Research letter

Epidermal melanocytes in segmental vitiligo show altered expression of E-cadherin, but not P-cadherin

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Dear Editor, Vitiligo is the most common pigmentation disorder, with a worldwide prevalence of 1%. The loss of melanocytes from the skin is the main clinical feature of patients with vitiligo, resulting in depigmented macules.[1] Vitiligo has been classified into two major forms: nonsegmental vitiligo (NSV) and segmental vitiligo (SV).[2–4] NSV lesions are generally bilateral or symmetrically scattered over the entire body. Onset may occur at any age, but most patients develop vitiligo before 40 years of age, and the depigmentation evolves over time.[2] SV is characterized by its unilateral distribution, early onset and rapid stabilization.[3]

The aetiopathogenesis of NSV and SV has not been fully elucidated, and it is possible that the two subtypes share common mechanisms for initiation of the disease, but diverge in the progression phase, as depigmentation rapidly stabilizes in SV, but not NSV.[5] There is much data to support an autoimmune phase in the extension of vitiligo, and mechanical and chemical trauma are known environmental stressors that accelerate disease appearance. However, the events that initiate the loss of melanocytes are still a subject of debate.

We aimed to explore cell adhesion defects in melanocytes before clinical lesions appear as an indicator of early events in the pathogenesis of vitiligo. We have recently shown that E-cadherin, which mediates the adhesion between melanocytes and keratinocytes in the epidermis, is discontinuously or heterogeneously distributed within (or lost from) melanocyte membranes of patients with NSV.[6] This alteration of melanocyte E-cadherin distribution is observed in biopsies distant from the depigmented macules, before clinical lesions appear, indicating that the alteration of E-cadherin distribution is an early event in the pathogenesis of vitiligo. Furthermore, we have shown that this primary defect, which affects adhesion, combined with stress conditions, leads to the disappearance of melanocytes in human epidermal reconstructed skin and mouse models.[6] Here we assessed whether alteration of E-cadherin is a common first step in the various subtypes of vitiligo by studying the distribution of E-cadherin in epidermal melanocytes of frozen skin biopsies from patients with SV and NSV, taken from clinically normal-looking skin.

Briefly, we classified each melanocyte based on its E-cadherin labelling (#610182; BD Biosciences, San Jose, CA, U.S.A.) by immunochemistry. Type I melanocytes correspond to a homogeneous membrane distribution of E-cadherin, whereas type II melanocytes correspond to heterogeneous distribution or lack of E-cadherin. We counted and analysed ≥ 50 melanocytes per patient from 10 controls, 10 patients with SV and 10 with NSV. Tyrosinase-related protein-2 (shown in red; SC-10451; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) was used as a marker for melanocytes. In control patients, E-cadherin (shown in green) displayed type I homogeneous membrane staining in 78% of melanocytes (Fig. 1a [panel 1]), with the remaining 22% showing type II staining (Fig. 1b). We observed a similar pattern of distribution at the melanocyte membrane (of control patients) for β-catenin (#610154; BD Bioscience), a major cytoplasmic partner of E-cadherin (Fig. 1a [panel 2], b). In contrast, melanocytes from patients with SV showed only 43% and 46% type I staining for E-cadherin and β-catenin, respectively (Fig. 1a [panels 7,8], b).

There was no statistical difference for E-cadherin and β-catenin staining between the two vitiligo subtypes; however, both were significantly different than in control patients (Fig. 1a [panels 1,2,4,5,7,8], 1c). Thus, our results demonstrate that melanocytes in both SV and NSV share the same altered distribution pattern for E-cadherin and β-catenin, which is different from that of melanocytes in patients without vitiligo. Moreover, we detected a similar number of suprabasal melanocytes in the buttock of normal epidermis in SV and NSV: 14% and 18%, respectively. These percentages are higher than the normal situation, with 5% of suprabasal melanocytes. We could not find any correlation between the E-cadherin staining and the number of suprabasal melanocytes according to the appearance of the vitiligo.

P-cadherin is the other type I cadherin expressed in melanocytes, and its gene is located in the same chromosomal region as E-cadherin in humans and various mammals.[7] We then examined whether a modification in melanocyte E-cadherin membrane distribution could have some *cis* influence on the distribution of P-cadherin (#610228; BD Bioscience). We found that P-cadherin was homogeneously distributed throughout the plasma membrane of melanocytes (Fig. 1a [panels 3, 6, 9], b) in both controls and patients with NSV and SV. Thus P-cadherin distribution was similar for both vitiligo subtypes and controls, despite its structural and functional similarity to E-cadherin.

