Decreased expression of NKG2D, NKp46, DNAM-1 receptors, and intracellular perforin and STAT-1 effector molecules in NK cells and their dim and bright subsets in metastatic melanoma patients

Katarina M. Mirjačić Martinović[a], Nada Lj. Babović[b], Radan R. Džodić[c,d], Vladimir B. Jurišić[f], Nikola T. Tanić[e] and Gordana M. Konjević[a,d]

Although natural killer (NK) cells play an important antitumor role, melanoma cells may affect their effector functions. In this study, we analyzed the expression of various receptors and effector molecules in NK cells and their subsets in metastatic melanoma (MM) patients compared with healthy controls (HCs). In HC and MM patients, we analyzed NK cell activity using a chromium release assay and the expression of CD107a degranulation marker, activating NKG2D, NKp46, DNAM-1, and inhibitory CD158a and CD158b receptors, IL-12R beta 1, IL-12R beta 2, intracellular interferon (IFN)-γ, perforin, and STAT-1 in CD3-CD56+ NK cells, and cytotoxic CD3-CD56^{dim} and immunoregulatory CD3-CD56^{bright} subsets by flow cytometry. MM patients compared with HC not only had significantly decreased NK cell activity, lower expression of CD107a, and impaired IFN-γ production but also had decreased expression of activating NKG2D, NKp46, and DNAM-1 receptors, which was followed by lower expression of perforin, STAT-1, and both IL-12R subunits in NK cells. In MM patients only, there was a positive correlation between NKG2D expression and degranulation capacity, as well as IFN-γ production in NK cells. Analysis of the expression of various parameters of NK cell effector functions between MM patients with different localization of distant metastases showed that patients in the unfavorable M1c subclass had decreased expression of NKG2D and NKp46 on NK cells compared with patients in the M1a+b group. Downregulated NKG2D, NKp46, and DNAM-1 receptors associated with impaired NK cell effector function are important biomarkers of advanced disease with a poor prognosis in melanoma patients. *Melanoma Res* 24:295–304 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Melanoma Research 2014, **24**:295–304

Keywords: cytotoxicity, DNAM-1, interferon-γ production, metastatic melanoma, natural killer cells, NKG2D, NKp46, perforin, STAT-1

Departments of [a]Experimental Oncology, [b]Medical Oncology, [c]Surgical Oncology Clinic, Institute of Oncology and Radiology of Serbia, [d]School of Medicine, University of Belgrade, [e]Department of Neurobiology, Institute for Biological Research 'Siniša Stanković', University of Belgrade, Belgrade and [f]Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia

Correspondence to Katarina M. Mirjačić Martinović, MD, MSc, Department of Experimental Oncology, Institute of Oncology and Radiology of Serbia, Pasterova 14, 11000 Belgrade, Serbia
Tel: +381 11 2067 290; fax: +381 11 2685 300;
e-mail: kmirjacic@sezam.net

Received 5 November 2013 **Accepted** 11 March 2014

Introduction

Melanoma, a potentially fatal form of skin cancer that arises from pigment cells, melanocytes, is characterized by a rapid progression to distant organs as well as by a limited efficiency of currently applied therapeutics [1]. Besides this, it has been shown, in both murine and human models, that melanoma cells are susceptible to natural killer (NK) cell-mediated cytotoxicity [2].

NK cells are a subset of lymphocytes that play a central role in the innate immune response toward tumors without previous sensitization. They directly kill target cells by releasing perforin and granzymes from their preformed granules and play an immunomodulatory role by producing various cytokines, primarily interferon (IFN)-γ [3]. CD107a, a membrane molecule of cytolytic granules of NK cells, is strongly upregulated on the surface of these cells after their stimulation by tumor cells and its expression is associated with perforin release. In this sense, CD107a has been described as a marker of NK cell cytotoxicity [4].

It is well known that numerous transcription factors including signal transducers and activators of transcriptions (STATs) regulate cytotoxicity and the immunoregulatory function of NK cells by affecting the transcription of perforin and IFN-γ genes [5]. However, there are only few data in the literature on the expression of molecules such as STAT-1 in NK cells of healthy individuals and especially in cancer patients [6].

In humans, NK cells are usually defined as $CD3^{-}CD56^{+}$ cells and they can be subdivided into two functionally and phenotypically different subsets on the basis of CD56 expression. Typically, $CD3^{-}CD56^{dim}$ NK cells, which constitute the majority of peripheral blood NK cells, express a high level of CD16, several types of inhibitory receptors for major histocompatibility class-I (MHC-I) molecules, and have a high level of perforin. In this sense, they play a key role in NK cell cytotoxicity. However, $CD3^{-}CD56^{bright}$ NK cells are more abundant in secondary lymphoid tissues, and they have a low level of CD16 and a much lower perforin expression than $CD56^{dim}$ cells. They produce abundant

0960-8931 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CMR.0000000000000072