Decreased expression of pSTAT, IRF-1 and DAP10 signalling molecules in peripheral blood lymphocytes of patients with metastatic melanoma

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ABSTRACT

Aims As numerous signalling molecules regulate effector functions of peripheral blood lymphocytes (PBLs) that have an important anti-tumour activity, the aim of this study was to analyse their level in patients with metastatic melanoma (MM) compared with healthy controls (HCs).

Methods Peripheral blood mononuclear cells (PBMCs) of 36 MMs and 28 HCs were analysed for the level of perforin, interferon-regulating transcription factor-1 (IRF-1), DAP10 and Src homology 2 domain-containing tyrosine phosphatase-1 by reverse transcriptase PCR, level of phosphorylated signal transducers and activators of transcription (pSTAT)-1, pSTAT-4, pSTAT-5 by western blot and interferon (IFN)-γ production by ELISA. The expression of activating NKG2D and inhibitory killer immunoglobulin-like receptors (KIR), CD158a and CD158b, on PBL, CD3−CD56+ natural killer (NK) cells and CD3+CD8+ cytotoxic T lymphocytes (CTLs), as well as the percentage of CD14+HLA-DR- cells in PBMC were estimated by flow cytometry.

Results Patients with MM, compared with HCs, had significantly lower level of cytotoxic molecule perforin and decreased IFN-γ production, as well as lower level of pSTAT-1, pSTAT-4, pSTAT-5 and IRF-1 signalling molecules in PBMC. Furthermore, MM had decreased expression of activating NKG2D receptor on PBL and NK cells and low level of its DAP10 signalling molecule contrary to no changes in KIR expression on all investigated cells. These results could be associated with increased percentage of immunosuppressive CD14+HLA-DR− myeloid-derived suppressor cells detected in patients with MM.

Conclusions The altered signalling molecules of PBL could represent biomarkers of impaired cytotoxic and immunoregulatory function of these cells, indicating melanoma-associated immunosuppression that facilitates tumour progression.

INTRODUCTION

Natural killer (NK) cells and CD8+ cytotoxic T lymphocytes (CTLs) exert their cytotoxic function against tumour cells by releasing perforin and granzymes from their granules and together with CD4+ T lymphocytes have an immunoregulatory role by producing many cytokines especially interferon (IFN)-γ.[1]

It is well known that numerous transcription factors such as signal transducers and activators of transcription (STATs) or interferon-regulating transcription factor-1 (IRF-1) play an important role in regulating and maintaining both innate and adaptive immune responses. STAT proteins regulate the proliferation and survival of lymphocytes, Th1 differentiation, as well as cytotoxicity and cytokine production by NK and T cells.[2 3] Furthermore, IRF-1 is a critical effector molecule in IFN-γ-mediated signalling and in the development and function of NK, NKT cells and CTL.[4–7] However, there are only a few data in literature about the level of these molecules in peripheral blood lymphocytes (PBLs) of patients with melanoma and healthy controls (HCs).[8]

The activating NKG2D, c-lectin-like receptor, is expressed by a variety of immune cells, mostly NK cells and CTL. This receptor upon binding to its ligands, the major histocompatibility complex (MHC) class I chain-related proteins A and B, and the UL-16 binding proteins, upregulated on transformed cells, in association with its DAP10 signalling molecule induces NK cell and CTL-mediated cytotoxicity against cancer.[9] In this sense, NKG2D-DAP10 receptor complex is fully sufficient to trigger NKG2D-mediated lymphocyte killing against tumour cells.[10]

Killer immunoglobulin-like receptors (KIRs) belong to the immunoglobulin superfamily, and they are responsible for the inhibition of NK cell-mediated lysis of normal cells that express MHC-I molecules. In this sense, according to the 'missing-self' hypothesis, the activation of NK cells occurs in contact with malignantly transformed cells that have lost MHC-I molecules and have therefore become susceptible to lysis. Other than NK cells, CD4 and CD8 T cells, as well as γδ T cells also express KIRs.[11] Depending on the sequence in their intracellular domain, KIRs are divided into inhibitory and activating receptors, although inhibitory KIRs are dominant. These KIRs display immunoreceptor tyrosine-based inhibition motifs, which are tyrosine-phosphorylated upon cross-linking with MHC-I molecules and recruit Src homology two domain-containing tyrosine phosphatases, SHP-1 and SHP-2, that dephosphorylate activating adaptor molecules primarily DAP10 and Vav-1 and stop activation.[12]

Melanoma is the most aggressive form of skin cancer due to its capacity to form metastases.[13] Aside from that, various experimental and clinical data indicate that melanoma cells are susceptible to NK cell and CTL-mediated cytotoxicity.[14] However, it is well known that tumour cells and numerous immunosuppressive cells in tumour microenvironment such as myeloid-derived suppressor cells (MDSCs) by producing cytokines,

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