

REVIEW ARTICLE

Research progress of NK cell immunodeficiency in immune escape of acute leukemia

Yuling Zhang¹, Zhong Yu², Hailiang Li^{2*}

¹ Gannan Medical University, Ganzhou 341000, Jiangxi Province, China.

² Department of Hematology, The First Affiliated Hospital, Gannan Medical University, Ganzhou 341000, Jiangxi Province, China. E-mail: gyfyllhl@163.com

ABSTRACT

NK cell immunodeficiency has a variety of manifestations and complex mechanisms in the tumor. NK cell immune deficiency is closely related to immune escape of acute leukemia. This paper demonstrates the immunological escape mechanism of acute leukemia from NK cell immune deficiency manifestation and cause.

Keywords: NK Cell; Activated Killer Cell Receptor; Inhibitory Killer Cell Receptor; MicroRNA-29b; UL16 Binding Protein-3

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1. Introduction

Acute leukemia (AL) is a malignant clonal disease of hematopoietic stem progenitor cells, with rapid onset and progression. The natural course of disease usually lasts only a few weeks or months. Although chemotherapy and immunobiotherapy have seen great advances, 50% to 70% of patients with acute myeloid leukemia (AML) still suffer from relapses, and so do 20% to 30% of children with acute lymphoblastic leukemia (ALL)^[1,2]. Part of the reason for relapse is the immune surveillance dysfunction of the body's innate immune cells. Therefore, in order to develop more effective anti-leukemia drugs, it is necessary to understand how leukemia cells evade innate immunity. NK cell is a kind of large granular lymphocyte, which plays a very important role in the anti-leukemia process. Its immune deficiency can cause leukemia cells to evade the attack of host immune system. In this paper, we review the manifestations and pathogenesis of NK cell immunodeficiency in acute leukemia.

2. Expression of NK cell immunodeficiency

2.1 Abnormal proportion and reduced number of NK cell subsets

NK cells account for about 5%–15% of peripheral blood mononuclear cells (PBMC). Immature NK cells have high expression of CD56 (CD56^{bright} NK cells) and high expression of NKG2A, but no expression of CD16. Mature NK cells have low expression of CD56, but no expression of NKG2A, namely, CD56^{dim} NK cells^[3]. Data showed that the percentage and absolute number of NK cells in peripheral blood of

AL patients decreased^[4-7]. It has been reported that the percentage of NK cells in various organs (spleen, bone marrow, blood and lymph nodes) of mice with leukemia is lower than that of mice without leukemia. The absolute NK cell count of bone marrow and lymph node was lower than that of non-leukemia mice. There is the selective reduction of CD27⁺CD11b⁺ double positive NK cells in various organs of mice with leukemia (the maturation process of mouse NK cells is CD27⁻CD11b⁻→CD27⁺CD11b⁻→CD27⁺CD11b⁺→CD27⁻CD11b⁺)^[8]. Rouce *et al.* reported that CD56^{bright}-CD16⁻NK cells in ALL patients were more than that in healthy people^[9]. However, Mundy Bosse and Rey *et al.* found that the reduction of total CD56^{bright} NK cells and CD56^{bright} CD16⁻NK cells in peripheral blood of patients with AML may be different from that of patients with ALL^[8,10]. In conclusion, NK cells in AL patients are not only abnormal in proportion, but also have trouble maturing, especially in AML patients.

2.2 Weakened NK cell killing ability

Activated NK cells can non-specifically kill tumor cells through the following pathways. (1) Directly kill tumor cells through FASL/FAS, perforin, granulation enzyme pathway. (2) FCγR(CD16) mediated the ADCC effect by binding to the FC segment of anti-tumor antibody^[4]. (3) Secretion of IFN-γ, TNF-α, TNF-β can produce anti-tumor effect. It was found that the B expression of perforin and granase decreased in NK cells of leukemia mice, and the intracellular IFN-γ content decreased after stimulation^[8]. Clinical studies have shown that both IFN-γ and TNF-α of NK cells in patients with leukemia are reduced, and their killing ability is weakened^[9,11].

