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Research paper

High Antigen Processing Machinery component expression in Langerhans cells from melanoma patients' sentinel lymph nodes

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ABSTRACT

Langerhans cells (LCs) from melanoma patients sentinel lymph nodes (SLN) are poor T cell activators mostly due to an immature immunophenotype. However Antigen Presenting Machinery (APM) role is unknown. We investigated HLA-class I APM components (Delta, LMP-7/10, TAP-1, Calnexin, Tapasin, β2-microglobulin and HLA-A,B,C) in LCs from healthy donors skin and melanoma patients SLN. APM component levels were low in immature epidermal LCs and significantly increased after maturation ($p < 0.05$); their levels were significantly high in SLN LCs ($p < 0.01$). APM component expression correlated with melanoma Breslow's thickness and SLN metastases: HLA-A,B,C level was significantly lower in SLN LCs from thick lesions patients compared with those from thin/intermediate lesions ($p < 0.05$); β2-microglobulin level was significantly higher in positive SLN LCs compared to negative ones ($p < 0.05$). Functionally, SLN LCs did not phagocytose exogenous antigens. These findings extend LCs knowledge indicating that they are not fully impaired by melanoma, contributing to design new LCs-based therapeutic approaches.

1. Introduction

Sentinel lymph node (SLN) represents the first draining node from a tumour site, where primary immune responses towards tumour antigens are expected to take place [1]. However SLN functions appear heavily altered in cancer patients and particularly in melanoma patients [2–5]. Melanoma cells secrete highly immunosuppressive factors, such as IL-10 and TGF-beta [6], negatively affecting SLN dendritic cells (DCs), the professional antigen presenting cells (APCs), which exhibit a complex of alterations, ranging from histo-pathological aberrations to immunophenotype and functional defects [2,3,7]. Thus, the severe melanoma immunosuppressive microenvironment may favour early metastases in SLN, which represent a crucial step in tumour progression [8]. In melanoma patients, SLN Langerhans cells (LCs) display an immature immunophenotype, characterized by low expression of CD83 and co-stimulatory molecules [9]. Consistently, they are also poor activators of T cells proliferation [10]. These defects may have significant impact on anti-melanoma immunity, because LCs are expected to present tumour antigens to cytotoxic $CD8^+$ T cells in SLN [11]. The phenotypic and functional features of SLN LCs are similar to those of immature LCs located in peripheral tissues, also characterized by the ability to up-take and process exogenous antigens, an attribute which is then lost by mature DCs [12,13].

Antigen processing activities have been reported to play a key role in immune responses against tumours. In particular, Antigen Processing Machinery (APM) component expression in tumour cells appears to influence cancer cell recognition by host's immune system [14], therefore representing an important topic in tumour immunotherapy, particularly in melanoma patients [15]. Although APM molecule expression has been investigated in many types of cancer cells, particularly melanoma and neuroblastoma [15,16], and in DCs originated *in vitro* from blood monocytes (moDCs) [17–19], they have not been explored in human LCs yet [20]. Indeed, LCs are the professional antigen presenting cells of the epidermis therefore the expression of APM

Abbreviations: APCs, antigen presenting cells; APM, Antigen Processing Machinery; β2-m, β2-microglobulin (β2-m, only in Table and Figures); DCs, dendritic cells; HLA, human leukocyte antigen; LCs, Langerhans cells; MHC, major histocompatibility complex; mAbs, monoclonal antibodies; moDCs, monocytes-derived DCs; SLN, sentinel lymph node

