Beneficial in-vitro effects of interleukin-2, interleukin-12, and their combination on functional and receptor characteristics of natural killer cells in metastatic melanoma patients with normal serum lactate dehydrogenase levels

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Considering tumor-mediated suppression of natural killer (NK) cells, the aim of this study was to investigate the in-vitro effects of interleukin (IL)-2 and IL-12, as immunostimulatory cytokines, on the functional and receptor characteristics of NK cells and their subsets in healthy control (HC) and metastatic melanoma (MM) patients. Peripheral blood mononuclear cells of 27 HC and 35 MM patients were stimulated *in vitro* with IL-2, IL-12, and their combination for functional and phenotypic analysis. IL-2, IL-12, and primarily their combination, significantly induced NK cell activity, CD107a degranulation marker, and perforin expression in NK cells and their subsets in HC and MM patients. Furthermore, the combination of IL-2 and IL-12 was significantly more efficient than IL-12 alone in the augmentation of NK cell cytotoxicity and CD107a expression. Also, IL-2 and IL-12 reciprocally upregulated each other's receptors, IL-2Rα and IL-12Rβ1/β2, on NK cells and their subsets in MM and HCs. In addition, the priming of NK cells with IL-2 before IL-12 treatment led to an increase in the expression of both IL-12 receptors. In contrast to IL-12, IL-2 increased activating NKG2D and DNAM-1, as well as inhibitory CD158a and CD158b KIRs. In addition, the cytokines investigated exerted a more potent effect on the increase in NK cell activity and the expression of various NK cell receptors in MM patients with normal lactate dehydrogenase (LDH) serum levels. Therefore, serum LDH could represent a predictor of response to cytokine immunotherapy in MM patients. The optimization of combined IL-2/IL-12 therapy is needed to enhance NK cell functions in MM patients stratified by their LDH levels. *Melanoma Res* 26:551–564

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Introduction

Human natural killer (NK) cells are important components of the innate immune system. They control tumors by releasing perforin and granzymes from their cytolytic granules as well as by producing many cytokines, primarily interferon-γ (IFN-γ) [1].

The balance between NK-cell-activating and NK-cell-inhibitory signals mediated by various receptors regulates NK cell effector functions [2]. NKG2D, as the most prominent activating c-lectin-like receptor, upon binding to stress-induced ligands constitutively expressed on transformed cells such as major histocompatibility complex (MHC) class-I-related molecules, MICA/MICB, and ULBP1–4, induces NK cell cytotoxicity against cancer [3]. DNAM-1 is a coreceptor expressed by almost all NK cells and its stimulation by interaction with various members of the nectin family on tumor cells leads to NK cell activation and target cell lysis [4].

In contrast to this, killer immunoglobulin-like receptors (KIRs) that belong to the immunoglobulin superfamily are responsible for the inhibition of NK-cell-mediated lysis of normal cells that express MHC class-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 class-I molecules and therefore become susceptible to lysis [5]. According to the length of their cytoplasmic tail, KIRs are classified into long (e.g. KIR2DL and KIR3DL), inhibitory, and short (e.g. KIR2DS and KIR3DS) stimulating receptors, although inhibitory KIRs are dominant [6].

In humans, $CD3^{-}CD56^{+}$ NK cells can be subdivided into two phenotypically, functionally, and developmentally different NK cell subsets on the basis of CD56 receptor expression. The mature $CD3^{-}CD56^{dim+}$ subset, besides the high expression of inhibitory KIRs and the CD16 activating receptor, also expresses abundant perforin and granzymes in their granules and is involved in NK cell

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