In conclusion, NSV and SV showed the same reduction in E-cadherin expression in melanocytes relative to normal

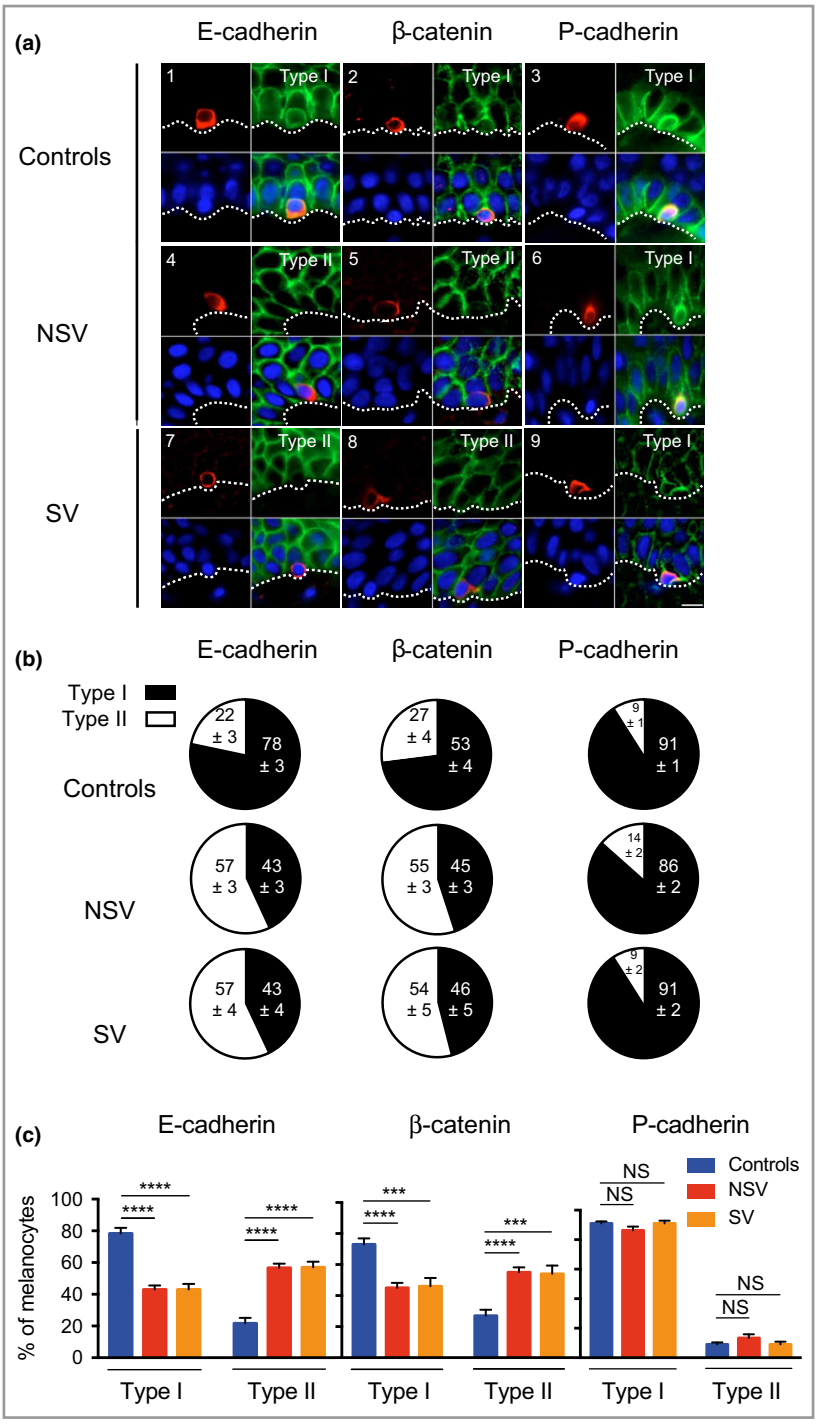

Fig 1. Loss of E-cadherin and β-catenin, but not P-cadherin, in nonsegmental vitiligo (NSV) and segmental vitiligo (SV). Biopsies were taken from clinically normal-looking skin of patients with SV (buttock area; five female, five male). The mean age was 29 years. The patients with NSV and controls were described previously.[5] (a) Immunofluorescence of controls and patients with NSV or SV for E-cadherin, β-catenin and P-cadherin. These markers are each shown in green, and tyrosinase-related protein-2 in red. The nuclei (blue) were counterstained with DAPI (4′,6-diamidino-2-phenylindole). The white dotted line indicates the basal membrane. Scale bar = 10 μm. (b) Average percentages of control, NSV and SV melanocytes showing type I and II staining for E-cadherin, β-catenin and P-cadherin. Ten biopsies for each condition were analysed. Patients provided signed, informed consent before the study, which was approved by the relevant local ethics committees (Bordeaux CNIL #1545937 v.0 and Rabat Hospitals). (c) Average percentages of control, NSV and SV melanocytes showing type I and II staining of E-cadherin, β-catenin and P-cadherin. ***$P < 0{\cdot}001$; ****$P < 0{\cdot}0001$; NS, not significant.

pigmented skin and a similar increase of suprabasal melanocytes in the epidermis. This finding indicates that the changes in cell adhesion mediated by E-cadherin in melanocytes are similar in both NSV and SV. This favours the cell adhesion defect theory, called melanocytorrhagy,[8] not only for the initiation step of NSV, as originally proposed, but also for the SV subtype. In addition, P-cadherin expression remained the same for both vitiligo subtypes and controls. Thus, alteration of E-cadherin distribution is an intrinsic defect of this protein and not a general defect affecting other cadherin molecules. A noticeable difference between SV and NSV was found in the architecture of the epidermis of the normally pigmented buttock skin: SV presents a notable increase of the rete ridges, and NSV shows acanthosis compared with healthy controls. This feature might impact melanocyte integrity differently and eventually participate in the progression of the disease.

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