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Tumor-infiltrating natural killer cells

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Abstract

Because of their potent antitumor activity and their proinflammatory role, natural killer (NK) cells are at the forefront of efforts to develop immuno-oncologic treatments. NK cells participate in immune responses to tumors by killing target cells and producing cytokines. However, in the immunosuppressive tumor microenvironment, NK cells become dysfunctional through exposure to inhibitory molecules produced by cancer cells, leading to tumor escape. We provide an overview of what is known about NK tumor in infiltration and surveillance and about the mechanisms by which NK cells become dysfunctional.

Introduction

Natural killer (NK) cells are cytotoxic innate immune cells of lymphoid origin that were first described in 1973 as "null" killer cells after the discovery that a non-T non-B lymphocyte subset could efficiently kill antibody-coated cells (1). Two years later, the ability of NK cells to kill various types of tumor cells was reported, and the term "natural" killer cell was coined (2). NK cells play a role not only in the antitumor response, but also in defense against microbial infection (3). NK cells express diverse activating and inhibitory receptors (4), and the balance between the signals mediated by these receptors determines the outcome of NK-cell activation.

NK cells express cell surface inhibitory receptors that recognize MHC Class I (MHC-I) molecules. The most important inhibitory receptors are the members of the killer cell immunoglobulin-like receptor (KIR) family (5), and the CD94/ NKG2A heterodimer. Each

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individual expresses a specific set of KIRs. In total, 17 KIR genes have been described in humans, two of which are pseudogenes (6, 7). Each of these genes is highly polymorphic, with a total of 1,110 confirmed polymorphisms (IPD-KIR Database, Release 2.9.0), and at least twice that number of polymorphisms that have been reported. KIRs can be classified on the basis of their structure and function, according to two factors: (i) the number of immunoglobulin-like (Ig-like) domains, either two (KIR2D-) or three (KIR3D-), and (ii) the presence of a long or a short intracytoplasmic tail, assigned by the letter "L" for "long" or "S" for short. Inhibitory KIRs usually possess a long cytoplasmic tail (KIR2DL) whereas activating KIR possess a short one (KIR2DS), except for the activating KIR2DL4 that has a long cytoplasmic tail. Two main KIR haplotypes, A and B, have been described. The KIR A haplotypes encompass inhibitory KIR plus the activating KIR2DS4, and the KIR B haplotypes are characterized by the expression of at least one of the following activating KIR: KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, or KIR3DS1. Inhibitory KIRs contain immunoreceptor tyrosine-based inhibition motif (ITIM) sequences in the intracytoplasmic tail responsible for the inhibitory signal. KIRs containing ITIM sequences are the most common type of KIRs and are the canonical inhibitory KIRs responsible for inhibiting NK cells as far as MHC-I molecules are concerned, together with CD94/NKG2A. Activating KIRs can associate with immunoreceptor tyrosine-based activation motif (ITAM)-bearing molecules (KARAP/DAP12/TYROBP; refs. 8, 9) to transmit an activating signal. These activating KIRs have been associated with the outcome of autoimmune diseases, pregnancy-associated disorders, infectious diseases and cancers (10, 11).

According to the principle of "missing self" recognition (12), NK cells can eliminate targets that do not express sufficiently large numbers of MHC-I molecules. The expression of these molecules is often lost during viral infection and neoplastic transformation, enabling the cells to escape $CD8^+$ T-cell immunosurveillance, which requires the presentation of antigens by MHC-I molecules (13). The down- regulation of these molecules renders the cells invisible to T cells, but with the loss of the main ligands for NK-cell inhibitory receptors (KIRs and CD94/NKG2A), NK cells are no longer inhibited and are more prone to activation via a plethora of activating receptors (14). The major activating NK-cell receptors are the natural cytotoxicity receptors (NCR) NKp30, NKp46, and NKp44, as well as CD16 and NKG2D. Other molecules, such as DNAX accessory molecule1 (DNAM1), 2B4, and NKp80, enhance NK-cell activity, mostly by acting as coreceptors. NK cells also express a large number of other inhibitory receptors regulating their activation status, including LILRB1 (also known as LIR1), CD161, KLRG1, SIGLEC7, SIGLEC9, PD-1, TIGIT, LAG3, and TIM3.

Human NK cells have heterogeneous phenotypes and functions. Two NK-cell subsets can be defined according to differential expression of the markers CD56 and CD16. The blood of healthy individuals contains two main NK cell populations: CD56dim CD16+ NK cells and CD56bright CD16− NK cells. The CD56dim subset is considered as a mature cytotoxic population accounting for most of the circulating NK cells, whereas the CD56bright subset is less mature, mostly immunomodulatory, and mainly located in secondary lymphoid organs (15). Following the stimulation of activating receptors, CD56dim NK cells can kill other cells directly, but without prior priming. In contrast, CD56bright NK cells are less cytotoxic, but produce larger amounts of cytokines upon exposure to environmental stimuli,

