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Commentary

Melanin 'dust' or 'ghost'?

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Abbreviations: FM, Fontana Masson; TEM, Transmission Electron Microscopy; VK, Von Kossa; WS, Warthin-Starry.

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Although epidemiological data would support a photoprotective role of melanin pigments in the skin, the detailed underlying mechanisms are still a matter of debate and some controversy. This confusion may derive in part from unreliable *in vitro* experiments and in part from the widespread, still largely unwarranted, assumption that melanin (eumelanin) provides the perfect protection against UV-induced photodamage because of its black colour evoking highly efficient light absorption. Understanding the detailed aspects of epidermal melanization, including chiefly pigment distribution, transfer from melanocyte to keratinocytes and fate under stressing conditions is therefore mandatory to devise efficient photoprotective strategies to counteract deficiencies in constitutional pigmentation. Another largely overlooked source of uncertainty is represented by the lack of adequate methods to assess melanin levels and distribution in the skin, besides the histological studies based on Fontana-Masson (FM) and Von Kossa (VK) stains. According to handbooks in histology (S1), there are two types of silver-reduction-based staining methods – argentaffinic or argyrophilic. All of them take advantage of the dark-brown to black stain of colloidal metallic silver produced by reduction in soluble and colourless silver (I) complexes. In the first class of reactions, it is the inner native reducer responsible for staining, such as indole residues or tyrosine-derived amines, while in the other case, the exogenous reducer is applied to the sample pretreated with the silver (I) solution. By these methods, it has been reported that while melanosomes in dark skin are resistant to degradation by lysosomal enzymes, remain intact throughout the epidermal layers and form supranuclear caps in keratinocytes and melanocytes, in lightly pigmented skin, melanosomes are degraded and only persist as 'melanin dust' in the suprabasal layers. This latter phenomenon in the upper epidermis (*stratum corneum*) has been implicated as an important contributory factor in carcinogenesis, as it compromises the photoprotection of the skin (S2). As emphasized by the Joly-Tonetti *et al.* (1), the 'melanin dust dogma' of skin pigmentation science can be prominently found in skin pigmentation reference books. The term is used here to refer to 'degraded particulate melanin granules that have reached, via epidermal stratification, the suprabasal layers of the epidermis, most particularly the *stratum corneum*'. The presence of a melanin dust is supported almost exclusively by the diffuse black staining associated with the FM reaction in the *stratum corneum*. Yet, no direct evidence, for example by ultrastructural analysis via transmission electron microscopy (TEM), has ever been obtained for the presence of a particulate or dust form of melanin in this layer of the epidermis.

To definitely verify the dogma of 'melanin dust' in the *stratum corneum*, these authors have now reported a careful review of the literature with a vis-à-vis of the FM and VK histochemical methods plus the Warthin-Starry (WS) stain on very thin cryosections of normal human skin and mouse pelage skin epidermis lacking melanocytes. Both WS and FM staining methods, originally introduced for staining of spirochetes, are currently largely used for tissue staining. While FM is the argentaffinic method, the status of the WS staining is blurred by the unclear role of hydroquinone, whether it directly reduces the silver adsorbed on melanin, or pre-reduces melanin. The results thus obtained were compared with a visual count of melanin transferred to keratinocytes by TEM using the ImageJ image processing program. By this approach, the authors were able to conclude that: (i) WS stain provides a much more sensitive and more specific melanin detection method than the commonplace FM stain and (ii) 'melanin dust', long purported to be present in the *stratum corneum*, is most likely an artifact due to non-specific silver deposition in the *stratum corneum*.

Interestingly, the WS stain was shown to highlight the immature colourless stage III melanosomes in amelanotic melanoma evidenced by TEM much more specifically than the FM staining (S3).

The conclusions by Joly-Tonetti *et al.* raise a number of important issues. *First*, what makes the WS stain different from FM and VK, despite a similar silver ion reduction chemistry? Chemically, the main difference lies in the acidic pH and the presence of hydroquinone (S4). It would thus be tempting to argue that progressive darkening in the FM reaction is due to the oxidation of colourless diffusible products of melanocyte activity leading to melanin particles and metallic silver at alkaline pH. This reaction would conversely be blocked by the acidic pH of the medium. The presence of small amounts of a reducing agent like hydroquinone may contribute to the stability of the staining preserving the high contrast with the background.

