In conclusion, we propose that involvement of the skin melatonergic system (SMS) in the pathogenesis of vitiligo is context dependent. Insufficient production of melatonin and/or of its powerful antioxidant metabolites (namely AFMK) would lead to development of vitiligo when oxida-

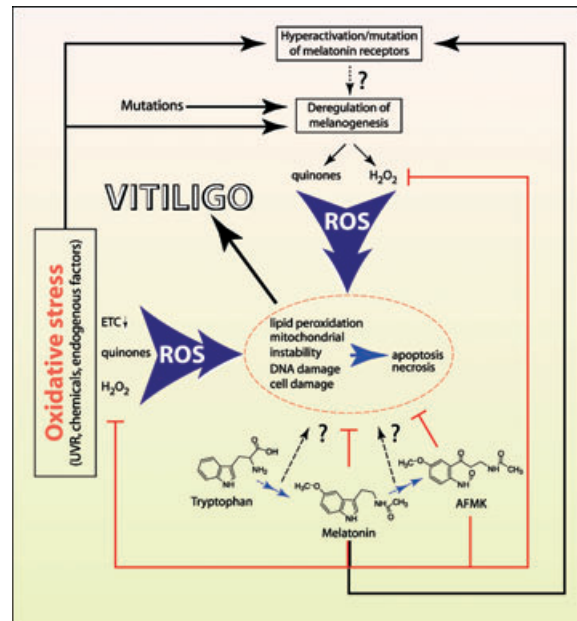


Figure 1. Hypothesis: possible role of SMS in vitiligo pathogenesis. Oxidative stress is now well recognized to be a major factor in vitiligo pathogenesis. Under physiological conditions, synthesis of both melanin and melatonin protect cells from negative effects of oxidative stress by serving as potent ROS-scavenging molecules. Melatonin and AFMK, powerful protectors of cellular and genomic integrity (19–21), can act not only directly as antioxidants but also indirectly through interaction with/or stimulation of anti-oxidative/cell protective pathways and stabilization of mitochondrial electron transport chain (ETC) (19–23). As melatonin synthesis and metabolism in skin likely provide an additional line of defense against oxidative damage (16,17), acquired or inherited inhibition of the SMS in conjunction with environmental damage (e.g. UVR and chemicals) or action of endogenous factors (inducing local oxidative stress or production of toxic intermediates of melanogenesis) might contribute to the triggering, severity and/or clinical course of vitiligo. From a historical point of view (8), we are including pathological activity of melatonin receptor as one of the melanin synthesis deregulators.

tive stress, cellular/subcellular or DNA damages occur and/or because of insufficient scavenging and/or buffering activity of the local anti-oxidative system. However, under certain conditions (e.g. overactive melatonin receptors) or improper production or distribution of reactive melatonin metabolites/precursors, a dysregulated SMS can amplify pro-oxidative and pro-apoptotic responses. Thus, our revised hypothesis integrates the emerging SMS concept (14–18) with the historical melatonin receptor theory (8) and the oxidative damage scenario of vitiligo pathogenesis (5–7).

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Commentary 1

The aetiology and pathogenesis of the skin-depigmenting disease vitiligo has long been the subject of both research and debate. Currently, the exact cause of vitiligo remains obscure, but many factors have been implicated in its development including infections, stress, neural abnormalities, melatonin receptor dysfunction, impaired melanocyte migration, genetic susceptibility, biochemical defects and autoimmunity (1,2). Ultimately, these different factors could act independently or together to yield the same effect, namely the disappearance of melanocytes from the skin and this is proposed in the convergence theory (1). For example, autoimmunity might arise as a secondary phenomenon following the self-destruction of pigment cells due to biochemical imbalances and this might then amplify the damage to melanocytes.

Autoimmunity involvement in vitiligo aetiology is supported by several lines of evidence. Predisposition to vitiligo appears to be associated with certain alleles of the major histocompatibility complex (MHC) class II antigens as well as with other autoimmune-susceptibility genes (3–5). Furthermore, the association of vitiligo with various autoimmune disorders, animal models of the disease and the positive response to immunosuppressive therapeutic agents emphasize the role of autoimmunity in the development of this disorder (6). Moreover, autoantibodies and autoreactive T cells against cutaneous melanocytes have been identified in patients with vitiligo (7,8). Circulating melanocyte-specific cytotoxic T lymphocytes expressing high levels of the skin-homing receptor cutaneous lymphocyte-associated antigen at frequencies correlating with both

the extent and activity of the disease have been detected in patients with vitiligo (8,9). In addition, perilesional T-cell clones derived from patients with vitiligo exhibit a predominant type 1-like cytokine secretion profile and also display anti-melanocyte cytotoxicity (10).

Autoantibodies that are able to destroy melanocytes *in vitro* by complement-mediated damage and antibody-dependent cell-mediated cytotoxicity and *in vivo* following passive immunization of nude mice grafted with human skin are found in patients with vitiligo (11,12). Furthermore, IgG purified from patients with vitiligo can destroy melanoma cells both *in vitro* and *in vivo* (13), and vitiligo anti-melanocyte IgG antibodies can induce HLA-DR and intercellular adhesion molecule-1 expression on and interleukin-8 release from pigment cells (14), changes that may enhance the antigen-presenting activity of the cells allowing antigen-specific immune effector cell attack resulting in the destruction of melanocytes.

Despite detailed studies that implicate autoreactive T cells and autoantibodies in vitiligo pathogenesis, the exact contribution that they play in the destruction of melanocytes during development of the disease remains to be determined. Of particular interest is whether aberrant immune responses are of primary origin or whether they constitute a secondary reaction. A genetic predisposition to immune dysregulation at the T- or B-cell level could lead to the production of autoreactive T cells or autoantibodies that destroy melanocytes. Alternatively, autoreactive T cells or autoantibodies against melanocytes could arise in response to a challenge to the immune system. For