2.3 Reduced NK cell proliferation^[5]

Immune cells' maintaining certain proliferation ability is the basis of maintaining normal immune function of the body. The percentage of NK cells in spleen mitosis of leukemic mice was lower than that of non-leukemic mice by flow cytometry. 24 h after injection of IL-15 (which can promote NK cell proliferation and differentiation), the NK cell proliferation capacity of all organs (spleen, bone mar-

row, blood, lymph node) was lower than that of non-leukemia mice^[8].

2.4 Abnormal NK cell receptor expression

Ho *et al.* analyzed the bone marrow of 78 newly diagnosed AML patients (46 cases < 60 years old, 32 cases ≥ 60 years old) and found that the receptor spectrum of NK cells was changed; except the activation receptors KIR2DL4, KIR2DS4, CD94/NKG2C, the expression level of other receptors including natural cytotoxic receptors (NKp30, NKp44, NKp46), killing immunoglobulin-like receptors (KIR2DL1, KIR2DL2, KIR3DL1) and activated receptors (DNAM-1, NKG2D) was lower than that of healthy people^[7]. Sanchez-Correa *et al.* found that in AML patients younger than 65 years old, the expression of DNAM-1 in NK cells of bone marrow and the levels of NKp30 and NKp46 also decreased, while the level of NKp46 in elderly AML patients (≥ 65 years old) also decreased^[12]. However, the expression of DNAM-1 and NKp30 was not different from that of healthy people. The reason of different results of these two studies is related to the different age distribution of patients in the two studies, but both of them indicate that the receptor spectrum of NK cells in leukaemia has changed, leading to the weakening of NK cells' recognition and activation ability.

NKG2A is a transmembrane protein with C-type lectin structure in the extracellular region and ITAM in the cytoplasmic region. CD94/NKG2A is a suppressor killer cell receptor, which can block NK cell-mediated cytotoxicity. Multiple studies have shown increased NK cell CD94/NKG2A expression in AML patients^[7,9,11,13]. Sandoval-Borrego *et al.* found that CD158b was overexpressed in NK cells of AML patients^[13]. For example, blocking CD158 recipient epitopes (CD158a, CD158b) can increase the killing activity of NK cells in AML patients^[14].

3. The mechanism of NK cell immunodeficiency

The mechanism of tumor immune escape has not been fully elucidated. The following viewpoints have been proposed: (1) tumor cells lack the components necessary to stimulate immune response; (2)

tumor antigen induces immune tolerance; (3) tumor cells induce apoptosis of immune cells or resist apoptosis; (4) malignant tumors directly or indirectly inhibit immune function^[15]. For AL immune escape, NK cell immune deficiency is one of the reasons. Recent studies have focused on the mechanism of NK cell deficiency leading to AML immune escape, mainly in the following aspects.

3.1 MicroRNA-29b mediates abnormal NK cell development

MicroRNA-29b is one of the three members of MicroRNA-29s family, and a large number of studies have confirmed that MicroRNA-29b is involved in tumor occurrence, migration and invasion^[16-18]. Mundy-Bosse *et al.* showed that the number of CD56^{bright} NK cells in peripheral blood of patients with acute myeloid leukemia (human NK cell de-

velopment process is shown in **Figure 1**) decreased^[18]. In order to find the cause, they used RT-PCR to measure the MicroRNA-29b of NK cells in leukemia mice, and found that CD11b⁺ NK cells (including the NK cells of middle and late stage mice): the expression of MicroRNA-29b in CD27⁺ CD11b⁺ NK cells and CD27⁻CD11b⁺ NK cells was higher than that in non-leukemia mice. NK cells with MicroRNA-29b knockdown were injected into leukemia mice with reduced WBC and improved survival. The same team also found increased MicroRNA-29b expression in CD56^{bright} NK cells from AML patients. Currently, the cause of MicroRNA-29b overexpression in leukemia NK cells is unclear and further research is needed, but many researchers have begun to develop MicroRNA-29b inhibitors to achieve anti-leukemia efficacy.