such as IL1β, IL2, IL12, IL15, and/or IL18 (16). The development of the two subsets is not yet fully understood, but the prevailing theory suggested that they correspond to two different steps of NK-cell maturation (17). We recently identified by single-cell profiling of human bone marrow NK cells that CD56bright CD127+ CD160– CD52+ cells, called NK0, can give rise to conventional NK2/CD56bright CD160+ CD52– cells and to NK1/CD56dim perforin^{hi} cells at steady state (Crinier et al., in press). NK cells were recently classified as belonging to the innate lymphoid cell (ILC) family, also encompassing helper-like ILCs (ILC1, ILC2, and ILC3) and lymphoid tissue inducer cells. Like ILC1, NK cells are capable of secreting type-1 cytokines such as IFNγ, TNFα,and granulocyte–macrophage colony-stimulating factor (GM-CSF). They are sometimes described as cytotoxic ILCs (18). ILC1 cells differ from NK cells principally in their lack of granule-dependent cytotoxicity. The other differences among these two cell types are relatively minor, and a degree of plasticity between the two subsets has been previously described (19–21). Most activating and inhibitory receptors expressed by ILC1s discovered to date are also expressed by NK cells (22), but these two subsets differ in terms of their localization in different organs. ILC1s are almost entirely absent from the bloodstream, instead being located mainly in the peripheral tissues, such as within the intestine (23). In terms of transcription factors, human ILC1s are described as EOMES−/lo T-bet+, whereas NK cells are described as EOMES+ T-bet+ (18). The conversion from cytotoxic NK to noncytotoxic ILC1 upon TGFβ signaling has been described in mice (19, 20), but there is still no clear evidence that a similar process occurs in humans. Nevertheless, the identification of poorly cytotoxic, cytokine producing, tissue-resident EOMES− T-bet+ CD49a+ NK cells in the human liver (24) may provide evidence of a possible bridge between NK cells and ILC1s in humans resembling that described in mice.

To perform their functions correctly, NK cells must migrate to inflamed tissues. NK cells express a broad repertoire of chemokine receptors to control this migration. Resting CD56bright CD16− NK cells described as expressing CCR2 [recognizing CCL2 (MCP1), CCL7 (MCP-3), CCL12, CCL13 (MCP4), CCL16 (HCC-4)], and CCR5 (recognizing RANTES, MIP1α, and MIP1β) are targeted to lymph nodes via CCR7 (recognizing CCL19 and CCL21). They preferentially express CXCR3 [recognizing CXCL4, CXCL9 (MIG), CXCL10 (IP10)] and CXCL11 (I-TAC/IP9) and exhibit higher levels of CXCR4 (recognizing CXCL12 (SDF1; refs. 25, 26), when compared to the CD56dim subset. By contrast, CD56dim NK cells uniquely express CXCR1 (recognizing CXCL8; refs. 25, 26)), CX3CR1 (recognizing CX3CL1; ref. 25) and ChemR23 [recognizing the peptide chemerin and the eicosapentaenoic acid-derived lipid mediator resolvin E1 (RvE1)]. CD56dim cells also express high levels of S1P5 (recognizing the bioactive lipid S1P) required for their egress from bone marrow and lymph nodes and also for their recruitment to inflamed tissues.

The ability of NK cells to kill cancer cells without prior sensitization results in these cells playing a role in tumor immunosurveillance, as demonstrated on multiple occasions. Evidence for the importance of NK cells in preventing tumor progression is the array of mechanisms used by tumor cells to decrease NK-cell immunosurveillance, and the requirement of these mechanisms for disease progression. In this review, we discuss NK-cell

infiltration into various types of tumor and the mechanisms underlying NK-cell dysfunction contributing to tumor evasion.

NK cell infiltration of solid tumors

At the tumor bed, NK cells may control tumor growth by interacting directly with tumor cells and through interplay with other immune cells (27). However, investigations of tumor biopsy specimens for several solid cancers have revealed little NK-cell infiltration into these tumors, although the methods used to identify tumor-infiltrating NK cells can be disputable. NKp46 cell-surface expression is a marker of human NK cells encoded by the NCR1 gene (28). NCR1 expression is to date the marker providing the most reliable information about the presence of NK cells. NCR1 expression is weaker than that of other specific genes, such as CD4, CD8, and CD19, suggesting that NK cells infiltrate solid tumors in smaller numbers than CD4+ T, CD8+ T, and B cells, respectively. However, the presence of NK cells within the tumor microenvironment (TME) may be associated with a good prognosis. Indeed, low NK-cell numbers are observed in primary head and neck squamous cell carcinoma (HNSCC) tumor sections (29) and were associated with the insufficient tumor rejection. Higher levels of NCR1 expression at the tumor site are associated with better survival (30). There is also a positive correlation between the infiltration of NK cells and CD8+ T cells into the TME and prolonged survival in patients with colorectal carcinoma (31). In gastric and esophageal cancers (32), the proportion of CD56dim NK cells infiltrating tumors gradually decreases with disease progression in patients.

Non–small cell lung carcinoma (NSCLC) samples display low levels of NK-cell infiltration and an overexpression of NK inhibitory receptors. NK cells mostly infiltrate the tumor stroma, and smaller numbers of infiltrating NK cells in lung cancer tissues are linked to a larger primary cancer, smoking history, and poorer patient prognosis (33). Another study on NSCLC reported NK cells to be less frequent in cancer tissues than in normal lung tissue. The low percentage of NK cells in tumors results principally from a smaller number of cells from the CD16+ NK-cell subset (34).

