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REVIEW

Advances in research on natural killer cell-associated tumor immune escape

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Abstract
Tumor immune escape plays an important role in carcinogenesis. Nature killer (NK) cells are human innate immune cells that do not need antigen sensitization prior to their activation. NK cells directly or indirectly kill tumor cells through cytotoxicity and immune regulation, but they are also involved in tumor immune escape. In this study, we reviewed the mechanism of NK cell-related tumor immune escape. The current knowledge on tumor cell alteration and the impact of inhibitory factors and the joint influence of other cells that lead to NK cell dysfunction and ultimately result in tumor immune escape have been researched and discussed. Understanding the potential mechanisms for restoration of the anti-tumor function of immune cells is of critical significance for discovering novel approaches for the treatment of tumors.
Keyword: tumor, immune escape, nature killer cell

Background

Tumor immune escape is a complicated multi-factor and multi-step process, which is among the ten characteristics of tumorigenesis. Immune escape is associated with an alteration of the tumor and the tumor microenvironment, as well as with suppression of the immune system. These are the necessary conditions for the tumor escape from the recognition and killing by the immune system, leading to proliferation, infiltration, recurrence, and metastasis [1-3]. Natural killer (NK) cells are specific immune cells involved in tumor immunity. They can not only directly kill tumor cells through cytotoxicity and immune regulation, but can also regulate the activities of other immune cells which indirectly kill tumor cells. Therefore, NK cells play an important role in tumor immune surveillance [4]. However, tumor cells escape the recognition and killing by NK cells through a variety of mechanisms. Thus, the restoration and enhancement of NK cell function is one of the most significant strategies for anti-tumor treatment. In this study, we reviewed the mechanism of immune escape of NK cells-related tumors.

1. Mechanism of tumor immune escape in carcinogenesis

In 2011, Hanahan and Weinberg [3] added four new features to the previously established six major tumor features: genome instability and mutation, pro-tumor inflammatory response, avoidance of immune attack, and abnormal cell energy metabolism, indicating immune escape is one of the most important tumor characteristics.

1.1 Tumor immune escape originated from the escaped tumor cells themselves

Tumor cells can evade the host's immune attack by the alteration of their own antigens, for example, by downregulating or not expressing major histocompatibility complex (MHC-I) molecules [5], MHC class I polypeptide-related sequence A (MICA), and MICB [6]. The low expression of adhesion molecules/co-stimulatory molecules related to T-cell activation prevents tumor cells from recognition by the immune system, whereas the high expression of negative-regulating costimulatory molecules, such as Fas ligand (FasL), programmed death ligand 1 (PD-L1), and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) [2] induces T-cell or NK cell apoptosis and inhibits the anti-tumor immune response. Tumor cells can also secrete immunosuppressive cytokines into the tumor microenvironment, such as the transforming growth factor-β (TGF-β), interleukin (IL)-10, and IL-4, which can inhibit anti-tumor immunity [2, 7, 8].

1.2 Tumor immune escape originates from the dysfunction of immune cells in the tumor microenvironment

Immune cells cannot kill tumors in the tumor microenvironment, which is affected by the activities of cytokines and tumor cells. Conversely, they promote tumor cell growth and metastasis. Cytokines in the tumor microenvironment induce the infiltration,

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differentiation, and aggregation of chemotactic inhibitory immune cell subsets. For example, Treg cells are an inhibitory cell subset in which Foxp3 is a transcription factor, and whose phenotype is $CD4^+CD25^+$ $Foxp3^+$. TGF-β can induce $CD4^+CD25^+$T-cells to express Foxp3 and downregulate Smad7 to transform the subsets into Treg cells [9]. Furthermore, CCL22 and CCL5 enhance the infiltration and enrichment of Treg cells in local tumors [9-10]. Additionally, Treg cells can inhibit NK activation and induce NK apoptosis by secreting TGF-β and β-galactoside-binding protein (β-GBP) [11-12]. It was also reported that CD18 adhesion was necessary for Treg cells to kill local immune cells in tumors, which ultimately lead to immune escape [13]. Regulatory B cells (Breg) also have a variety of phenotypes; for example, the tumor necrosis factor-α (TNF-α) secreted by Breg that can inhibit tumor immunity [14]. Tumor-infiltrating myeloid cells suppress the anti-tumor function of immunocompetent cells. Tumor-associated macrophage (TAM) accumulates in tumors and promotes their growth. Cytokines that are present in the tumor microenvironment, such as interferon-γ (IFN-γ) and TNF-α, can promote the polarization of TAM to M2 type macrophages and obtain immunosuppressive properties [15-16]. Myeloid-derived suppressor cells (MDSCs) are a subgroup of immature myeloid cells with suppressive properties. MDSC inhibits anti-tumor immunity by inducing T-cell apoptosis via suppressing T-cell receptor signal [17-18], producing IL-10, and TGF-β, inhibiting the function of NK cells and promoting the polarization of TAM [19] and infiltration of Treg cells to the tumor.

