Determining the Impact of NK Cells on Each Stage of Cancer Progression

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ABSTRACT

Natural Killer (NK) cells are crucial regulators of anti-tumor immunity, but their activity is importantly shaped by the tumor microenvironment across diverse cancer types. This study investigates how variations in cytokine and chemokine presence, ROS dynamics, and effector-to-target (E:T) ratios influence NK cell phenotype and cytotoxic function in Lung, Breast, Ovarian, Gastric, Renal, Colon & Colorectal cancers. Using experimental killing essays and patient data, this paper demonstrates that higher E:T ratios and a predominance of mature NK cells are closely linked to increased cancer cell death. This data also suggests that anti-inflammatory cytokines (IL-12, IFN-γ) enhance NK cell function while immunosuppressive cytokines (IL-10, IL-6) and elevated ROS levels suppress cytotoxicity. Single-cell and pathway data highlight how the balance of these molecules vary across tumor microenvironments. The findings support the development of NK cell based therapies aimed at restoring cytotoxicity and anti-tumor immunity, providing insight into the interplay between cytokines, ROS, and NK cell activity.

Keywords: *Natural Killer (NK) cells, effector to target (E:T), cytokines, reactive oxygen species (ROS), cytotoxicity, tumor microenvironment, apoptosis*

INTRODUCTION

Cytotoxic Functions of NK Cells

Natural Killer (NK) cells are a vital part of the immune system and play a critical role in tumor progression. Unlike other lymphocytes such as T cells, which require antigen specific receptors to act, NK cells can directly kill cells. T cells need these receptors to precisely identify which specific antigens presented on Major Histocompatibility Complex (MHC) molecules to bind to, ensuring a targeted response. NK cells, part of the innate immune system, are instead regulated through a balance of activating and inhibitory receptors to detect abnormal cells, and often are in direct interaction with these cells, triggering cell death through distinct cytotoxic mechanisms. This gives NK cells a unique advantage of providing a rapid defense mechanism before the T-cell mediated response develops (1).

The core process by which NK cells enact their killing mechanisms happens through a process called cytotoxicity. **Cytotoxicity** refers to the capacity of immune cells to kill cells. In the context of cancer, cytotoxicity is especially important because it allows NK cells to kill growing tumor cells and suppress tumor growth. However, NK cell activity is often inhibited due to a suppressive tumor microenvironment and immune evasion strategies by tumor cells to avoid death. Studies have shown that reduced NK cell activity or numbers in certain cancers correlate

with increased tumor growth and increased cancer development, highlighting the importance of NK cell cytotoxicity. This raises the question: **How does NK cell activity shape cancer tumor progression across different tissues or environments?** To understand how NK cell activity influences tumor progression, it's crucial to examine their primary cytotoxic mechanisms. First, this review will explore the primary cytotoxic mechanisms employed by NK cells, particularly apoptosis and necrosis, and their implications for tumor progression.

Apoptosis: Programmed Cell Death

A main mechanism of eliminating target cells is through apoptosis, a programmed intracellular self-destruction that removes abnormal cells without causing damage or inflammation. This occurs through two main pathways, the perforin/granzyme pathway or the Fas/Fas ligand (FasL) pathway, and results in the shrinking of cells controllably.

The primary intrinsic pathway, perforin/granzyme, is activated when NK cells recognize a target cell. Upon activation, NK cells release perforin, a protein that inserts into the target cell membrane and forms pores, allowing other molecules to pass through. These pores allow the entry of granzymes: enzymes released by NK cells that trigger apoptosis by activating caspases or caspase-independent pathways, leading to DNA damage and mitochondrial dysfunction. Caspase activation directly drives cell death and dismantling of the cell, while other functions within the apoptotic pathway help ensure the process remains contained, preventing damage to surrounding tissue (1,2).

NK cells can also induce apoptosis extrinsically through the Fas/Fas ligand (FasL) pathway. The Fas receptor, also known as a death receptor, is found on the surface of lymphocytes and other immune cells. When FasL binds to Fas, the protein, FADD and procaspase-8 are recruited, forming the death-inducing signaling complex (DISC). Within the DISC, procaspase-8 is cleaved to form caspase-8 , which then triggers a cascade of activating additional caspases. This cascade results in features of apoptosis, dismantling the target cell without any further inflammation (1,2).

Necrosis: Cell Tissue Death

When apoptosis is evaded, NK cells can induce necrosis, a type of cell death resulting from injury, infections, or environmental stress. Necrosis leads to the rupture of the cell membrane, releasing intracellular content and triggering inflammation that can cause damage to tissues and neighboring cells. This process involves a destruction of cell membrane integrity, following a release of DAMPs, like ATP or HMGB1. These DAMPS create an inflammatory response, inducing phagocytosis which eliminates the target cells. Additionally, chronic necrosis can also contribute to tissue damage, and may support tumor progression (1,2,3). Reactive oxygen species (ROS) play an important role in this process. NK cells generate ROS during immune attacks, which can directly damage cell membranes, protein, and DNA, contributing to necrosis in target cells. When regulated, ROS normally helps to enhance the immune system by

supporting pathogen killing and activating signaling pathways. However, excessive buildup or overproduction of ROS levels may impair NK cell function (5).