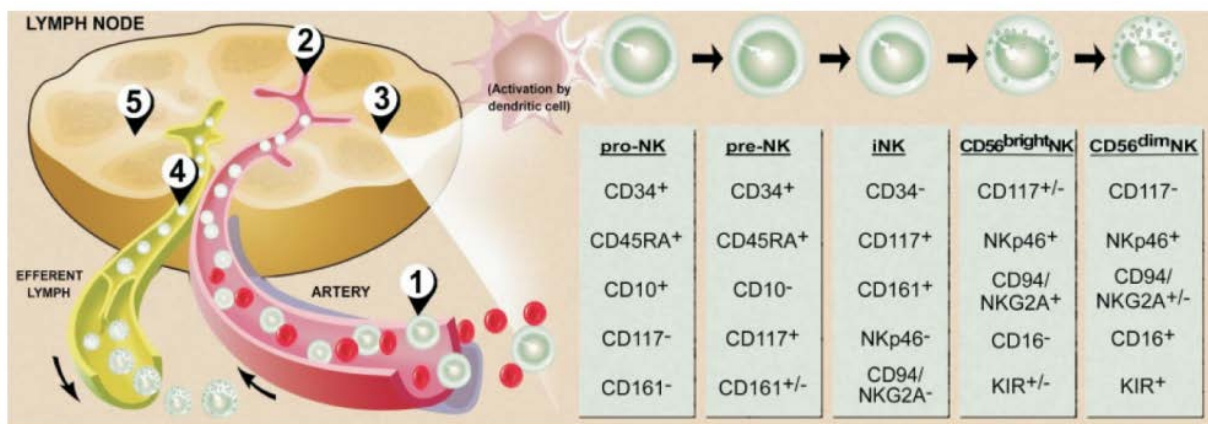


Figure 1. Pattern of human NK cell development process^[3].

3.2 Leukemia cells inhibit NK cells

3.2.1 CD200 binds to CD200L of NK cells

CD200 belongs to the immunoglobulin sub-family, which is expressed on the surface of various immune cells and is increased in solid tumors such as bladder cancer and lung cancer. Damiani *et al.* analyzed 244 AML patients and found that CD200 (also known as OX2) was expressed in leukemia cells of 56% AML patients, and CD200 expression was higher in patients with secondary leukemia^[19]. CD200 on the surface of leukemia cells can bind to CD200L on the surface of NK cells and inhibit the cytotoxicity and cytokine secretion of NK cells. It has been reported that the remission rate of patients with CD200⁺ is lower than that of patients with CD200⁻, which is associated with poor prognosis of

AML patients^[19-21].

3.2.2 Leukemic cells secrete IL-10

IL-10 is a negative regulator, mainly secreted by macrophages and dendritic cells. Stringaris *et al.* found that the killing ability of NK cells in patients with leukemia was weakened^[11]. In order to find the mechanism of NK cell inactivation, they co-cultured NK cells from healthy donors with acute myeloid leukemia cells (NK-AML) for 24 h. The expression level of IL-10 in the supernatant of NK-AML culture medium was significantly increased. Therefore, it is believed that leukemia cells of patients with AML can secrete IL-10, which has a significant immunosuppressive effect on NK cells.

3.2.3 Expression of NKG2D weakens NK cell function

NKG2D is generally expressed in NK cells and T cells. Tang *et al.* found that U937 and THP-1 cell lines not only express MHC I molecular related gene A of NKG2D ligand, but also express NKG2D^[22]. If NKG2D expression of U937 and THP-1 cell lines is blocked, NK cells of patients were activated by NKG2D ligand of U937 and THP-1 cell lines, showing degranulation and increased expression levels of CD107a, IFN- γ and TNF- α , namely, the expression of NKG2D in leukemia cells weakened the immune surveillance function of NK cells.