2. Correlation between NK cells and tumor immune escape

NK cells are a type of human innate immune cells, which are not restricted by MHC-I and antigen sensitization and are considered an important component for the tumor immunity function [20, 21]. The expression of activatory and inhibitory receptors on the surface of NK cells determines its activated state. Generally, activated NK cells can induce tumor cell apoptosis by releasing perforin and granzyme. They also augment the expression of death receptors (ligand of the tumor necrosis factor family) which mediate tumor cell apoptosis. Additionally, the secretion of effector factors (IFN-γ and TNF-α) is induced, and the CD16 is upregulated to activate antibody-dependent cytotoxicity that kills tumor cells [22]. However, in the tumor microenvironment, tumor cells can promote NK cell apoptosis by expressing abnormal molecules and secreting inhibitory factors that inhibit NK cell activation. Moreover, NK cell phenotype transformation can suppress the T-cell function, ultimately leading to tumor immune escape.

2.1 Abnormal molecules can be expressed on the tumor cells that inhibit NK activation and promote NK cell apoptosis

Importantly, tumor cells can express NK-activated receptor-related ligands, such as NKG2D ligand [23] and NKp30 ligand (BAG6) [24]. These ligands can protect tumor cells from metalloproteinase-mediated cleavage to form soluble ligands [25-27]. In addition, these ligands bind to receptors and promote sensitivity and internalization of NK cells, thereby reducing the NK cell activity. Tumor-related antigens can increase the expression of NK inhibitory receptors [28]. For example, Sun C *et al.* [29] found that infiltrated $CD56^{dim}$NK cells in hepatocellular carcinoma (HCC) patients had overexpression of the NK inhibitory receptor NKG2A, which induced NK cell depletion. In addition, tumor cells can escape the immune response by expressing FasL or downregulating Fas; they can also promote NK cells apoptosis by inducing NK cells to express Fas. Saito *et al.* [30] reported that the expression of Fas in NK cells around the tumor and the apoptosis rate in that area were significantly increased in gastric cancer patients.

2.2 Tumors can secrete inhibitory factors that inhibit NK cell activation

TGF-β [31], prostaglandin E2 (PGE2) [32], and indoleamine 2,3-dioxygenase (IDO)[33] are inhibitory cytokines of NK cells in the tumor microenvironment. Among them, TGF-β is one of the main inhibitors of NK cells, which is involved in immune escape. On the other hand, increased serum TGF-β levels have been detected in many tumor patients. Our previous study also showed that the content of TGF-β1 in the peripheral blood serum of the of HCC patients was significantly higher than that of normal people. Furthermore, TGF-β was reported to have an acute effect on the cytokine expression and cytotoxicity of NK cells [31]. It is noteworthy that TGF-β can inhibit the IFN-γ production in NK cells by activating the downstream Smad2 and Smad3 signaling pathways of TGF-βRI/II, downregulating the transcription factors T-bet and E4BP4 [34]. Additionally, by reducing granzyme B and perforin, TGF-β suppresses the cytotoxicity of NK cells. TGF-β also inhibits the expression of genes in non-coding regions by downregulating NKG2D [35] and thus mediates immune escape. Tumor-derived exosomes (TEXs) can inhibit the activation and killing ability of NK cells by actively releasing immunosuppressive molecules derived from tumor cells [36, 37]. Szczepanski *et al.* [38] found that TEXs downregulated the expression of the NK cell activation receptor NKG2D via its TGF-β content. Champsaur *et al.* reported that TEXs carried MHC-I molecular related proteins (MICA, MICB, and ULBPs) on the surface of tumor cells [39-40].