Together, apoptosis and necrosis represent two distinct strategies for eliminating harmful cells: apoptosis provides a controlled non-inflammatory technique to preserve tissue health, while necrosis is more forceful and can potentially damage tissue when controlled death is evaded.

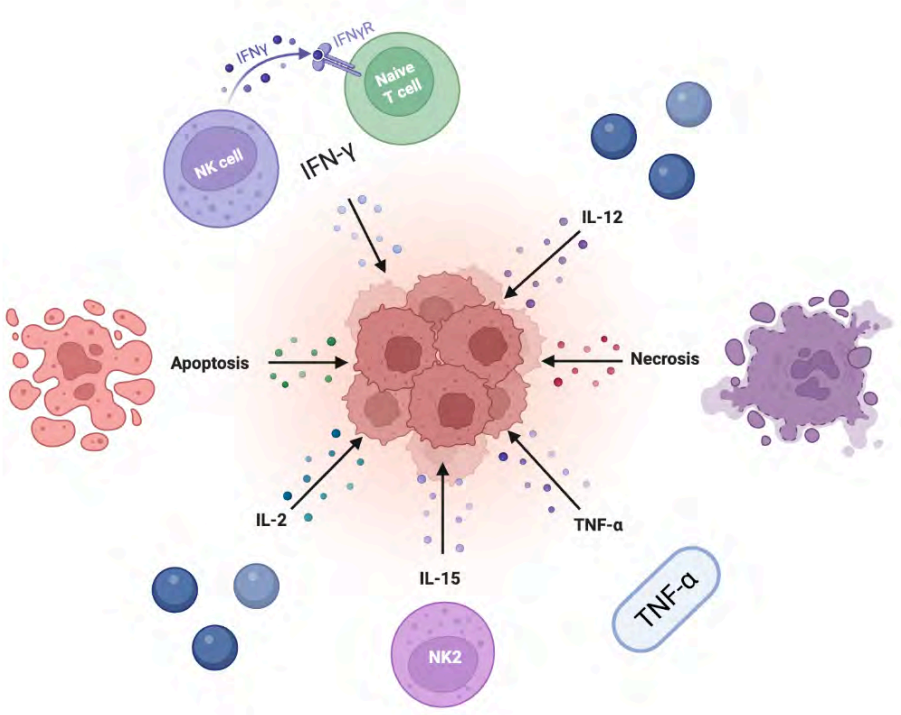

Figure 1. NK Cell Death
Details the mechanisms by which NK cells can kill. Through apoptosis (left), necrosis (right), and pro tumor killing cytokines (IL-2, IL-15, IL-12, TNF-α, and IFN-γ), NK cells eliminate tumor cells. These cytokines are especially important, as they amplify NK cytotoxicity and promote tumor suppression. **Made in www.biorender.com**.

Ultimately, these cytotoxic functions are directly related to the cell cycle and regulation of tumor cells. Within the cell cycle, those that are dividing and growing rapidly are labeled as cancer cells. These cells cause stress signals like MICA or MICB which can activate NK cells or reduce MHC-I molecules, causing NK cells to attack tumor cells. NK cells arrest tumor growth, as they can target cells in the G1 or S phase, inhibiting further growth and removing these cancerous cells. Not just by killing cells, but through the secretion of cytokines too, which also induce arrest, and stimulate immune responses, the cell cycle is regulated. However, as tumors

evolve, they may evade NK cell recognition and bypass checkpoints through altering their ligands or other methods, leading to the complex relationship between NK cells and cancer (3, 21).

Cancer Progression: ROS, Cytokines, Tumors

As tumors progress and evolve, their microenvironment changes accordingly, often causing harmful conditions for NK cells. One critical factor shaping NK behavior is the presence of **ROS**. ROS, generated by tumors and immune cells, act as important molecules, helping to regulate cell growth and immune activity. In balanced amounts, ROS promotes NK and T cell activation (6). However, once ROS levels exceed a threshold, they begin to have harmful effects, including cell death through apoptosis, necrosis, and promoting tumor development.

In cancer cells, these ROS levels are normally elevated due to increased metabolic activity and hypoxic conditions within the tumor microenvironment. Higher levels of ROS contribute to DNA damage, triggering the start of unchecked tumor proliferation. At the same time, ROS modulate signaling pathways, such as NF-κB, HIF-1α, and STAT3, which promote the survival of tumor cells. On the immune side, excess ROS can suppress NK cell function by downregulating activation receptors and inducing NK cell apoptosis, inhibiting the ability to kill target cells (7,8). This not only lowers the clearance of tumor cells, but also promotes immune evasion and metastasis, as tumor cells continue to create an immunosuppressive environment.

