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Mechanisms of immune evasion of human neuroblastoma

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Abstract

Neuroblastoma (NB) is a common neuroectodermal tumor of childhood with low response to conventional therapy in patients with advanced stage disease. Among novel strategies, immumotherapy has attracted much interest. However, scanty information is available about the immunogenicity of human NB.

Here, we review our data showing that human NB may evade the control mediated by T cytotoxic lymphocytes and natural killer (NK) cells through multiple mechanisms:

(i) downregulation of HLA class I molecules and antigen processing machinery components
(ii) downregulation of activating ligands for the activating immunoreceptor NKG2D expressed by cytotoxic T lymphocytes and NK cells.

Additional mechanisms of immune evasion used by human NB cells are discussed.

Keywords: Neuroblastoma; Immunotherapy; NK cells

1. Introduction

A tumor cell is immunogenic when it can stimulate the host immune system to evoke responses to tumor-associated antigens (TAAs). Molecules of the major histocompatibility complex (MHC) class I and class II, together with costimulatory molecules, are required in the induction and maintenance of T cells activation.

The main effector cells of the immune system involved in tumor cell recognition are:

(1) $CD8^+$ T cells, which specifically recognize through their T cell receptor (TCR) peptides presented by antigen presenting cells (APC) in the context of MHC class I molecules and mediate cytotoxic functions; (2) NK cells, innate effectors, which exert their cytotoxic activity through a diverse repertoire of

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activating and inhibiting receptors that recognize specific ligands on the surface of the target cells. (3) $CD4^+$ T lymphocytes and macrophages are other important players of anti-tumor immune responses, mainly through the production of cytokines and inflammatory mediators.

Neuroblastoma is a common extracranial pediatric tumor with low response to conventional therapy in patients with advanced stage disease [1]. Novel alternative strategies have emerged to circumvent such treatment failures and among them immunotherapy has attracted much attention.

The initiation of a successful T and/or NK-mediated immune response depends on different factors, including antigen, MHC, ligands for activating NK immunoreceptors and costimulatory molecules.

2. Antigen-processing pathway associated to MHC class I

Processing and presentation of tumor antigens by MHC class I molecules requires three major steps [2]:

(i) Tumor antigen degradation into 8–10 amino acid peptides operated by the proteasome and the immunoproteasome [2].
(ii) Peptides translocation into the lumen of the endoplasmic reticulum, driven by the transporter associated with antigen processing (TAP) in conjunction with the chaperons tapasin, calnexin, calreticulin and Erp-57 [2].
(iii) Loading of the peptides onto mature MHC class I molecules in the endoplasmic reticulum, with subsequent transfer of the peptide-MHC class I molecules to the cell surface [2].

Thus, cytotoxic T-lymphocytes can recognize the antigenic peptide associated to HLA class I molecules through their T cell receptor (TCR) and induce lysis of the tumor cell [2].

Many of the inhibitory receptors (KIR) expressed by natural killer cells (NK) recognize MHC class I molecules and provide protection for the cells that express normal amount of HLA class I molecules on the surface [3]. In contrast, downregulation of HLA class I due to viral infection or transformation, together with expression of specific ligands for activating receptors, enhances target cell sensitivity to NK cell-mediated cytotoxicity [3].

3. Neuroblastoma immune recognition by cytotoxic T cells

Different tumor-associated antigens, which can be recognized by cytotoxic T cells, have been characterized both in NB cell lines and primary tumors [4]. They include the family of the embryonic genes MAGE, BAGE and GAGE, the disialoganglioside GD2, the CD56 molecule and some others [4].

The majority of human tumors often present loss or downregulation of MHC class I and II molecules [5]. The mechanisms responsible for these defects can be summarized as follows:

- Total human leukocyte antigen (HLA) class I loss attributable to β_2-microglobulin (β_2-m) mutation [5].
- Total HLA class I downregulation related to multiple defects including altered binding of regulatory factors to the β_2-m free heavy chain (HC) gene enhancer element and abnormalities in the expression or function of different components of the HLA class I antigen processing and presentation pathways [5].
- Selective loss or downregulation of HLA class I allospecificities [5].

It has been demonstrated that NB cell lines display low to absent expression of MHC class I and II molecules [6]. Moreover, defects in β_2-m, β_2-m free HC and TAP1 molecules, which can be corrected by γ-interferon (γ-IFN) have been described in NB cell lines [7]. However, no information is available about the expression of β_2-m, β_2-m free HC and TAP1 as well as the antigen processing machinery (APM) components in primary tumors. In ongoing studies, we are performing a systematic analysis of the expression of APM components in NB primary tumors and NB cell lines, utilizing a panel of newly developed antibodies (mAb) and a recently developed intracellular staining technique. In preliminary experiments expression of β_2-m, β_2-m free HC,