3.2.4 Secretion of TGF- β induces NK cell in-activation

TGF- β is a polypeptide that regulates tumor proliferation, growth, migration and invasion. Hong *et al.* reported that there were different forms of TGF- β exosomes in AML patients and they were higher than the control group, and the proportion was different at diagnosis, induction chemotherapy and after induction chemotherapy, but they were always present, which could inhibit NKG2D expression of NK cells, and the addition of TGF- β antibody could reverse the inhibition of NK cells^[23]. The authors suggest that TGF- β exosomes may predict the presence of minimal residual disease (MRD). Rouce *et al.* found that TGF- β was the most obvious cytokine in the supernatant of leukemia cells cultured in ALL patients, and TGF- β could be used to reconstruct NK cell functions such as cytokine secretion and degranulation^[9]. Further experiments demonstrated that TGF- β mediated the decline of NK cell immune surveillance function through TGF- β /SMAD signaling pathway, leading to the escape of leukemia cells. TGF- β /SMAD is expected to be the target of novel therapies to restore the anti-leukemic toxicity of NK cells by inhibiting this pathway.

3.2.5 UL16 binding protein-3

UL16 binding protein-3 (ULBP3) is a kind of NKG2D ligands, which is distributed in colorectal adenocarcinoma, gastric adenocarcinoma and lung adenocarcinoma tissue. Soluble ULBP3 exists in serum of colorectal adenocarcinoma and gastric

adenocarcinoma and is considered as a potential tumor marker^[24]. Jiang Q *et al.* reported that serum sULBP3 level in the initial AML group was significantly increased and negatively correlated with the proportion of NK cells^[25]. Vitro experiments showed that exogenous ULBP3 protein affected the number and cytotoxicity of NK cells through the mechanism of apoptosis.

3.2.6 Expression of indoleamine 2, 3-dioxygenase 1

Indoleamine 2, 3-dioxygenase 1 (IDO1) is an enzyme that can degrade tryptophan to canisurine, and is associated with pathological inflammatory response and tumor immune escape. Activation of IDO1 can inhibit T cells, NK cells and promote the proliferation of regulatory T cells. Studies have shown that TIM-3 of NK cells binds to GAL-9 on the leukemia cell membrane of AML patients and releases IFN- γ , which induces increased expression of IDO1 in leukemia cells, and IDO1 reduces the degranulation activity of NK cells, thereby avoiding NK cell killing^[26]. Ma Jinfeng reported that Gal-9 expression was increased in AML patients, and Tim-3 expression level in NK cells of medium-high risk AML patients was higher than that of low-risk AML patients^[27]. These two studies demonstrate that the Tim-3/Galectin-9 pathway mediates immune escape of AML tumor cells by down-regulating the NK cell immune response through IDO1, influencing patient outcomes.

3.2.7 Inducing NK cell to express receptor activator for NF- κ B (RANK)

Tumor necrosis factor family RANKL not only regulates bone metabolism and immune cell function, but also affects the survival of dendritic cells and the ability to stimulate T cells, and promotes tumor cell metastasis. Schmiedel *et al.* reported that 68% of AML patients expressed RANKL in their peripheral blood leukemia cells^[28]. On the one hand, leukemia cells of RANKL⁺ inhibit NK cells directly or by secreting a variety of active substances (TNF, IL-6, IL-8 and IL-10) through RANKL signal; on the other hand, the active substances secreted induce NK cells to express RANK. RANKL on leukemic cells can bind to NK cell RANK. When

RANKL is blocked by Denosumab, the secretion of TNF, IL-6, IL-8 and IL-10 in leukemic cells is significantly reduced and the NK cell inhibition is weakened. In conclusion, a vicious circle is formed between leukemia cells and NK cells, which leads to NK cell inactivation and leukemia cells evade immune surveillance.