The relationship between ROS and NK cells is complex, but essential for understanding tumors. While NK cells are essential for eliminating abnormal or stressed cells, they are extremely sensitive to chances and stresses in the tumor microenvironment. At controlled levels, this makes ROS act as a positive regulator by enhancing NK cell activation and cytotoxicity. Chronic ROS, however, shifts the balance toward tumor progression. Adaptive responses which normally help microenvironments, such as dendritic cell activation or T cell recruitment, are also impaired by the tumor microenvironments. This role demonstrates the importance of ROS as a regulator between tumor progression and mediating the immune system (9,10)

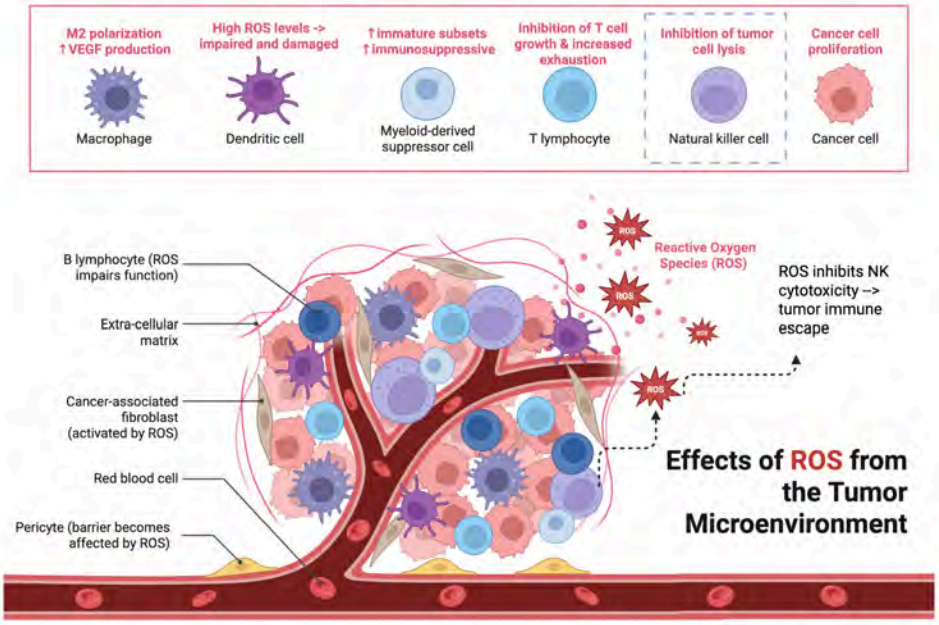

Figure 2. Effects of ROS
Reactive Oxygen Species (ROS) impairs multiple cell functions including macrophages, dendritic cells, myeloid-derived suppressor cells, T lymphocytes, NK cells, cancer cells, and B lymphocytes. ROS also activates cancer-associated fibroblasts, alters pericyte barrier function, and interacts with the extracellular matrix and red blood cells. Elevated ROS levels promote an immunosuppressive microenvironment that favors tumor progression. **Made in www.biorender.com.**

Beyond ROS, NK cell function is also profoundly regulated by another class of signaling molecules: cytokines and chemokines, which influence behavior, activation, etc. Cytokines such as IL-2, IL-15, TNF-α, IFN-γ play important roles when it comes to cytotoxicity. They are signaling molecules that help facilitate the body's immune and inflammation responses, influencing the function of NK cells and their ability to recognize and eliminate target cells. Chemokines, such as CXCL9 and CXCL10, help direct NK cells towards tumor sites, ensuring communication within the cell. While these cytokines and chemokines facilitate NK function, tumors can resist NK attack by altering these molecules by secreting inhibitory cytokines or downregulating activating ligands (11,12). This causes a reduced number of NK cell subsets and can suppress receptors on NK cells, therefore inhibiting their function and causing tumor progression (1).

