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Cover Page Footnote
This scientific material is part of a lager retrospective study of a PhD thesis, currently under development by the main author, M.D., Ph. D. Student at the Titu Maiorescu University of Bucharest, Faculty of Medicine with Prof. Cristiana Tanase, M.D., PhD., as thesis coordinator. All authors have read and approved the final manuscript and also declare that received no funding for publishing this material and that there are no conflicts of interest.

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Review

Natural killer (NK) cells and their involvement in different types of cancer. Current status of clinical research

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Abstract Natural killer cells are the main agents of innate immunity. Since 1970, various studies have repeatedly confirmed their involvement in decreasing local tumor growth and also decreasing the risk of metastasis, due to their cytotoxic effects and also through the release of immunostimulatory cytokines such as IFN-gamma. In the 1990s, several studies demonstrated the existence of certain inhibiting and stimulating receptors of these cells, leading to the concept of "induced self", thus explaining why tumors with MHC-1 are destroyed and autologous cells without it are saved out. Recognition and destruction of tumor cells by the NK cells are the result of complex interactions between inhibiting and activating factors. This paper, based on extensive research of currently available studies, summarizes the mechanisms employed by the NK cells to destroy the cancer cells, thus highlighting their role in the risk of tumor recurrence as well as their use and handling in certain types of immunotherapy.

Keywords: natural killer, cells, cancer, action, mechanisms

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Introduction

In 1975, interest was raised by the identification of certain cells with lymphocyte morphology, present in both humans and mice, cells with the ability to destroy modified cells without being previously activated (1). The difference between B and T lymphocytes is represented by the existence of a primary entity that does not require activation (2).

Karre and colleagues introduced the "missing self" hypothesis that basically stipulates that these cells have the ability to detect and destroy cells with MHC-1 deficiency (major histocompatibility complex) (3). In the 1990s, several studies demonstrated the existence of certain inhibiting and stimulating receptors of the NK cells, leading to the concept of "induced self", thus explaining why tumors with MHC-1 are destroyed and autologous cells without expression of MHC-1 are saved out (4).

Consequently, these cells can identify and destroy a wide range of abnormal cells (tumor cells, virally infected cells, cells coupled with antibodies, cells under a certain degree of stress), preserving healthy "self" cells (5). NK cells represent 5-20% of the mononuclear peripheral blood cells, usually defined as CD16+, CD 56+, CD3- and are found in the liver, peritoneal cavity, placenta and the uterine mucosa (6).

Depending on the density of CD16 and CD56 present on the surface, NK cells can be divided into two subpopulations: CD56 dim (moderate presence of CD56, predominantly displaying CD16 – and with high cytotoxic potential) and CD56 bright (CD16 presence greatly reduced, reduced cytotoxicity but with high cytokine production after activation) (7). Recently, a new NK cell marker – NKp46- has been discovered in humans and mice (8, 9).

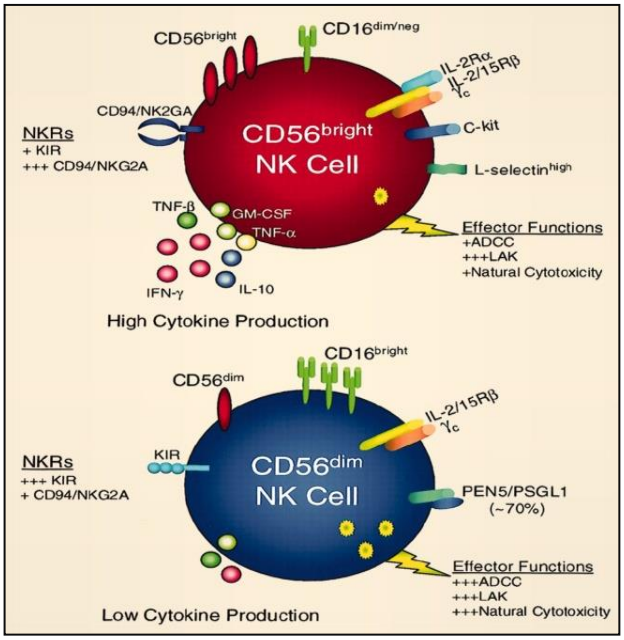

Figure 1. The High and Low-Cytokine Production (schematic representation) (9).

Discussion

- *The role of NK cells in cancer*

Growth and tumor invasion are the result of interactions between the tumor and the surrounding tissues, by initiating angiogenesis and the involvement of the immune system (innate and acquired). Clinicopathological signification of these processes is given by the infiltration of the tumor with lymphocytes. Lymphocytes T CD8+ and NK cells are representative agents of the anti-tumoral immunity (10).