An analysis of RNA-sequencing (RNA-seq) data for melanomas from The Cancer Genome Atlas (TCGA) showed that patients with metastatic cutaneous melanoma had better survival rates if their tumors showed signs of NK cell infiltration (35). Moreover, in patients with melanoma, higher NK-cell numbers were found to be correlated with the presence of protective stimulatory DCs in the tumor, patient responsiveness to anti–PD-1 immunotherapy, and better overall survival (36). Single-cell RNA-seq analysis on NK cells isolated from human melanoma metastases showed that NK cells were present in melanoma metastases, even in patients who failed to respond to checkpoint blockade (37).

We have also analyzed the various RNA-seq datasets available from the TCGA database of more than 10,000 tumor samples for 33 different types of cancer to assess NK-cell infiltration (Fig. 1). Informative genes significantly overexpressed by NK cells were selected to establish a NK-cell signature and to infer the abundance of these cell types in the tumor samples analyzed. A 13-gene signature, CD160, CD244, CHST12, CST7, GNLY, IL18RAP, IL2RB, KLRC1, KLRC3, KLRD1, KLRF1, PRF1, and XCL2 (38), to which NCR1 was added, was used as a metagene for NK-cell identification. For comparison, we also studied

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infiltration of the CD8+ cytotoxic T-cell subset, which is also involved in tumor elimination. The T-cell metagene was defined as an 8-gene signature—CD3D, CD3E, CD8A, CD8B, KLRC1, KLRK1, GZMH, and CCL5—with the signature matrix file LM22 obtained with CIBERSORT (39) by selecting the genes with the highest weights for the population of interest. The specificity of the list was then validated with an immune cell compendium (40). These results revealed that the presence of tumor-infiltrating NK cells varies across tumor types and pave the way for the use of treatments targeting NK cells and/or T cells in specific cancer conditions, as most of the tissues with marked NK-cell infiltration were also infiltrated with T cells.

Dysfunctional NK cells

Several mechanisms operating within the TME alter NK cell function (Table 1; Fig. 2). NK-cell IFNγ production and the proportion of CD56bright cells have been shown to be significantly lower in patients with prostate cancer than in controls (41). In an analysis of samples from patients with gastric cancer, the expression of activating and inhibitory receptors on NK cells was shown to be similar in tumor tissues and the surrounding nontumor tissues. However, the effector functions of NK cells were impaired, as these cells had lower levels of IFNγ, TNFα, and Ki-67 in gastric cancer (42). The percentages of tumor-infiltrating NK cells were significantly lower than in controls and were correlated with tumor progression and poor overall patient survival. In hepatocellular carcinoma (HCC) tissues, NK cells have poor cytotoxicity and an impaired capacity to produce IFN γ (43). In human liver cancer, tumor-infiltrating NK cells display mitochondrial alterations (smaller size and fragmentation) not observed in peripheral NK cells outside the tumor (44). This fragmentation is correlated with lower cytotoxicity and NK-cell counts, resulting in impaired tumor control and evasion from NK-cell immunosurveillance, which was found to have a direct negative impact on patient prognosis. A microarray expression assay on tumor slices from chemotherapy resistant breast cancer samples showed a downregulation of NK cell–mediated cytotoxicity genes (45). In another study, the proportion of NK cells in mammary tumors was found to be similar to that in paired healthy mammary tissues. However, NK cells in malignant and healthy tissues had different phenotypes, with CD56 bright cells being the more prevalent in the tumor than in healthy control tissues (46).

Sustained exposure to high concentrations of NKG2D ligands (NKG2DL) is often but not always associated with a systematic decrease in NKG2D expression and the induction of tolerance in NK cells, leading to an evasion of immune surveillance (47, 48). The cumulative levels of these ligands in plasma have been implicated in the inhibition of NK-cell activity and shown to correlate with disease progression and tumor load in several types of cancer, including HNSCCs and melanoma (29, 49, 50). However, the impact of the chronic interaction between NK cells and NKG2DLexpressing cells remains a matter of debate. For instance, the soluble variant of the murine high-affinity NKG2D ligand MULT1 was found to have the opposite effect on NK cells, stimulating tumor rejection and increasing NKG2D membrane expression in an in vivo melanoma model (51). Of note, a subset of acute myeloid leukemia (AML) cells from patients lacks NKG2D ligand expression and can overcome NK cell–mediated killing, with repercussions for disease progression (52).

In breast cancer samples and studies of pancreatic cancer cells, NK cells have been shown to have low levels of expression of the activating receptors NKp30, NKG2D, NKp46, DNAM1, and CD16, and high levels of expression of inhibitory receptors, such as NKG2A, this profile being correlated with an impairment of NK-cell cytotoxicity (46). NK cells from patients with chronic B lymphocytic leukemia (B-CLL) display a small but significant downregulation of NKp30 (53, 54), leading to a poorer prognosis. Patients with AML have an NK-cell phenotype in which the NKp30 (55) and NKp46 (55, 56) receptors are weakly expressed. High levels of NKp46 expression at diagnosis were found to be correlated with a better prognosis in patients with AML (57). Patients with CLL have higher levels of soluble ligands for NKp30 and NKG2D than healthy individuals, which can lead to the suppression of NK-cell cytotoxicity and the promotion of CLL evasion from NK cells (58). The α -disintegrin and metalloproteinases ADAM 10 and 17 expressed on the surface of tumor cells have been reported to participate in the shedding of B7-H6 (59). The presence of the soluble form of the NKp30 ligand B7-H6 has also been associated with lower levels of NKp30 expression on NK cells in various cancers (60–62). CD16 is a key receptor in antigen-dependent cell cytotoxicity (ADCC) and has been shown to be less strongly expressed in patients with breast cancer than in healthy individuals (63). In patients with melanoma, the major infiltrating NK-cell subset is the CD56 dim CD16 − subset (64). The metalloprotease ADAM17 is a regulatory checkpoint of the CD16A receptor in NK cells. Upon activation, it can induce CD16A cleavage, hampering its signaling and NK-cell function. The proportions of the activating receptor DNAM1 (CD226) and the adhesion molecule CD96 on NK cells are significantly lower in pancreatic tumor samples than in control samples, and the downregulation of these molecules is associated with tumor histologic grade and lymph node metastasis (65).