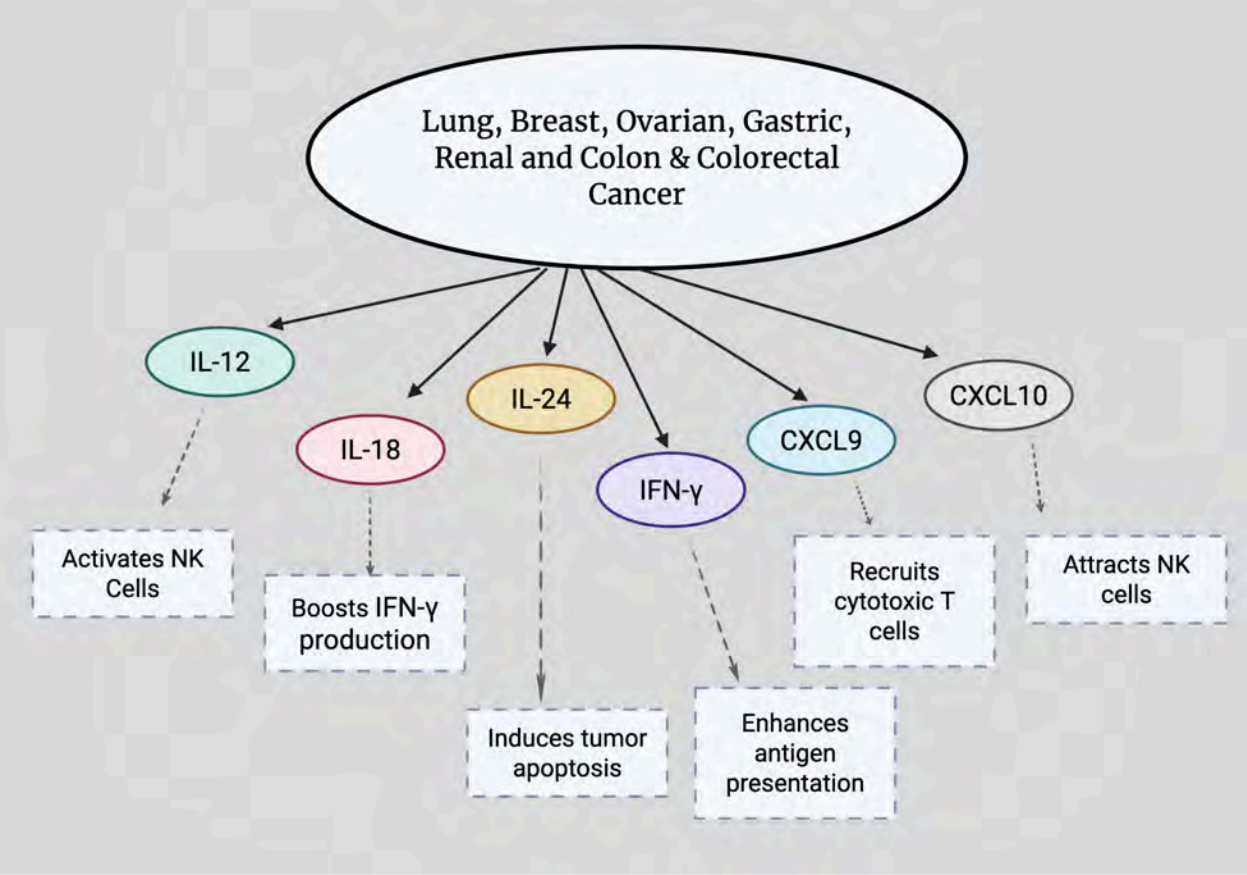

Figure 3. Cytokines and Chemokines of Cancer
Positive roles of various cytokines (IL-12, IL-18, IL-24, IFN-γ) and chemokines (CXCL9, CXCL10) in lung, breast, ovarian, gastric, renal and colon & colorectal cancers. Cytokines regulate NK cell activity, while chemokines drive Nk cell movement. Both interact with ROS pathways to influence cancer progression. **Made with www.biorender.com.**

NK Cell Numbers and Subsets Across Cancer Types

The abundance and state of NK cells play an important role in cancer progression and survival. Examining NK cell numbers and their subsets across different cancers provides insight into how these immune cells can either suppress or promote tumor growth.

Across cancers and progressive stages, reduced NK cell numbers are associated with poor survival rates and enhanced tumor growth. In contrast, higher NK cell numbers have led to improved survival in lung, colorectal, and many other cancers. However, there is one exception: **t**riple-**n**egative **b**reast **c**ancer (TNBC). In TNBC, a higher number of NK cells correlates with worse outcomes, as NK cells actually aid tumor progression. This paradox is because of an immature subset of CD56^bright NK cells. In healthy conditions, CD56^bright NK cells are less cytotoxic but are important for secreting cytokines such as TNF-α and IFN-γ, which help promote immune responses (13, 27). Within the TNBC tumor microenvironment, however, these cells fail to perform their role. Their immaturity exhibits reduced cytotoxicity due to low granzyme

and perforin expression and also secrete cytokines in a way that favors tumor growth and immune evasion. While normally regulatory, this NK subset becomes one of the main factors in poor survival for TNBC.

This complex relationship between NK subsets and tumor outcomes highlights the importance of not just NK cell abundance, but NK cell maturity and function. In regards to therapeutic strategies, approaches that target ROS pathways or modulate cytokine signaling may help shift NK cells toward a more tumor-suppressive and cytotoxic phenotype.

Tissue Microenvironments

Key Cancer Types: *Lung, Breast, Ovarian, Gastric, Renal, Colon & Colorectal*

The study of major solid tumors, including lung, breast, ovarian, gastric, renal and colon/colorectal cancers, highlights how distinct tissue microenvironments influence NK cell activity and disease progression. Lung cancer, the leading cause of cancer-related deaths, develops within a highly immunosuppressive microenvironment with factors that impair immune cells and NK cells that are critical for early tumor clearance (17, 20). In breast cancer, particularly hormone receptor-positive and HER2-positive subtypes, NK cells often show reduced cytotoxicity and altered states (14). Colorectal cancer progression reflects both genetic mutations and a microenvironment enriched in suppressive cytokines and metabolic stress, limiting NK maturation and infiltration (15). Gastric and ovarian cancers, frequently diagnosed at advanced stages, feature hypoxic and cytokine rich niches that limit NK response, though ovarian tumors paradoxically show high NK cell activity along suppression. Lastly, in renal cell carcinoma (kidney cancer) inflammatory and stress-related signals combine with immune cell infiltration to shape NK cell function. As explored in this paper, each of these cancerous functions depends heavily on the microenvironment's ability to sustain or suppress NK cell activity.