Some studies have shown a link between the number of lymphocytes and survival in certain types of cancer, as the lower the number of NK liver cells the more advanced the neoplasm. These results suggest that the metastasis is due to ineffective antitumor liver mechanisms because of a low number of NK cells (11, 12). Studies were extended in order to explain the decline in the number of the NK cells in advanced cancer stages, demonstrating that the tumor allegedly inhibits the NK activating receptors and stimulates the

inhibitory receptors, thus conducing to metastatic expansion (13).

These results have defined the concept of the tumor cell associated with the NK cell phenotype – NK humoral infiltrates (14-16). In certain cancers, not only NK cells present in the tumor may have different phenotypes or display a decrease in cytotoxicity but also the NK cells in the peripheral blood may show the same changes (studies done on patients with metastatic malignant melanoma have ascertained the reduction of NK cell activity and a reduction of IFN gamma production as well as an increase in CD16 in the detriment of the CD56 bright). Although the molecular mechanisms responsible for reducing receptor activity in peripheral blood are not yet known, the hypothesis is that the increase in the soluble serum values of tumor receptors would inhibit the cell receptors, contributing to a diminished NK cell activity. It is certain that the NK cells have a tumor fighting potential but, taken in consideration the results mentioned above, work must continue towards fully understanding and activating this potential (17).

- *Mechanisms of action for natural killer cells*

NK cells recognize target cells via receptors on the cell surface, which can be inhibited or stimulated by various conditions. For NK cells to be active, the receptors must be stimulated. Until now we know 3 types of NK cell action:

1. Throughout perforin-granzime system. Releasing of these cytotoxic beads is the fastest and the most effective mechanism for cell lysis. Trials made on perforin-deficient mice have shown a reduced ability of tumor lysis, suggesting that perforins are indispensable for NK cell cytotoxicity. Several studies have shown the importance of perforins in tracking the cancer relapse risk. The role of granzimes is not understood yet (18);

2. The induction of apoptosis is done by the TNF ligand family. This mechanism is slower (several hours) and less efficient. It needs the presence of TNF ligands on the cellular surface, ligands that will latch onto the Fas receptors on the surface of the target cells (19);

3. Through the activity of the IFN-gamma, the activated NK cells secrete numerous cytokines (IFN-g, TNF-alpha, IL-10, IL-13 etc.). Among IFN effects, the following must be highlighted: inhibits cell proliferation in vitro and indirectly slows tumor growth in vivo by stimulating anti-angiogenic factors; increases the sensitivity of tumor cells to the action of perforins and apoptosis; elimination of sarcoma and metastasis induced by methylcholanthrene (carcinogenic chemical agent); stimulates the dendritic cells, by which it indirectly contributes to tumor control, by means of T-lymphocytes (20).

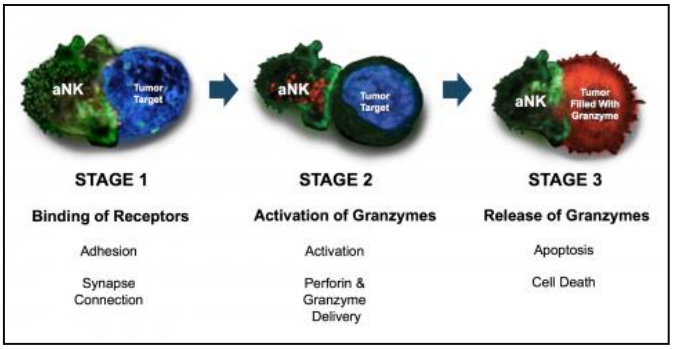

Figure 2. The schematic view of the mechanism of action and innate killing of a NK cell (with permission from http://nantkwest.com/platform/).

- *The role of NK cells in limiting the tumor growth and metastasis*

The current advances in this field have established some facts. Among them:

✓ It has been shown in mice that the tumor cytotoxicity depends on the presence of cell surface ligands. There is little available information about the mechanisms of NK cell migration in tumors, while it is established that selectins play a role in this process (21).

✓ Mice with a low NK cell count have a higher predisposition to chemical induced neoplasms, hence a role of NK cells in tracking risk of developing malignancies can be considered (22).

✓ Experiments on animals have shown the ability of NK cells to inhibit the development of lung metastasis following treatment with IFN.

✓ An 11-year study on humans has shown an increased risk of developing malignancy in patients with low activity of NK cells (for example, patients with hereditary colorectal adenocarcinoma and metastatic melanoma have an altered mechanism of perforins (23).

