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### Viewpoint 3

Vitiligo has, for a long time, lacked a clear classification, and even the origin of the name is controversial, indicating that many aspects of vitiligo are still unclear. The definition of the disease reaches back to the dawn of the last century (1). A step forward was taken with the consensus provided by the Vitiligo European Task Force (2). Several theories have been proposed to explain the disappearance of functional melanocytes, when even the concept of 'disappearance' is a matter of debate (3–5). Several morphological, functional or metabolic alterations, apparently unrelated, have been described. One of the problems is that researchers work separately and have rarely tried to reproduce the data published by others and to combine these with their own results.

*In vitro* studies demonstrated intrinsic cellular damage, providing a clear indication for the involvement of apparently healthy melanocytes. Metabolic damage, characterized by increased susceptibility to chemical/physical stress, high H<sub>2</sub>O<sub>2</sub> intracellular level, low catalase activity, faulty activity of the mitochondrial respiratory chain and defective assembly/activity of TRP1, all have been demonstrated (6–11). The increased cellular death cannot be a simple effect of the *in vitro* manipulation, but mirror the *in vivo* experience of the Koebner phenomenon.

For us the main questions are: Is vitiligo due to a specific defect of the melanocytes? How do melanocytes disappear? Are the other epidermal cells affected? Is systemic involvement possible?

Current publications underline two facts: (a) even non-epidermal cells are affected; and (b) localized or even systemic oxidative stress occurs (12–14).

If the oxidative stress participates in vitiligo pathogenesis, further questions remain open: where does oxidative stress arise from? Is it generated by a unique cause? How could it be related to the large variety of observed alterations, such as pro-inflammatory cytokines and the production of keratinocyte growth factors (15–17)?

If the epidermal reactive oxygen species (ROS) hyperproduction were only due to the oxygen burst of the infiltrating polymorphonuclear cells (PMN) or to the excessive release of TNF- $\alpha$ , this is inconsistent with ROS overproduction even *in vitro*, where both PMN and TNF- $\alpha$  are absent (16,18).

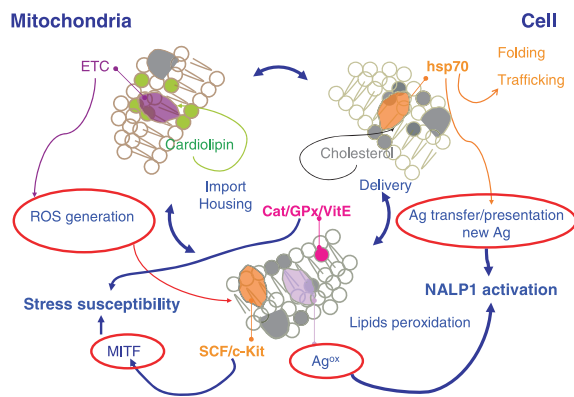
An effort has to be made to identify a potential unifying process that can account for the described cellular impairments. The genetic approach suggests a wide range of potential, defective checkpoints, but the differences between the studied populations make it difficult to interpret the data (19–22). The genetic analyses indicate a possible pathogenic pathway mainly in those patients with vitiligo (25–40%) with an apparent auto-immune contribution. The description of the 'inflammosome' (23,24) as a central element of the disease rather suggests an increased susceptibility towards hazardous stimuli of the skin in patients with vitiligo. However, this concept did not clarify what activates the immune system in the first place, and why melanocytes are the main target.

Yet another question is whether the observed alterations could be the consequence of a more generalized impairment. On the basis of our results, we speculate that the membrane plays a central role. The lipid composition and the insufficient saturation of fatty acids compromise the membrane tridimensional structure, giving rise to altered intramembrane protein assembly on the plasmatic as well as on the mitochondrial membrane (25). Consequently, receptor activity and activity are affected (26–28). Lipid remodelling is an important mechanism for targeting proteins within cells and for controlling their activity. The fluidity of the membrane is due to the cholesterol content and the pattern of fatty acids of the phospholipids. Cholesterol ensures the correct structure and function of the glycolipid- and cholesterol-enriched *lipid rafts*, where the receptors, and the associated protein kinases, can move and cluster. The

final effect will be the control of the intracellular signal transduction (28). An alteration of the PI3K pathway has recently been reported (29) and we have observed defective ERK1/2 and JNK activation in vitiligo fibroblasts (Picardo, unpublished data).

We have recently demonstrated that, in vitiligo cells, plasmatic membranes exhibit an increased cholesterol content, leading to a loss of fluidity. An additional consequence is the inappropriate membrane embedding of the proteins with the exposure of new antigens. The inner mitochondrial membrane is characterized by the dimeric phospholipid cardiolipin. Any damage, oxidation or altered synthesis, to cardiolipin would have a negative impact on the biochemical activities associated with the inner membrane (30–33). The respiratory chain is thus the main target of a cardiolipin deregulation. The first effect will be the high free radical species production, which will take part in the membrane lipo-peroxidation and in an additional free radical production.

A last point. Until now attention has been mostly focused on resident epidermal melanocytes. However, these have a span of life that well precedes their migration into the epidermis. The melanocyte originates from the hair follicle niche, differentiates and migrates to epidermis whilst maintaining a reservoir of undifferentiated cells inside the bulge. Thus, the niche allows the continuous production of new melanocytes, avoiding cell exhaustion and senescence. Vitiligo may represent a localized senescence process through a mechanism similar to the 'free radical-mediated greying', associated with a loss of progressive melanocytes from the hair bulb. The presence, or absence, of a functioning follicular melanocyte reservoir may be the deciding factor in the successful melanocyte graft. The ability to answer to specific growth factors (i.e. Stem Cell Factor [SCF], and the c-Kit/SCF axis appears to be broken in vitiligo) may determine the continuous production of differentiated and functioning epidermis-committed melanocytes (34).



**Figure 1.** Possible unifying pathomechanism.

In summary, what do we suggest? The initial membrane alteration, mainly involving cholesterol and cardiolipin, may represent the unifying mechanism for different alterations that involve different cell types and that characterize vitiligo (Figure 1).

How could this happen? The altered cardiolipin composition affects stability and activity of the respiratory chain, increases the rate of ROS production, and promotes membrane lipo peroxidation (11). Consequently, the loss of unsaturated fatty acids, specifically susceptible to oxidants, affects the membrane fluidity, already conditioned by the cholesterol content. The possible damage of lipid rafts may affect the correct expression of or signal transduction by growth factor receptors. The oxidative process, as such, may also alter cellular proteins, thus generating new, immunogenic antigens. The last event may contribute to or start the immune activation. According to other experimental models, the effectiveness of cardiolipin components or lipoic acid supplementation, supports this hypothesis (35,36).

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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- $\alpha$ ) 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