Despite their distinct origins, these diverse cancer tissue microenvironments share several critical features that collectively undermine the immune system and NK cell function. Some commonalities are increased levels of ROS, nutrient deprivation, acidic pH, and immunosuppressive cytokines, such as IL-10. These factors collectively work to suppress NK cell activity by reducing resources necessary for cytotoxicity and altering the balance within the cell.

The diverse tumor microenvironments profoundly influence the dynamics of NK cells, impacting their activation, proliferation, and maturation at different stages of progression. In early stages, NK cells are more effectively recruited with higher cytotoxicity due to a higher number of cytokines and activating ligands on malignant cells. However, in later stages, as the tumor progresses, the environment becomes increasingly suppressive and reductions in NK cells start to appear, causing impairment and less mature phenotypes (16, 18,19).

Within these varying tumor microenvironments, the ratio of NK cells to target cells is a critical determinant of patient survival rates, and this ratio is significantly influenced by the microenvironmental conditions. High NK to target ratios are often associated with a controlled early stage, yet diminish as tumors begin to evade detection and change their microenvironment. The difference in this ratio is driven by processes involving cytokines and chemokines, and involves differing rates of NK cell expansion and proliferation. In more aggressive cancers, factors like TGF-β can reduce overall NK cell numbers and create immature subsets with reduced cytotoxicity.

As mentioned before, immature subsets can be marked by high expression of CD56^bright. Others include low CD16, receptors like NKG2A, or a lack of killer immunoglobulin receptors (KIRs). These subsets are more likely to have decreased cytotoxicity, however they excel at releasing cytokines and help to regulate the immune system (13, 22). But, with tumor progression, the prevalence of these subsets are enhanced. Increased immature NK cells across tumor microenvironments generally correlates with reduced antitumor immunity, impaired direct killing of cancerous cells, increased immune evasion, and poorer outcomes with more aggressive tumor behavior.

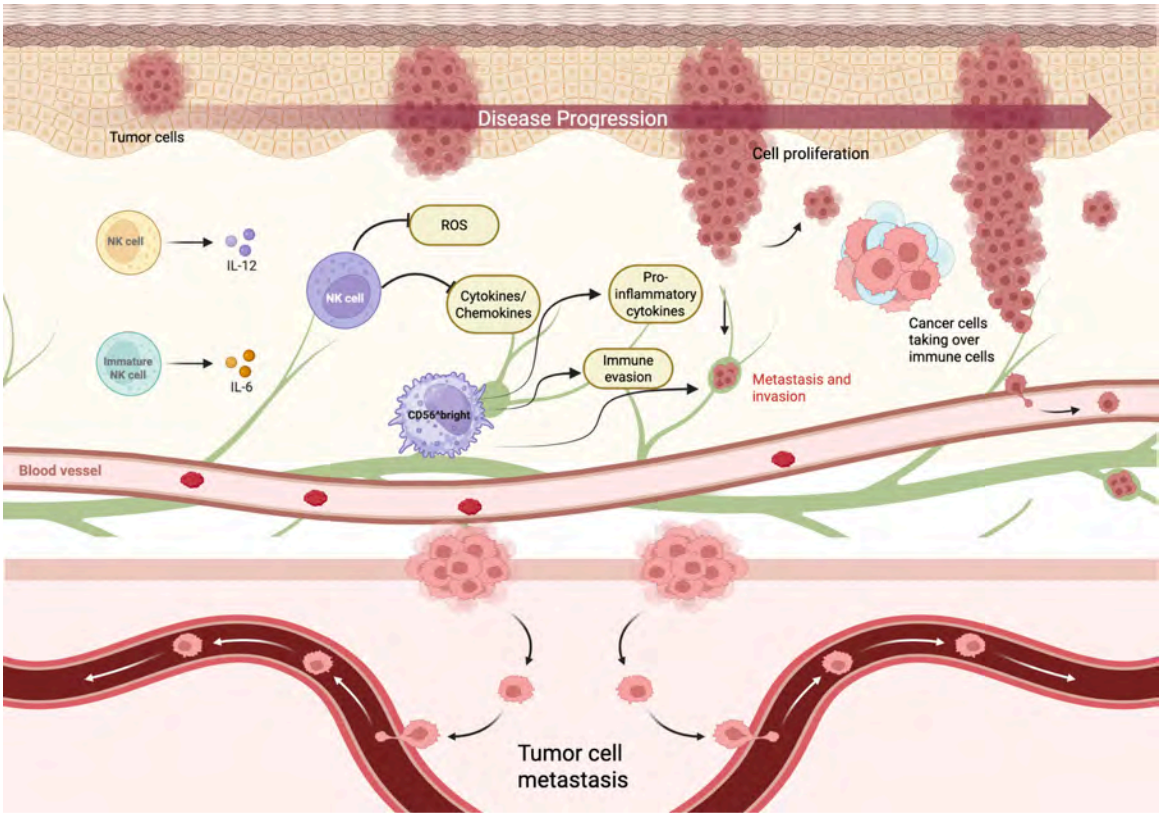

Figure 4. Disease Progression

The top panel illustrates cell proliferation, where tumor cells multiply to form a larger mass. This process is influenced by the interaction between various immune cells (immature/mature NK