PD-1 has been well characterized as an exhaustion marker on T cells but can also be expressed on NK cells (66). Its expression on NK cells has been called into question (67), but the likelihood of PD-1 detection on NK cells may be highly dependent on the choice of reagents (68), and functional analyses often demonstrate the functionality of PD-1 on NK cells. Indeed, the PD-1+ NK cells infiltrating head and neck tumors have an exhausted phenotype. PD-1 is overexpressed on the surface of NK cells from digestive cancers, and PD-1/PD-L1 antibody blockade restores NK function in vitro (69). Intratumoral TIM3+ PD-1+ NK cells from patients with various cancers have defective IFNγ and granzyme B secretion and are less cytotoxic than TIM3−PD-1− NK cells (70). In colon tumor samples, levels of PD-1 and CTLA4 expression are very low on tumor-infiltrating NK cells. However, levels of TIGIT expression are significantly higher on NK cells in the tumor than on those outside the tumor, and this feature is associated with NK-cell exhaustion (71). CD155 acts as a ligand of both the stimulatory receptor DNAM1 and the inhibitory receptor TIGIT. It has been found to be overexpressed in various types of cancer, and its interaction with DNAM1 can induce its downregulation (72–74).

The inhibitory receptor NKG2A and its ligand HLA-E are frequently overexpressed in many types of tumor. In HCC (75) and colorectal carcinoma (76), HLA-E overexpression is correlated with NK-cell exhaustion and poor prognosis. Moreover, NKG2A blockade has been shown to enhance antitumor immunity by promoting both NK-cell and CD8+ T-cell effector functions in mice and humans (77).

NK cells are particularly important for metastasis prevention (78), and NK-cell dysfunction is associated with an increase in cancer metastasis in various cancer types. In breast cancer, GSK3β (glycogen synthase kinase-3) inactivation has been linked with NK-cell dysfunction and is considered to be responsible for metastasis formation (79). In gastric cancer, NK-cell dysfunction, in terms of lower levels of activating receptor expression on circulating NK cells, is correlated with tumor progression (80). In addition, mouse models with less efficient NK-cell migration to the tumor due to a lack of heparanase (81) display higher rates of metastasis, and the induction of NK-cell invasion of the tumor by the deletion of atypical chemokine receptor 2 (ACKR2), which competes with chemokine receptor CCR4, causes a significant decrease in metastasis (82). Similar results were obtained in a study in which TRAIL activation by dose-dependent ONC201 (an orally active antitumor agent) administration increased NK-cell invasion and cytotoxicity, reducing the rate of metastasis formation (83).

The tumor microenvironment affects NK cell functions

The tumor microenvironment can sustain tumor growth and influence immune escape and cancer progression. Most solid tumors contain regions of permanent or transient hypoxia due to changes in vascularization and restricted access to nutrients and oxygen. Hypoxia has been demonstrated to limit NK-cell activity (84). The fragmentation of NK-cell mitochondria is one of the mechanisms by which hypoxia decreases NK-cell survival and function (44). Other studies in digestive tumors reported the production of abundant H2O2 in the TME, linked to a decrease in the infiltration of CD56dim NK cells (32). Indeed, cytotoxic CD56dim cells are more susceptible to H2O2-induced apoptosis than CD56bright cells. Another mechanism of tumor evasion has been demonstrated for platelets. Platelet cloaking of tumor cells can promote the shedding from the tumor cell surface of the NKG2D ligands MICA and MICB, which are released into the TME and may decrease NKG2D expression on NK cells (85). Moreover, NK-cell exposure to the H2O2 produced within the TME impairs ADCC activity and decreases CD56dim NK-cell infiltration (32). Indoleamine 2, 3-dioxygenase 1 (IDO1) is a metabolic enzyme that converts the essential amino acid tryptophan into downstream molecules known as kynurenines. IDO1 is overexpressed in various tumors and is responsible for immunosuppression and NK and T-cell inhibition (86, 87). The TME also affects NK-cell functionality through the production of soluble factors. TGFβ is a major immunosuppressive cytokine that decreases the antitumor activity of NK cells. In glioblastoma, for example, TGFβ has been described as an agent potentially capable of decreasing the expression of the major NK-cell activating molecules (88). In acute lymphoblastic leukemia, the coculture of healthy donor NK cells with ALL blasts results in a suppression of the cytotoxic activity of the NK cells through the release of soluble factors, including TGFβ1 (89). Patients with lung and colorectal cancers have higher levels of TGFβ1 in their blood than healthy volunteers, which has been shown to decrease the level of NKG2D expression on NK cells (90). STAT3 is a key regulator of the transcription of genes encoding factors involved in cell growth and differentiation, including TGFβ, and is constitutively activated in various primary tumors and cell lines. In esophageal squamous cell carcinoma, STAT3 signaling also plays a key role in IL6 and IL8 secretion by tumor cells, leading to the downregulation of activating receptors (NKp30 and NKG2D) on the surface of NK cells, impairing their function (91). Prostaglandin-E2 (PGE2) is a small

