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Letter to the Editor

Hypoxia-Inducible Factor-1α and CD271 inversely correlate with melanoma invasiveness

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Abstract: Melanoma is characterized, among other features, by microenvironmental factors and by an altered apoptotic machinery. Melanoma cell response to a hypoxic environment is transcriptionally regulated by the Hypoxia-Inducible Factor (HIF)-1α. p75 neurotrophin receptor ($p75^{NTR}$), also called CD271, mediates apoptosis in several cell systems. The purpose of this study was to analyze the expression of HIF-1α and CD271 in melanomas at different phases of progression, as evaluated by histology and reflectance confocal microscopy (RCM). By RCM, 41.67% tumors were characterized by the presence of a population of dendritic and pleomorphic cells (D+P), corresponding to *in situ* melanoma; 25% exhibited a predominantly round-cell (RN) proliferation with histologic features of superficial melanoma, and 33.33% showed the presence of cells aggregated in nests (DN), typical of invasive melanoma. HIF-1α was scarcely detected in D+P and in RN melanomas, while it was highly expressed in DN tumors. By contrast, CD271 positive cells were mostly detected in D+P population, and barely observed in the other subtypes. This work demonstrates that CD271 expression inversely correlates with hypoxia in melanoma, and that the two markers may be used in the future as diagnostic/prognostic tools for this neoplasm.

Key words: CD271 – Hypoxia-Inducible Factor-1α – melanoma – reflectance confocal microscopy

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Background

Melanoma is the most aggressive form of skin cancer and, if disseminated through the dermis, has a poor prognosis with a high mortality rate. Microenvironmental factors including varying concentrations of oxygen play an active role in determining the malignant potential in response to oncogene activation by controlling cell differentiation, growth and survival. In particular, hypoxia increases the mutation frequency, promotes survival of cells with lower apoptotic potential, alters the expression of genes involved in cell cycle regulation and cytokine production and impacts treatment outcome and patient survival (1). The cellular response to a hypoxic environment is transcriptionally regulated by the hypoxia-inducible factor (HIF)-1α (2). In melanoma, hypoxia may be essential for melanocyte transformation, and HIF-1α has been shown to promote melanoma metastasis (3).

The CD271 ($p75^{NTR}$) neurotrophin receptor belongs to the tumor necrosis factor receptor superfamily and mediates apoptosis in many cell settings. CD271 was first isolated from a melanoma cell line, and it is currently used to characterize melanoma initiating cells (4). We have previously shown that melanoma cell lines express the CD271 receptor and mediate a number of functions *in vitro* (5).

Questions addressed

The purpose of this study was to analyse the expression of HIF-1α and CD271 in melanomas at different phases of progression.

Experimental design

We evaluated 12 skin samples derived from patients with different melanoma subtypes (Table S1), as selected by histology and reflectance confocal microscopy (RCM). Detailed methods are reported in the supporting information.

Results

RCM allows to distinguish different subtypes of melanoma that well correlate with the histologic counterpart (6). 5 (41.67%) of the 12 melanomas were characterized by the presence of a population of dendritic cells (D+P subtype), mostly located within the epidermis, whereas 3 (25%) exhibited a predominantly round-cell proliferation (RN subtype) with a pagetoid-like infiltration characterized by cell aggregates. The remainder 4 cases (33.33%) showed a proliferation of cell aggregated in nests within the dermis, with little epidermal involvement (DN subtype) (Fig. 1a). Haematoxylin and eosin (H&E) staining appeared to be consistent with the RCM features, in that in D+P type, melanoma cells were mostly located within the epidermis, close to the epidermal junction; in RN, a pagetoid infiltration was observed throughout the epidermis, and in DN, melanoma was predominantly detected in the dermis (Fig. 1b).

Because a high content of HIF-1 α is consistently detected in metastatic melanoma, we evaluated its expression in the three melanoma subtypes. HIF-1α was expressed in all melanomas, though at different levels (Fig. 2a). In D+P melanoma, few positive cells were confined to the epidermis. In RN tumors, a stronger HIF-1α positivity was observed in epidermal aggregates, but still within the epidermis. On the other hand, in DN melanomas, most dermal infiltrate expressed HIF-1α (Fig. 2a). Two melanomas (16.66%) expressed HIF-1α in <50% of cells (score 2), seven tumors (58.33%) displayed HIF-1α staining in 50–75% of cells (score 3), and in three melano-