cells) and cytokines (IL-6, IL-12, and pro-inflammatory cytokines). Cancer cells evade the immune system (immune evasion), and take over immune cells. This causes metastasis and the evasion of surrounding tissue and blood vessels. The bottom panel, labeled “Tumor cell metastasis” depicts cancer cells spreading to a new location in the body, making it more harmful. **Made in www.biorender.com.**

Therapeutic Implications

Given their potent cytotoxic capabilities and inherent safety profile, NK cell-based therapies are emerging as promising cancer treatments, offering significant benefits over traditional immunotherapies. CAR-NK cells (NK cells with Chimeric Antigen Receptors) can be used effectively, as clinical trials have shown fewer adverse side effects compared to CAR T-cell therapy. The CAR enables NK cells to recognize tumor-specific antigens directly, activating NK cell killing through perforin/granzyme release and cytokine secretion, enhancing tumor clearance. Unlike CAR T-cell therapy, CAR-NK therapy is associated with lower rates of side effects like cytokine release syndrome and neurotoxicity, making it a safer option. Recent strategies also focus on enhancing NK cell function directly through genetic engineering to improve tumor recognition and resistance to suppression. Furthermore, optimizing NK cell therapies involves combining them with antibodies or other immunostimulatory agents to activate dendritic and T cells, thereby fostering lasting immune protection (23).

Cytokine-Based Therapies and Checkpoint Blockade in NK Cell Immunotherapy

NK cell immunotherapy also leverages distinct strategies to enhance NK cell activity, notably through cytokine-based therapies and immune checkpoint blockade.Cytokine therapy aims to enhance NK cell proliferation, activation, and cytotoxic function by restoring activation signals and growth that may have been impaired in cancer patients. Key cytokines include IL-2, IL-12, IL-15, and IL-2, which act through various mechanisms: IL-2 promotes NK cell survival and proliferation, IL-12 stimulates IFN-γ production, IL-15 is important for NK cell development and cytotoxic activity, and IL-21 enhances granzyme and perforin expression, boosting tumor killing. Among these, IL-2 and IL-15 have advanced into early phase clinical trials, while IL-12 and IL-21 remain primarily in pre-clinical stages (11, 24)

Immune checkpoint blockade, effective for T cell activation in cancer, is now being extended to target NK cell specific inhibitory pathways that dampen NK cytotoxicity. Blocking these pathways releases NK cells from inhibitory signals, restoring their ability to kill tumor cells. Some targets include PD-1/PD-L1, a pathway that suppresses T and NK cell activity; KIR blockade,which dampens NK cytotoxicity; and NKG2A, which limits NK cell activation. Other inhibitory receptors include TIM-3, TIGIT, and CD96, which contribute to immune evasion and NK cell exhaustion. While most of these approaches remain in pre-clinical trials, some anti–PD-1/PD-L1 and anti-NKG2A antibodies have advanced with certain stages already being approved in immunotherapy and being further evaluated for specific benefits related to NK cells (25).

METHODS AND MATERIALS

Data Acquisition

Publicly available datasets were obtained through searches on the National Center for Biotechnology Information (NCBI) database. To ensure a collection of relevant material, searches were conducted using keywords such as “NK cell,” “cell cycle”, “progression,” “cytokines,” and the names of specific cancer types, including: breast, colorectal, lung, and ovarian. These keywords were chosen to focus on studies that investigated the role of natural killer (NK) cells in cancer progression, and their involvement in cell cycle regulation and cytokine signaling.

Data Selection

Datasets were evaluated and selected based on several criteria. These datasets were required to focus on at least one of the specific cancer types (breast, colorectal & colon, lung, ovarian, gastric, or renal). Additionally, datasets were only selected if they included information on survival versus death data, as it provided the opportunity to link NK cell presence to patient outcomes. Finally, datasets were prioritized if they contained specific information on NK cells like frequency, activity, and their relationship with cytokine presence. Studies which did not meet these criteria or lacked detail on NK cells were excluded from this paper and further analysis.

Data Analysis

Following the selection, datasets were analyzed to evaluate the link between NK cell activity and tumor progression. Within each dataset, NK cell activity was categorized as high or low, and compared to determine how these categories relate to tumor size, disease progression, and patient survival. Additionally, the presence of specific cytokines (e.g., IL-15, IFN-γ) was recorded, and examined to assess their correlation with tumor size and progression. The analysis also considered the number of NK cells, or lack thereof, and how it influenced tumor growth across various cancer types. By comparing NK cell activity and cytokine presence across multiple datasets, a consistent pattern was identified regarding the role of NK cells in regulating cancer progression.