Second, what determines the deceptive melanin dust-like response to FM in the *stratum corneum*? As the diffuse black

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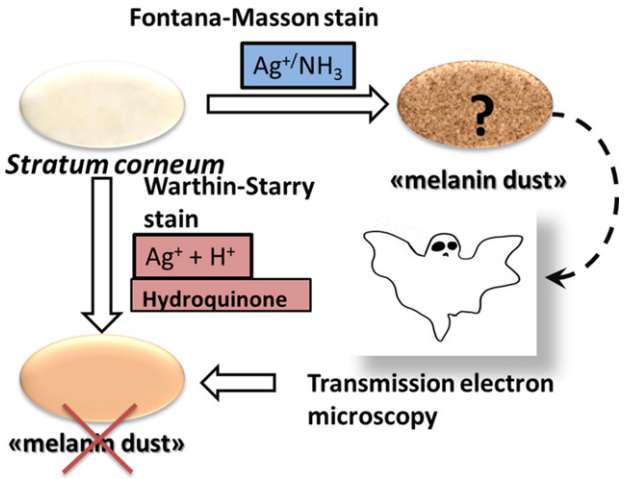

Figure 1. Response of the melanin staining silver-based Fontana-Masson (FM) and Warthin-Starry (WS) procedures in the keratinocytes (KC) of the *stratum corneum*. A false-positive response traditionally attributed to the so-called melanin dust is obtained by the FM stain, but not with the WS method. Thus, the 'melanin dust' observed turns out to be a real 'ghost'. The response of the WS are validated by direct observation through transmission electron microscopy.

staining is clearly not due to melanin, as evidenced by TEM analysis, the fact that the FM staining of the squames removed from the *stratum corneum* is negative for melanin clearly indicates an interference by other tissue components capable of reducing silver nitrate in ammonia but not in acids, as in the WS method.

Moreover, and most importantly, the authors of the article under analysis did not answer the key question in the title of their work, that is, what is the fate of epidermal melanin? The process of melanin degradation was tenaciously investigated by the late Professor Jan Borovansky, who remained sceptical about the identity of the so-called melanin dust as the products of partial degradation of the pigment (2). There are some pathways of removal and deposition of skin melanin, with the participation of phagocytic cells (3), and a putative transfer (and perhaps a parallel degradation) of the pigment to the lymph nodes and visceral organs (4). Another possibility is that melanin is totally degraded *in situ* by keratinocytes and other cells via extensive oxidative breakdown by H_2O_2 and UV activation. But what does 'total degradation' mean? Is the histochemical criterion proper? We hoped that the ImageJ-supported WS staining will provide useful insights, although perhaps one has to look for the answer deeper under the skin. To summarize, the paper by Joly-Tonetti *et al.* (1) concerns the 'real melanin ghost'; that is, it concerns the problem of what happens to melanin itself during its journey through the epidermal layers of the skin. Is it possible to detect any 'epidermal melanin ghosts', or rather does melanin totally disappear? It is noted that 'melanin ghost' is a term coined for the remains of pigmented cells of pathogenic fungi (such as *Cryptococcus neoformans*) which can be observed in the material after hydrolysis in HCl. In TEM pictures, the melanized cellular wall resembles the shape of the original cell (5), so it is rather the ghost of the cell which is observed. Now, it seems that melanin 'dust' in the epidermis is in fact a 'ghost' (Fig. 1) but maybe in a quite different sense than in fungi. In addition, the availability of improved methods for melanin detection may be of help to address important related issues, such as: (i) the fate and the actual toxicity of ectopic melanin which is generated as a side effect of chemotherapy-induced alopecia (6), (ii) the origin and fate of splenic melanin and visceral melanin (7), (iii) the presence or absence of melanin in the lymph nodes, which may turn out of importance in the intra-operative analysis of metastasizing (8) and (iv) determination of pathogenic microbial melanin as a factor of virulence (9).

Author contributions

MdI, AN, DMW and PMP surveyed literature and wrote the manuscript.

Conflict of interest

The authors declared that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Data S1. Supplementary references.

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