3.3 TLR4⁺ MSC inhibits NK cells

Mesenchymal stem cells (MSC) are non-hematopoietic pluripotent cells that can be isolated from bone marrow and adipose tissue and can secrete a variety of cytokines and chemokines to promote tumor growth and metabolism. Lu *et al.* isolated MSC from bone marrow of lung cancer (LC) patients, AML patients and healthy people, and co-cultured MSC from healthy people with Hela cells (named CM^{Hela}-MSC)^[29]. It was found that LC-MSC, AML-MSC and CM^{Hela}-MSC were all highly expressed TLR4mRNA. Further experiments showed that intravenous injection of TLR4⁺ MSC into C57BL/6J mice could induce decrease of peripheral blood NK cells. Co-culture of TLR4⁺ MSC and NK cells reduced the expression level of NKG2D receptor in NK cells, and CytoTox 96 non-radioactive cytotoxicity test showed that NK cell toxicity decreased. When TLR4 function was blocked, NK cell function recovered. This study demonstrates that TLR4 plays a key role in MSC-induced NK cell function inhibition.

3.4 Immunological synapse defects in NK-AML cells

NK cells and tumor cells can form an immunological synapse (IS), also known as supermolecular activation cluster (SMAC). NK cell immune synapse can be divided into two types. One is inhibitory synapse, in which actin recombination is blocked and dissolved particles are not transferred to the synapse. The second is the dissolved synapse, in which the actin skeleton is reorganized and the dissolved particles are transferred to the synapse (requiring NK cell receptor (NCRs) polarization). It has been reported that the decrease of soluble particles in the immune synapse of NK-AML cells, and almost no aggregation of CD3 ζ (a marker of NCRs polarization) in the immune

synapse of NK-AML cells is the cause of the deficiency of the immune synapse. Researchers have found that lenalidomide can increase the dissolved particles in the immune synapse of NK-THP1 and NK-HLA60 cells, while the activation and inhibitory receptors of NK cells are not changed^[30]. However, whether lenalidomide can increase the soluble particles in the immune synapses of NK-AML and NK-ALL cells is unknown and needs further study.

3.5 IFN- γ inhibits NK cells in tumor micro

Type II interferon, or γ -interferon (IFN- γ), is produced mainly by activated Th1 cells and almost all CD8⁺T and NK cells. Previous studies have shown that IFN- γ has antitumor activity, while other studies have shown that IFN- γ has pro-tumor activity, but the reason for this contradictory result remains unclear. Bellucci *et al.* reported IFN- γ or the supernatant of NK cells can induce leukemic cells to up regulate programmed death-1 ligand (PD-1)^[31]. If PD-L1 is blocked by antibodies, the cleavage ability of NK cells is increased. If JAK inhibitors are used, the cleavage ability of NK cells is also increased. Therefore, the researchers believe that IFN in the tumor microenvironment- γ activate JAK1 and JAK2 signaling pathways, and then up regulate PD-L1; PD-L1 binds to programmed death-1 (PD-1) of NK cells, weakening the anti-tumor activity of NK cells.

4. Conclusion

Since one of the causes of AL relapse is immune system dysfunction, understanding the causes of immune system dysfunction is the basis for finding more effective immunobiotherapy. As an important part of the immune system, the deficiency of NK cells is one of the causes of leukemia immune escape. NK cell immune deficiency is manifested in many aspects, including abnormal number of NK cells, maturation disorder, reduced killing ability, reduced proliferation ability, decreased activation receptor expression and increased inhibitory receptor expression. The mechanisms of NK cell immune deficiency include: (1) MicroRNA-29b mediates changes in NK cell dysplasia; (2) leukemia cells inhibit NK cells through various mechan-

isms; (3) TLR4⁺ MSC inhibits NK cells; (4) NK-AML cell immunological synapse defects; (5) IFN- γ in tumor microenvironment inhibit NK cells. With the deepening of research, drugs targeting the above targets have been developed, and some have entered clinical research. NK cell-based immunotherapy is expected to become one of the effective means to treat AL.

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