molecule that is upregulated in various cancers and can decrease NK-cell cytotoxicity (92, 93). Various cell types residing in the TME can downregulate the immune response. These cells include fibroblasts, stromal cells, regulatory T cells (Tregs) and the so-called myeloid-derived suppressor cells (MDSC). Tregs exert immunosuppressive effects by inhibiting CD8+ T cells and NK cells directly or via the secretion of TGFβ and IL10. Treg cells also influence NK-cell inhibition in cancer. High Treg cell numbers are correlated with poorer NK-cell function in patients with gastrointestinal cancer (94), and MDSCs from patients with cancer significantly inhibit NK cell–mediated ADCC by producing nitric oxide (95).

Concluding remarks

NK cells are involved in the immune response to tumors, participating in their elimination. This has led to their consideration as potential targets for cancer immunotherapy. However, malignant transformation often affects the expression or function of inhibitory and activating NK-cell receptors, with a direct impact on NK-cell cytotoxicity. Various mAbs directed against key checkpoint ligands and their receptors have been designed and produced for preventing NK-cell inactivation via the blocking of inhibitory receptors. Examples include antibodies directed against NKG2A, such as monalizumab, and against TIGIT, which have already entered clinical trials. Other therapeutic approaches aim to increase the efficacy of tumor cell recognition by NK cells. In this case, antibodies against tumor antigens, such as EGFR, can enhance ADCC by the immune cells. Another strategy aiming to prevent the shedding of the NKG2A ligands MICA and MICB has shown promising results in humanized melanoma models (96). Recent investigations have also focused on increasing the infiltration and recruitment of immune cells to the tumor site through the development of antibodies against soluble factors secreted by the tumor, such as TGFβ. Anti-TGFβ antibodies have already progressed to clinical trials for various solid cancers, where they are being tested alone and in combination with anti–PD-1 agents (97). An ability to assess immune cell infiltration into tumors and the receptor repertoires of the infiltrating immune effector cells would improve predictions of clinical response and make it possible to design personalized immunotherapies based on a better stratification of patients with cancer. The factors critical to the success of future treatments thus include the development of ways of increasing tumor infiltration and rescuing the function of immune effector cells, such as with NK cell engagers (98). In the case of NK cells, combination therapies addressing both these issues and the targeting of adaptive immune effectors may be particularly beneficial.

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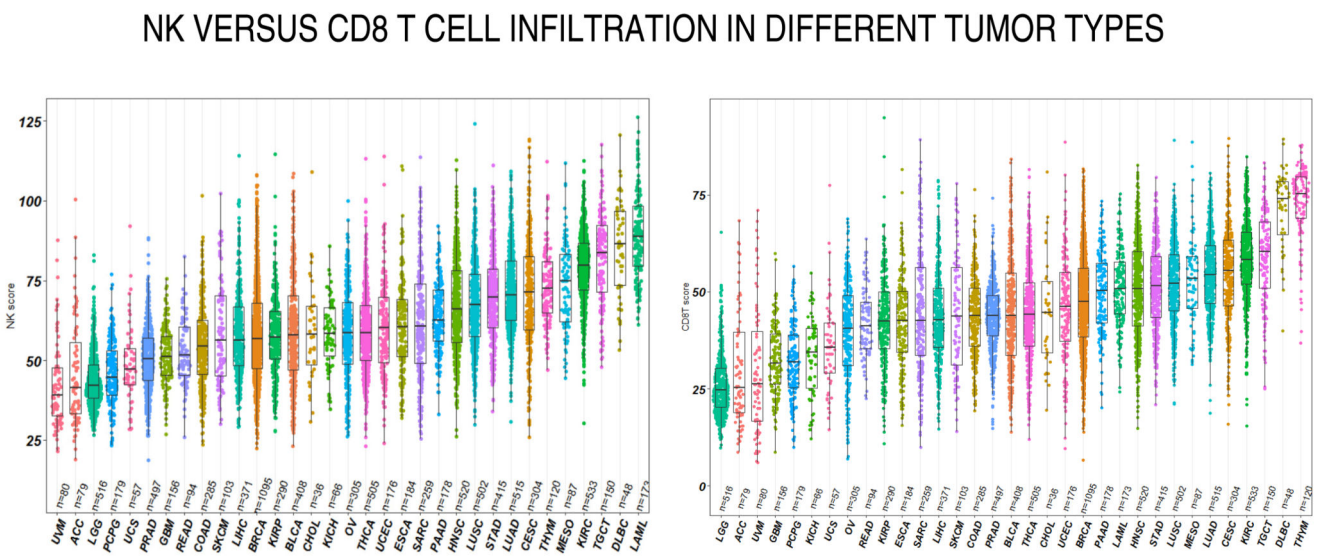

Figure 1. Comparison of NK cell immune infiltration and CD8 T-cell infiltration in various cancer tissues.