✓ Following administration of tumor cells in mice, NK cells released IFN, which stimulated dendritic cells, promoting a strong anti-tumor response of lymphocytes T CD8+ (24).

- *The involvement of NK cells in the anti-tumor management*

Although we know the NK cells advantage over the T lymphocyte cells in the anti-tumor fight, their therapeutic potential yet remains unexplored. Research on the scientific mechanisms of enhancing or inhibiting NK cells as well as methods to make tumor cells receptive to the cytotoxic activity of the NK cells led to the development of numerous genetic and pharmacological methods to increase NK cell activity:

✓ Cytokine administration

The potential of the IL-2 to enhance NK cytotoxicity was observed in vitro. This finding led to conducting clinical trials on patients with metastatic melanoma and renal carcinoma. Trials in primates have shown increased systemic toxicity of IL-2, so that Berger used IL-15 with IL-2 like properties, considering appropriate the intermittent administration of IL-15 (25).

✓ Monoclonal antibody therapy

Administration of monoclonal antibodies to target the tumor may induce rapid degranulation of NK cells and cell lysis. The efficiency of the monoclonal antibodies anti-CD20 (Rituximab), anti-Her2 (Transtuzumab™), receptors for epidermal growth factor (Cetuximab™) is due partially to the cytotoxicity of NK cells antibody-addicted (26-28).

✓ Blocking the inhibitory receptors

Blocking the inhibitors receptors Ly49 increases tumor activity both in vivo and in vitro. Current experiments test antibodies which block KIR (post-transplant of hematopoietic stem cells) (29).

✓ Vaccinating the tumor with dendritic cells is currently in the (early) experimental stage (30).

- *Ongoing clinical studies on NK cells*

✓ Study of the Combined Therapy for Pancreatic Cancer (Fuda Cancer Hospital, Guangzhou). This study focuses on finding the differences in behavior of advanced pancreatic cancer patients that received both irreversible electroporation (IRE) and immunotherapy of nature killer (NK) cells versus patients that received only immunotherapy of nature killer (NK) cells without irreversible electroporation (IRE). It is in progress since 2016;

✓ Intraperitoneal Natural Killer Cells and INCB024360 for Recurrent Ovarian, Fallopian Tube, and Primary Peritoneal Cancer (Masonic Cancer Center, University of Minnesota, USA). This is a single center phase I trial designed to determine the maximum tolerated dose (MTD) of the oral IDO inhibitor INCB024360 when administered as part of a larger regimen of intraperitoneal (IP) delivery of haplo identical donor NK cells and IL-2after a non-myeloablative cyclophosphamide/ fludarabine (Cy/Flu) preparative regimen for the treatment of recurrent ovarian, fallopian tube, and primary peritoneal cancer;

✓ Natural Killer Cells Plus IL-2 Following Chemotherapy to Treat Advanced Melanoma or Kidney Cancer (National Cancer Institute, USA)- This study determines the ability of the administration of autologous natural killer (NK) cells plus aldesleukin (IL-2) following a non-myeloablative lymph depleting preparative regimen to mediate tumor regression in patients with metastatic melanoma or kidney cancer, to determine the rate of repopulation of the natural killer

cells in treated patients and to find the overall toxicity of this treatment regimen;

✓ NK Cell Infusions with Trastuzumab™ for Patientswith HER2+ Breast and Gastric Cancer (National University Hospital, Singapore). This study focuses on the ability of Transtuzumab, a monoclonal antibody against HER-2 positive breast or gastric cancer, to deliver cytotoxic effects. It is used in combination with immunotherapy in treating HER2- positive tumor cells. This study will determine the response of the expanded activated autologous NK cells administered after Trastuzumab in patients with HER2-positive breast or gastric cancer.

✓ Natural Killer Cells and Bortezomib™ to Treat Cancer (National Institutes of Health Clinical Center, USA). This ongoing study is centered on the idea that pre-administration of Bortezomib™ makes NK cells more sensitive to TNF-related apoptosis-inducing ligand (TRAIL), as in vitro studies have already confirmed. This study will determine if there are the same effects in vivo as well.

✓ NK White Blood Cells and Interleukin in Children and Young Adults with Advanced Solid Tumors (National Cancer Institute, USA). This study will determine the safety and efficacy of administration of activated NK cells in solid tumors at children and young adults.

Conclusions

NK cells could be real weapons in the anti-tumor fight and could be employed to induce an optimal immune response against cancer. A better understanding of the molecular mechanisms of action of the NK cells provides the way for the development of new strategies to manipulate these cells in the fight against cancer.

Acronyms and abbreviations:

NK: Natural Killer; MHC-1: major histo-compatibility complex;

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