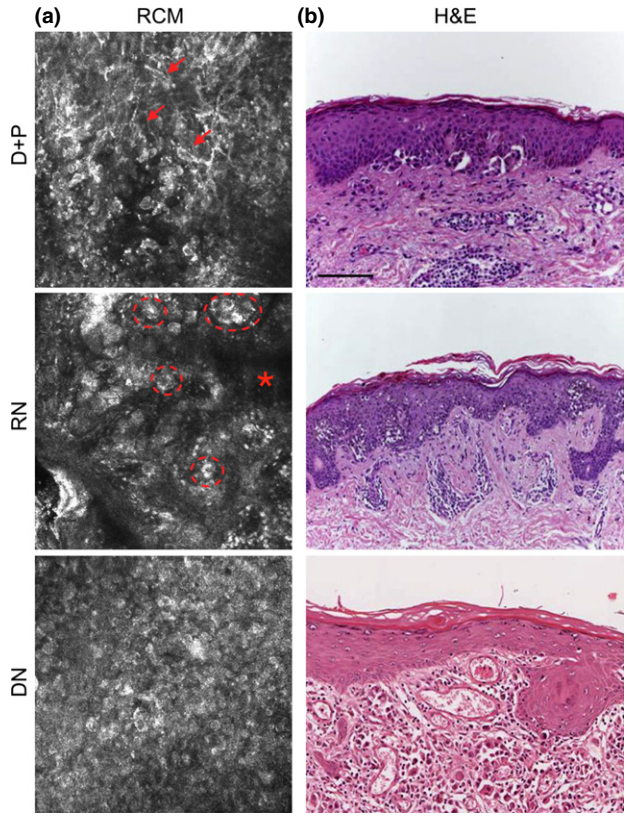

Figure 1. Melanoma subtypes. (a) Representative reflectance confocal microscopy (RCM) features. Dendritic cell (D+P) melanoma: presence of dendritic cell populations in the epidermis (red arrows). Round-cell (RN) melanoma: presence of roundish cells scattered throughout the epidermis and in small aggregates (red-dashed circles). The dermal papilla is marked by an asterisk. Dermal nest (DN) melanoma: presence of cerebriform nest located at the dermal level, constituted by small cells. RCM image = 1 × 1 mm. (b) Representative histopathology (H&E staining). Dendritic cell melanoma: thin by Breslow's index, horizontal growth pattern with atypical cells close to the dermo-epidermal junction. Round-cell melanoma: horizontal growth pattern with pagetoid infiltration throughout the epidermis. Dermal nest melanoma: thick tumors infiltrating the entire dermis. Scale bar = 70 μm.

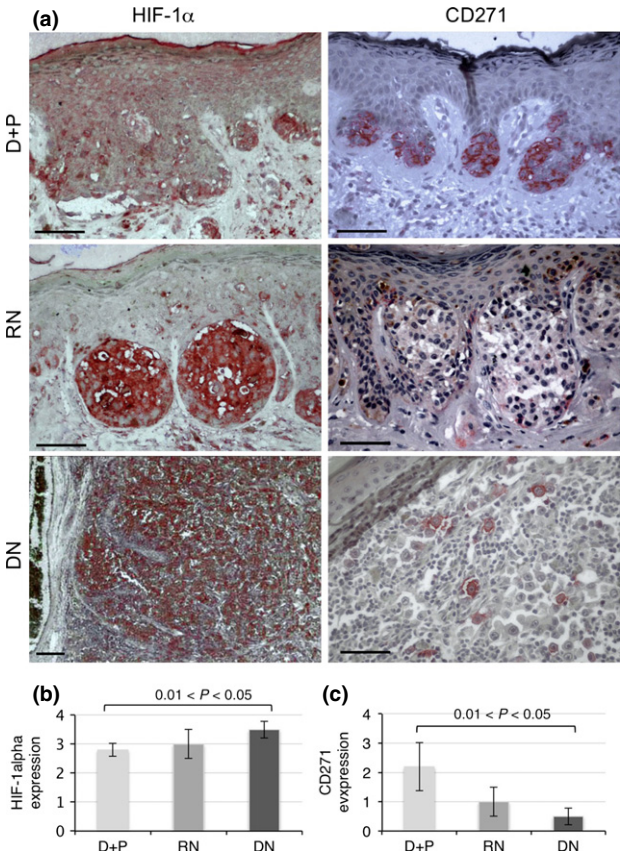

Figure 2. HIF-1α and CD271 expression in melanoma. (a) HIF-1α and CD271 staining of D+P, RN and DN lesions. Scale bar = 50 μm. Expression was scored 0–4 and the average positive cell was calculated for each individual melanoma. Student's *t*-test was used to determine the significant difference between the average expressions of (b) HIF-1α and (c) CD271.

mas (25%), the majority of cells (more than 75%, score 4) were HIF-1α positive (Table S2a). In DN melanomas, no lesion scored between 0 and 2. In RN melanomas, lesions scored between 2 and 4. On the other hand, in 50% melanomas, HIF-1α staining scored 3 and in the remaining 50% scored 4 (Table S2a). While D+P and RN melanomas mean score was 2.8 and 3, respectively, the highest mean score (3.5) of HIF-1α positivity was observed in the DN group. Indeed, statistical difference was observed only between D+P and DN groups (Fig. 2b).