2.3 In the tumor microenvironment, NK cell phenotype transformation can inhibit the T-cell function

In tumor immunity, NK cells can effectively kill tumor cells which have escaped clearance by $CD8^+$ T-cells (CTL). However, the phenotypic transition of NK cells was found to lead to a decrease in the number of T-cells and inhibition of the immune response in tumors [41]. In lung, colorectal, and breast (squamous cell carcinoma) cancers, NK cells with phenotypic transformation not only had impaired killing function, but were also characterized vascular endothelial growth factor (VEGF) overexpression, which promoted tumor development [41].

In addition, NK cell-related MDSC, TAMs, and tumor-associated fibroblasts (CAFs) are closely related, and their mechanisms of action are associated with the expression of secreted or binding inhibitory factors. Li *et al.* [42] established that the impairment of NK cells was related to MDSC proliferation in HCC, and the membrane-bound TGF-β1 on MDSC is was the key molecule that mediated the MDSC function. Of note, Balsamo *et al.* [43] found that CAFs can inhibit the expression of NK cells in melanoma activating the receptors NKp30 and NKp44 by releasing PGE2. In that respect, Li T *et al.* [44] found established that in HCC, CAFs caused NK cell dysfunction through the production of PGE2 and IDO.

3. Traditional Chinese Medicine (TCM) theory concerning NK cell-related tumor immune escape

TCM scholars have extensively studied and acquired a deep understanding of tumor treatment. Among them, the theories of the "holistic concept" and the "two-way regulation" have certain significance in explaining NK cell-related tumor immune escape. The surface receptors of NK cells have both "Yin" and "Yang" properties. For example, its inhibitory receptors (NKIR), such as NKG2A, transmit negative signals, and its activating receptors (NKAR), such as NKG2D, provide positive signals. Therefore, the balance of "Yin" and "Yang" is the key to determine the immunosuppressive or killing effect of NK cells.

Some scholars have found that Chinese herbs/prepared medicines also exerted a two-way anti-tumor regulation effect on NK cells. Guixiang *et al.* [45] reported that qi-tonifying and spleen-invigorating drugs can enhance the activity and killing ability of NK cells. In addition, Han *et al.* [46] evidenced that extracts from *Astragalus complanatus* R. Br. upregulated the expression of NKG2D and NKp44, and promoted the secretion of IFN-γ. In contrast, Luo *et al.* [47] revealed that Yupingfeng powder reduced the secretion of TGF-β and IL-10 and relieved NK-cell immunosuppression, leading to the prevention of immune escape and improved killing effect on tumors.

In recent years, research on tumor immune escape has been intensified. Immunomodulation drugs have been used in clinics, such as PD-1/PD-L1 and CTLA-4 monoclonal antibodies, which have achieved promising results in melanoma and non–small-cell lung cancer. However, these monoclonal antibodies still have undesired side effects, a high drug resistance rate, and limited survival prolongation. TCM focuses on the holistic concept, which is based on the notion that by regulating tumor cells and their microenvironment and enhancement of immune cell function, the number and activity of NK cells could achieve optimal therapeutic effects [4]. We previously also found that Xiaoliusan, invented by Professor Wu Zhengxiang, a famous Chinese medicine doctor, significantly inhibited tumor growth and proliferation, and regulated tumor immunity. The mechanism of Xiaoliusan action is exerted via promotion of T-cell proliferation and NK cell activation, increase in the number of NK cells, overexpression of NKG2D, decrease in the serum exosomal content, and downregulation of TGF-β1 expression [48-49]. However, few TCM reports on the mechanism of NK cell-associated tumor escape have been published to date. Since NK cell-related tumor cell escape is critically involved in tumorigenesis, it may attract considerable research interest of TCM and Western medicine scholars in the future due to its potential for anti-cancer treatment.

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