Supplementary Data Sources

In addition to publicly available data sets, online academic journals (Journal of Immunology and PubMed) were also accessed to obtain supplementary data tables and figures, including NK cell to cancer cell ratios and cytokine levels. These supplementary sources provided additional details on NK cell to cancer ratios and cytokine levels, which were later integrated into the overall analysis. Furthermore, using Reactome and the Human Cell Atlas, information on the role of cytokines and their association with the microenvironment was

obtained, providing additional data. Together, these sources strengthened the validity and findings, confirming observed patterns across data types.

Statistical Analysis

Lastly, a 2 way ANOVA test was performed to assess the independent and combined effects of cancer type and effector to target (E:T) ratios on NK cell-mediated cytotoxic response. This analysis evaluated how varying tissue microenvironments and E:T ratios contribute to differences in NK cell effectiveness across gastric, ovarian, renal, and colon cancers. Results are summarized in Table 2, showing cancer type and E:T ratio as statistically significant factors influence NK cell cytotoxicity.

RESULTS

NK Cell Ratio and Tumor Cell Death Across Cancers

Across experimental killing assays of gastric, renal, ovarian, and colorectal carcinomas, the results demonstrate that effector to target (E:T) ratios are a strong determinant of cancer cell lysis (26) . High E:T ratios are associated with increased killing and more robust cytotoxicity. At a 40:1 ratio, in gastric cancer, ligand-mismatched NK cells showed increased levels of target cell death, with over 80% lysis. This positive correlation continued as the E:T ratio decreased: both ovarian and renal cancer cells showed reduced NK cell killing at 20:1 and 10:1 ratios. Colon cancer cells, by contrast, presented the lowest rates of lysis across ratios, exhibiting either greater resistance or more immune evasion.This consistent decrease in cancer cell killing, seen as the number of NK cells continue to drop, shows that it’s not just important to have NK cells present, but also to have them in close contact with tumor cells in the tumor environment. These patterns indicate that the best treatment outcomes occur when there is a higher percentage of mature and effective NK cells relative to cancer cells.

Table 1. Cancer Type, Effector to Target Ratio, and Response Value. Primary data derived from Re et al, 2006 (26).

Cancer	E_T Ratio	Response
Gastric Cancer	40:1	0.8200
Ovarian Cancer	40:1	0.7900
Renal Carcinoma	40:1	0.7500
Colon Cancer	40:1	0.4000
Gastric Cancer	20:1	0.8200
Ovarian Cancer	20:1	0.6500
Renal Carcinoma	20:1	0.5700
Colon Cancer	20:1	0.3200
Gastric Cancer	10:1	0.8000
Ovarian Cancer	10:1	0.4300
Renal Carcinoma	10:1	0.5000
Colon Cancer	10:1	0.2900

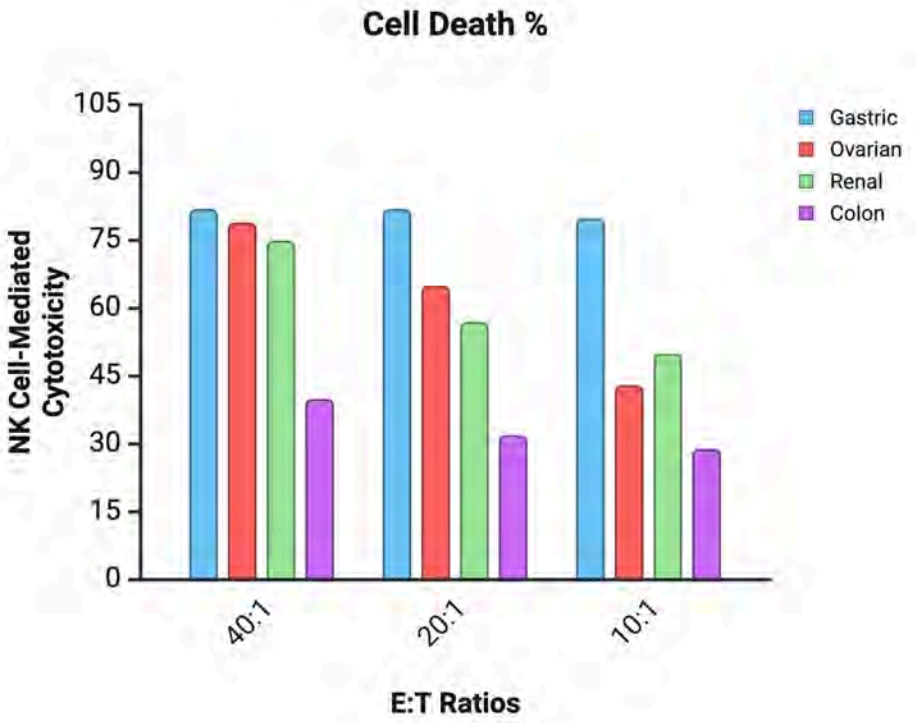

Figure 5. NK cell-mediated cytotoxicity E:T ratio across cancer types

The graph displays the percentage of cell death induced by NK Cells (effector) across four different cancer cell lines: Gastric, Ovarian, Renal, and Colon. The cytotoxic percentage was measured at three different effector: target (E:T) ratios: 40:1, 20:1, and 10:1. This data indicates NK cytotoxicity is most effective against gastric cancer, and less effective against colon cancer. Additionally, NK cell cytotoxicity generally decreases as E:T cell decreases. **Made in biorender.com**