RNA data from The Cancer Genome Atlas were analyzed by selecting a set of significantly differentially expressed genes as the NK-cell and CD8 T-cell signatures. The NK-cell signature was composed of the following genes: CD160, CD244, CHST12, CST7, GNLY, IL18RAP, IL2RB, KLRC1, KLRC3, KLRD1, KLRF1, PRF1, XCL2, and NCR1. The CD8 T-cell signature was composed of the following genes: CD3D, CD3E, CD8A, CD8B, KLRC1, KLRK1, GZMH, and CCL5. Expression values were summed to obtain the NK score and the CD8 T score. Cancer tissues are ranked by increasing median of log2-normalized expression. **ACC**, adrenocortical carcinoma; **BLCA**, bladder urothelial carcinoma; **BRCA**, breast invasive carcinoma; **CESC**, cervical squamous cell carcinoma and endocervical adenocarcinoma; **CHOL**, cholangiocarcinoma; **COAD**, colon adenocarcinoma; **DLBC**, lymphoid neoplasm diffuse large B-cell lymphoma; **ESCA**, esophageal carcinoma; **GBM**, glioblastoma multiforme; **HNSC**, head and neck squamous cell carcinoma; **KICH**, kidney chromophobe; **KIRC**, kidney renal clear cell carcinoma; **KIRP**, kidney renalpapillary cell carcinoma; **LAML**, acute myeloid leukemia; **LGG**, brain lower grade glioma; **LIHC**, liver hepatocellular carcinoma; **LUAD**, lung adenocarcinoma; **LUSC**, lung squamous cell carcinoma; **MESO**, mesothelioma; **OV**, ovarian serous cystadenocarcinoma; **PAAD**, pancreatic adenocarcinoma; **PCPG**, pheochromocytoma and paraganglioma; **PRAD**, prostate adenocarcinoma; **READ**, rectum adenocarcinoma; **SARC**, Sarcoma; **SKCM**, skin cutaneous melanoma; **STAD**, stomach adenocarcinoma; **TGCT**, testicular germ cell tumors; **THCA**, thyroid carcinoma; **THYM**, thymoma; **UCEC**, uterine corpus endometrial carcinoma; **UCS**, uterine carcinosarcoma; **UVM**, uveal melanoma.

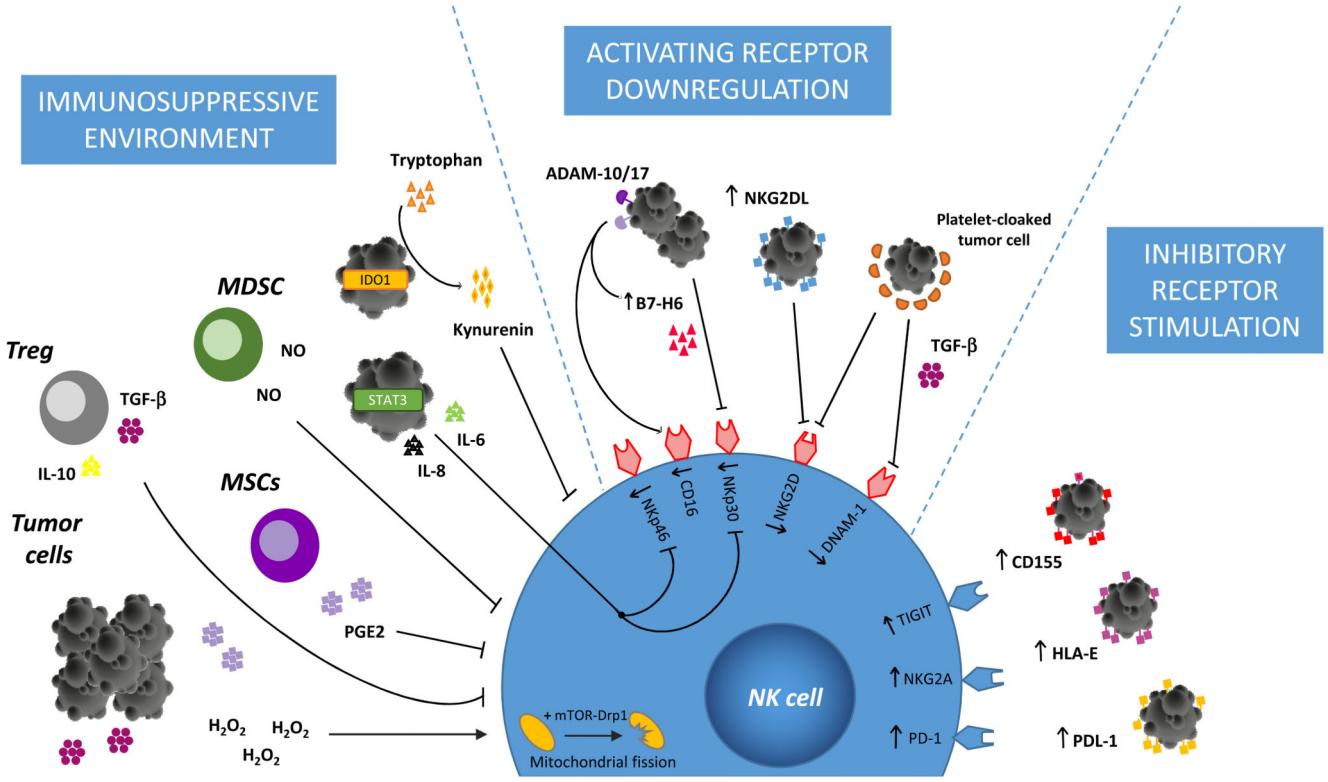

Figure 2. Mechanisms involved in NK cell dysfunction in the tumor bed.