The role of CD271 in melanoma is still controversial. Furthermore, CD271 regulates HIF-1α stabilization, and hypoxia induces the intramembrane proteolysis of CD271 (7). Thus, we evaluated CD271 expression in relation to different melanoma invasiveness. In D+P melanomas, CD271 was strongly expressed in the intra-epidermal tumors, namely in the rete ridges close to the epidermal junction. In RN melanomas, only few positive cells were detected in the epidermis. Finally, in DN melanomas, only scattered melanoma cells expressed CD271 (Fig. 2a). The expression score was very low or nearly absent in RN and DN, respectively, whereas it was higher in D+P melanomas. In particular, 4 melanomas (33.33%) failed to show CD271-positive cells; CD271 staining was <25% (score 1) in 4 melanomas (33.33%); in 1 case (8.33%), CD271 positivity scored 2 and in 2 cases (16.66%) positive staining scored 3 (Table S2b). Finally, only 1 case of melanoma scored 4. While DN and RN mean score was 0.5 and 1, respectively, in D+P type, CD271 expression mean score was 2.2. Examination of the different regions of melanoma subtypes revealed higher expression value of CD271 in D+P melanoma cells. On the other hand, the expression level significantly decreased in RN type to be almost undetectable in DN melanoma (Fig. 2c).

Conclusions

In the present study, we found that HIF-1α is scarcely expressed in the less aggressive type of melanomas (D+P) to increase in pagetoid melanomas within the epidermis (RN) and become highly present in tumors invading the dermis (DN). These data are in agreement with the notion that hypoxia, through HIF-1α, regulates a set of invasive genes, while HIF-1α silencing favours the increased expression of the melanocytic marker MLANA (8). Furthermore, HIF-1α induces alterations of microRNA associated with proliferation in melanoma cell lines (30). By contrast, CD271 was markedly expressed in the slow-growing, superficial melanomas (D+P), while it progressively decreased in pagetoid melanomas (RN) to nearly disappear in DN, nodular type tumors. Consistently, CD271 is expressed in normal melanocytes and in nevi, while it is not detected in invasive melanomas (9). We postulate that CD271 is

progressively lost during melanoma progression and that this finding may be related to the pro-apoptotic role of this receptor, which would allow to eliminate neoplastic cells in the less aggressive melanomas. With more data available, HIF-1α and CD271 could be used as valuable tools for the diagnosis/prognosis of melanoma.

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Author contributions

AM, RB and GP designed the study; FT, CL and FP performed the research; AM, RB, GP and CP analysed the data; AM, RB, GP and CP wrote the paper.

Conflict of interest

The authors have declared no conflicting interests.

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Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Data S1. Methods.

Table S1. Population study and RCM features of melanoma subtypes.

Table S2. Absolute and relative frequencies of melanoma subtypes and biomarker staining. HIF-1α (a) and CD271 (b) expression was evaluated in the three types of malanomas, as selected by RCM.

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Letter to the Editor

Characterization of the major bacterial–fungal populations colonizing dandruff scalps in Shanghai, China, shows microbial disequilibrium

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Abstract: Dandruff is a scalp disorder characterized by the formation of flaky white-yellowish scales due to an altered proliferation and differentiation status; a disrupted barrier function; a decrease in the level of hydration and of natural moisturizing factors (NMF) in the scalp, with a persistent and relapsing inflammatory condition. It was recently reported that an imbalance between bacterial and fungal species colonizing the scalp of French volunteers was associated with dandruff condition. The purpose of the present study was to analyze the major bacterial and fungal species present on the scalp surface of Chinese volunteers and to investigate possible region-related variation in the microbiota linked to dandruff condition. The data obtained from the Chinese populations were highly similar to those obtained in France, confirming that dandruff scalps are associated with a higher incidence of *Malassezia restricta* and *Staphylococcal* sp. The ratios of *Malassezia* to *Propionibacterium* and *Propionibacterium* to *Staphylococcus* were also significantly higher in the dandruff volunteers as compared to normal volunteers, suggesting that equilibrium between the major bacterial and fungal taxa found on the normal scalps is perturbed in the dandruff scalps. The main difference between the French and Shanghai subjects was in their *Staphylococcal biota*. The results obtained in China and in France suggest that targeting one particular *Malassezia* sp. by antifungals instead of using large spectrum antifungals and rebalancing the dandruff scalp microbiota could be common approach to improve dandruff condition in the two countries.

Key words: Chinese population – dandruff – disequilibrium – microflora profiling – skin physiology

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