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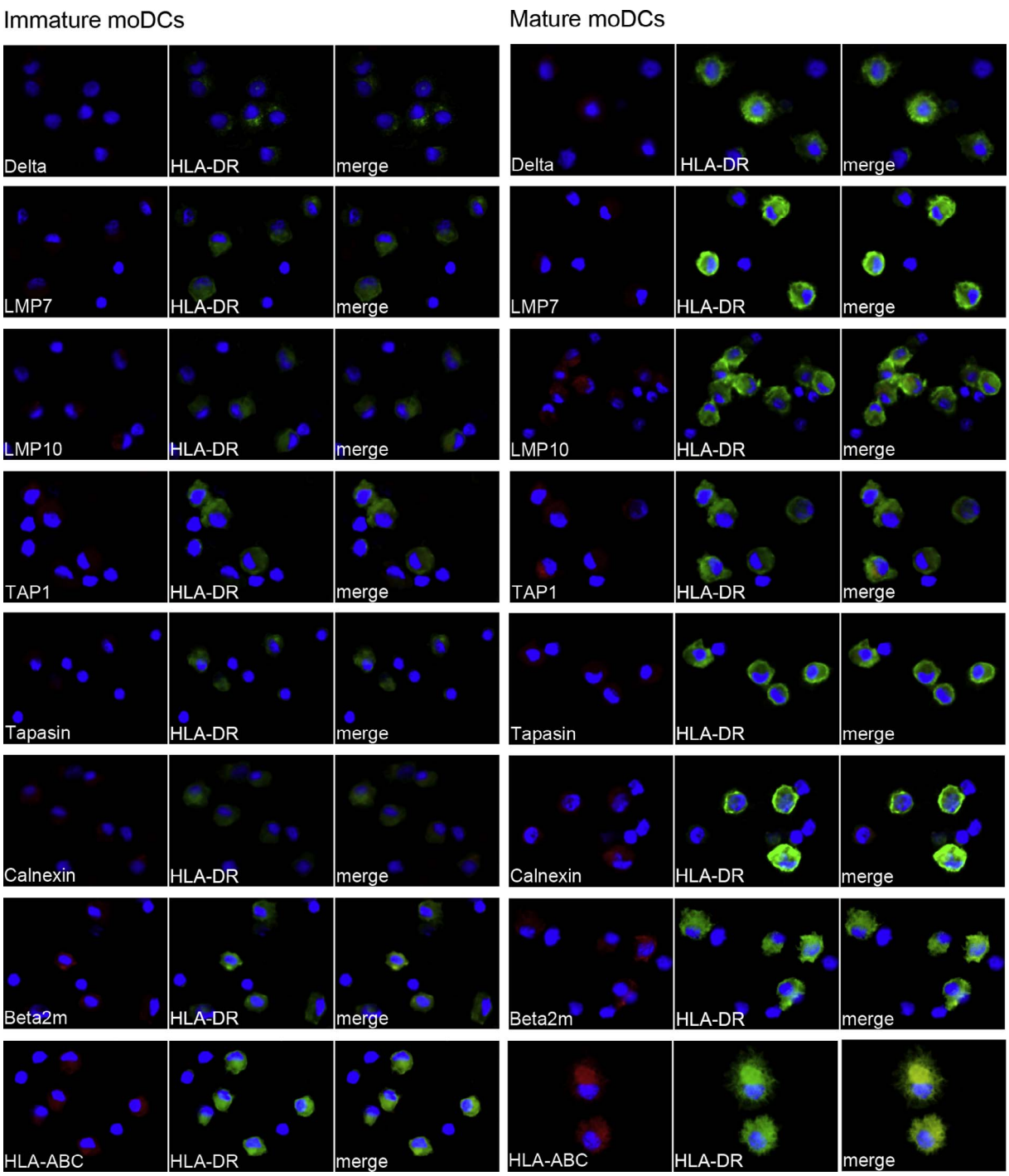

Fig. 1. HLA-class I APM components in immature and mature moDCs. Double fluorescent immunocytochemistry analyses were performed on immature and LPS-matured monocyte-derived dendritic cells (moDCs) deposed on slides by centrifugation (cytospin) and fixed in cold acetone. The fluorescent immunocytochemistry labelling was performed with the indicated antibodies, revealed with Alexa Fluor 594 (*red*) and 488 (*green*), respectively. Co-expression of antigens is indicated as merge (*yellow/orange*). Nuclei were labelled with Hoechst dye (*blue*). Details from representative images are shown. Immature moDCs (left panels) expressed a faint APM cytoplasm signal for Delta, LMP-7 and Tapasin, which increased in LPS-matured moDCs (right panels). LMP-10, TAP-1 and Calnexin were detected both in immature and mature moDCs, although their expression increased with maturation. Beta2-microglobulin and HLA-ABC expressed a strong plasma-membrane signal both in immature and matured moDCs (*Original magnification x400*). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

components may be important for initiating melanoma antigen-specific immunity.

Taking advantage of a panel of APM component-specific monoclonal antibodies (mAbs) we have developed, we have first investigated APM component expression level in immature and mature moDCs from healthy PBMC; then in fresh epidermal immature LCs and in epidermal derived migrated mature LCs from healthy skin. Finally we have measured APM component expression level in LCs from SLN of melanoma patients and we have correlated these values with the Breslow thickness of primary melanoma tumours and with the presence of metastases in SLNs.