Tumor cells can modify the extracellular environment by producing immunosuppressive cytokines, such as TGFβ, which decrease NK-cell functionality and infiltration. The tumor cells can also affect NK functionality by promoting hypoxic conditions leading to an activation of the intracellular protein mTOR-DRP1 causing mitochondrial fragmentation in the NK cell. Mesenchymal stem cells (MSC) and tumor cells produce prostaglandin-E2 (PGE2), which decreases NK cytotoxicity. Regulatory T cells (Treg) can directly suppress NK-cell function or indirectly influence NK-cell inhibition by secreting IL10 and TGFβ. Myeloid-derived suppressor cells (MDSC) can inhibit NK-cell ADCC via the secretion of nitric oxide (NO). Tumor cell–secreted IL6 and IL8 impair the activity and function of NK cells via STAT3, suppressing expression of the NK cell–activating receptors NKp30 and NKG2D. In many human tumors, a downregulation of the expression of the activating receptors NKp46, NKp30, NKG2D, DNAM1, and CD16 has been observed on NK cells, with a direct negative impact on NK-cell activation and antitumor function. Moreover, tumor cells have been found to overproduce and shed the ligands of these activating receptors (e.g., NKp30 ligand B7-H6 and NKG2D ligands MICA and MICB), inducing an inhibition of activating signaling pathways. The metalloprotease ADAM17 expressed on the surface of tumor cells in addition to participating in the shedding of B7-H6 is also able to induce the cleavage of the activating CD16. Tumor cells are able to attract platelets, which in turn promote the release of NK2GDL and TGFβ, contributing to the suppression of NK-cell function. Tumor cells can also overexpress ligands (e.g., CD155, PD-L1, and HLA-E) of NK

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inhibitory receptors and increase the expression of some of these receptors, such as TIGIT, PD-1, and NKG2A, leading to alterations of the function of infiltrating NK cells.

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Table 1

Mechanisms of NK cell dysfunction.

Mechanism	Cause	Evidence	Cancer type	Species	Ref.
Loss of expression/function of activating NK cell receptors	NKG2D downregulation	Downregulation of NKG2D on peripheral NK cells from patients	Breast cancer	Human	Mamessier, *et al.* 2011 J Clin Invest.
		Decrease in NKG2D surface expression on circulating NK cells in patients	Lung and colorectal cancer	Human	Lee, *et al.* 2004 J Immunol
	Impairment of NKG2D-dependent activation by NKG2DL	Removal of shed NKG2DL ligands from the plasma of HNSCC patients restores NK cell function *in vitro*	Head and neck cancer	Human	Weil, *et al.* 2017 Front Immunol
		Tumor cells can attract platelets *in vitro,* promoting the release of NK2GDL and supressing NK cell function	Ovarian cancer and melanoma	Human	Cluxton, *et al.* 2019 Plos One
		Elevated levels of soluble ULBP2 ligand are associated with poorer prognosis	Melanoma	Human	Paschen, *et al.* 2009 Clin Cancer Res
		Lack of NKG2DL expression by a subset of AML cells from patient leads to tumor evasion	Acute myeloid leukemia	Human	Paczulla, *et al.* 2019 Nature
		Increase in MICA and TGFβ1 levels in the plasma of HNSCC patients impaired NKG2D-dependent cytotoxicity against tumor cells	Head and neck cancer	Human	Klöß, S. *et al.* 2015 Oncoimmunology
	NKp46 downregulation	Downregulation of NKp46 on peripheral NK cells from patients relative to healthy donors	Acute myeloid leukemia	Human	Fauriat, *et al.* 2007 Blood
					Stringaris, *et al.* 2014 Haematologica
		Patients with higher levels of NKp46 expression at diagnosis had a better prognosis than patients with NKp46 downregulation	Acute myeloid leukemia	Human	Chretien, *et al.* 2017 Oncoimmunology
	NKp30 downregulation	Downregulation of NKp30 on peripheral NK cells from patients	Breast cancer	Human	Mamessier, *et al.* 2011 J Clin Invest.
		Slight but significant decrease in NKp30 expression on peripheral NK cells	Chronic lymphocytic leukemia	Human	Veuillen, *et al.* 2012 J Clin Immunology
		Decrease in NKp30 expression on NK cells isolated from the peripheral blood of patients	Acute myeloid leukemia	Human	Fauriat, *et al.* 2007 Blood
		Patients from the poor prognosis group had lower levels of NKp30 expression on peripheral NK cells	Chronic lymphocytic leukemia	Human	Costello, *et al.* 2012 Immunology
	Impairment of NKp30 activation by soluble NKp30 ligands	Impairment of tumor recognition through metalloprotease-mediated shedding of B7-H6, leading to an increase in blood B7-H6 levels	Melanoma	Human	Schlecker, *et al.* 2014 Cancer Research
		Soluble B7-H6 levels in the patients' blood correlated with the downregulation of NKp30 expression and a poorer prognosis	Neuroblastoma	Human	Semeraro, *et al.* 2015 Science Transl. Medicine

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Mechanism	Cause	Evidence	Cancer type	Species	Ref.
			Ovarian carcinoma	Human	Pesce, *et al.* 2015 Oncoimmunology
			Hepatocellular carcinoma	Human	Mantovani, *et al.* 2018 Hepatology
	DNAM-1 (CD226) downregulation	Decrease in the percentage of $CD226^+$ $CD96^+$ NK cells associated with tumor histological grade and lymph node metastasis	Pancreatic cancer	Human	Peng, *et al.* 2016 Oncotarget
	CD16 downregulation	Low levels of CD16 expression on $CD56^+$ cells in all patient cohorts	Breast cancer	Human	Petricevic, *et al.* 201 3 J Trans Med
		$CD56^{dim}$ $CD16^-$ NK cells are the dominant subset in the tumor microenvironment	Melanoma	Human	Vujanovic, *et al.* 2019 Front Immunol
Overexpression/overstimulation of inhibitory receptors	PD-1 expression	Increase in PD-1 expression on peripheral NK cells indicates poorer survival in esophageal and liver cancers	Digestive cancer	Human	Liu, *et al.* 2017 Oncogene
		Intratumoral $Tim\text{-}3^+PD\text{-}1^+$ NK cells from patients less cytotoxic than Tim-3−PD-1− NK cells	Late-stage cancer	Human	Seo, *et al.* 2017 Nat Commun
	PD-1 ligands overexpression	PD-L1 ligation of $PD\text{-}1^+$ NK cells decreased their activation status	Head and neck cancer	Human	Concha-Benavente, *et al.* 2018 Cancer Immunol Res.
	NKG2A	NKG2A is overexpressed on peripheral NK cells and associated with impaired NK cell effector function	Acute myeloid leukemia	Human	Stringaris, *et al.* 2014 Haematologica
		NKG2A expression is influenced by factors from cancer nests and contributes to NK cell exhaustion	Hepatocellular carcinoma	Human	Sun, *et al.* Oncoimmunology 2016
	NKG2A ligand overexpression	Higher levels of HLA-E expression are correlated with a poor prognosis in HCC patients	Hepatocellular carcinoma	Human	Sun, *et al.* Oncoimmunology 2016
		Patients with HLA-E overexpression had the lowest long-term survival	Colorrectal carcinoma	Human	Zhen, *et al.* Medical Oncology
	TIGIT	High TIGIT expression was correlated with NK cell exhaustion in tumor-bearing mice and patients with colon cancer	Colon cancer	Human	Zhang, *et al.* 2018 Nature Immunol
	TIGIT ligand overexpression	Interactions with CD155 ligand-expressing target cells lead to reduction of DNAM-1 expression on NK cells	Ovarian cancer	Human	Carlsten, *et al.* 2009 J Immunol
		Overexpression of CD155 was correlated with more proliferative cancer cells and reduced overall patient survival	Breast cancer	Human	Li, *et al.* 2020 J Immunol Res
		Overexpression of CD155 in primary cancer cells plays a role in tumor cell invasion and is associated with poor patient prognosis	Lung cancer	Human	Nakai, *et al.* 2010 Cancer Sci
NK cell inhibition by extracellular stimuli	Hypoxia	Induces mitochondrial fragmentation, which decreases NK cell survival and function	Liver cancer	Human	Zheng, *et al.* 2019 Nature Immunol

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Mechanism	Cause	Evidence	Cancer type	Species	Ref.
		H_2O_2 produced within tumor microenvironments is inversely correlated with NK infiltration and impairs activity	Esophageal cancer	Human	Izawa, *et al.* 2011 Cancer Immunol Immunother,
	TGF-β production	Infiltrating NK cells express low levels of activation receptors due to TGF-β-mediated inhibition	Glioblastoma	Human	Close, *et al.* 2019 Clinical and Experimental Immunology
		TGF-β/SMAD signaling pathway was constitutively activated in ALL-NK cells	Acute lymphoblastic leukemia	Human	Rouce, *et al.* 2016 Leukemia
		High concentrations of TGF-β1 present in the plasma of cancer patients were found to cause NKG2D downregulation	Lung and colorectal cancer	Human	Lee, *et al.* 2004 J Immunol
	Prostaglandin-E2 secretion	PGE2 produced by tumor-associated mesenchymal stem cells mediates NK cell inhibition	Lung cancer	Human	Galland, *et al.* 2017 Cell Reports
		PGE2 promotes thyroid cancer progression by inhibiting NK cell maturation and cytotoxicity	Thyroid cancer	Human	Park, *et al.* 2018 Frontiers in Immunology
	Interleukin-dependent STAT3 activation	Tumor cells activated the STAT3 signaling pathway, increasing IL-6 and IL-8 production, and inhibiting the activity and function of NK cells	Esophageal cancer	Human	Wu, *et al.* 2019 J Exp & Clin Cancer Research
NK cell inhibition by immunosuppressive cells	Regulatory T cells (Treg)	Inverse correlation between NK cell activation and expansion of the Treg cell population in tumor-bearing patients	Gastrointestinal stromal tumor	Human	Ghiringhelli, *et al.* 2005 J Exp Med
	Myeloid-derived suppressor cells	Autologous MDSC cells produce nitric oxide (NO), which inhibits NK cell-mediated ADCC	Melanoma, HNSCC and breast cancer	Human	Stiff, *et al.* 2018 Clin Cancer Res