Table 2. Statistical results from ANOVA evaluation of NK cell mediated cytotoxicity and effect of E_T Ratio and cancer type/tissue.

	Sum of Squares	DF	Mean Squares	F	P value
Cancer Type	0.34603	3	0.11534	18.571	0.0019357
E_T Ratio	0.0686	2	0.0343	5.5224	0.04362
Error	0.037267	6	0.0062111		
Total	0.4519	11			

Statistical analysis using two-way ANOVA evaluated the impact of cancer type and E:T ratio on NK cell-mediated cytotoxicity across these models. The results revealed that cancer type has a greater effect ($F=18.57$, $p=0.0019$) than E:T ratio ($F=5.52$, $p=0.043$) on cytotoxic response. The low p-value for cancer type ($p < 0.01$) indicates a significant difference in NK cell cytotoxicity among different cancer tissues. The E:T ratio also is statistically significant ($p < 0.05$), indicating that while it still has an effect, it is smaller than that of cancer type. Together, these findings highlight the importance of both the cancer tissue and effector to target concentration.

Table 3. Survival Correlation

Cancer Type	NK Cell Subset	Survival Correlation
Lung Cancer (13)	CD57	Patients with >5 TINK (Tumor infiltrating NK) had better survival, risk of death higher with <5 TINK
Triple Negative Breast Cancer (27)	CD56^bright	Higher levels of CD56^bright NK cells correlate with poor survival

Life/Death Outcomes: NK Cell Maturity and Survival

Quantitative analysis has revealed a critical link between the presence of tumor infiltrating NK cells, specifically mature and cytotoxic subsets, and outcomes across various cancers. In lung cancer, those with subsets of the CD57+ (mature) NK cell demonstrate significantly greater survival rates. This association supports models in which the presence of effective NK cells promotes the immune system and deters tumor progression. These findings additionally show that measuring the number of NK cells or analyzing their phenotypes can help predict how lung cancer patients will respond to treatment. By contrast, in breast cancer patients, despite increased infiltration of NK cells, these outcomes did not mirror those of lung or colorectal cancers. Here, most NK cells were CD56^bright, an immature phenotype. These cells show decreased expression of granzyme and perforin, and led to poor tumor cell killing in these patients. As shown by Table 3, their infiltration correlates with poor survival. In colorectal cancer, a different dynamic appeared: although mature NK cells were present, their effectiveness was dulled by metabolic dysfunctions, most notably mitochondrial impairment leading to reduced granzyme levels. These observations illustrate that survival is not just tied to NK cell presence, but to their maturity and cytotoxicity within the specific tumor tissue as well.

Cytokines and Chemokines Across Cancers/Stages

Across all cancer types analyzed, NK cell function and their effectiveness was influenced by the cytokines and chemokines present in each tumor microenvironment, and at each stage of progression. High levels of cytokines, particularly in early stage tumors, such as IL-12, IL-18, IL-24 and IFN-γ generally supported greater cytotoxicity and NK cell proliferation, creating an environment free of tumor cells. The presence of such cytokines often coincides with increased expression of chemokines like CXCL9 and CXCL10, helping the migration of NK cells into tumorous tissue. However, as shown from the data, as cancers progressed or adapted to pressure, the landscape of cytokines and chemokines began to change, shifting towards a weaker immune system. Some signs of this include: rising TGF-β and IL-10 concentrations, more attraction of immature subsets, and inhibition of NK cell growth and cytotoxicity. The transition going from working to suppressive cytokines and chemokines was especially present in advanced breast cancer and late stage colorectal tumors, where overlonged exposure led to impaired growth, function, and an immature subset of NK cells. These altered phenotypes further affected the environment by releasing immunomodulatory cytokines, thus promoting tumor evasion and further immunosuppression.

IL-10 Expression and Immune Cell Diversity

Figure 6B below (left UMAP) shows the quantitative expression of IL-10 across different cell clusters, highlighting the key role of IL-10 as an immunosuppressive cytokine. IL-10 suppresses NK cell function and affects the tumor microenvironment by depending on immune response in many cancer types, not just renal cell carcinoma. Figure 6C (right UMAP) shows the diversity of immune and stromal cells within the tumor, illustrating the complexity of the microenvironment and emphasizing the importance of understanding which cells produce or respond to cytokines for interpreting NK cell activity.

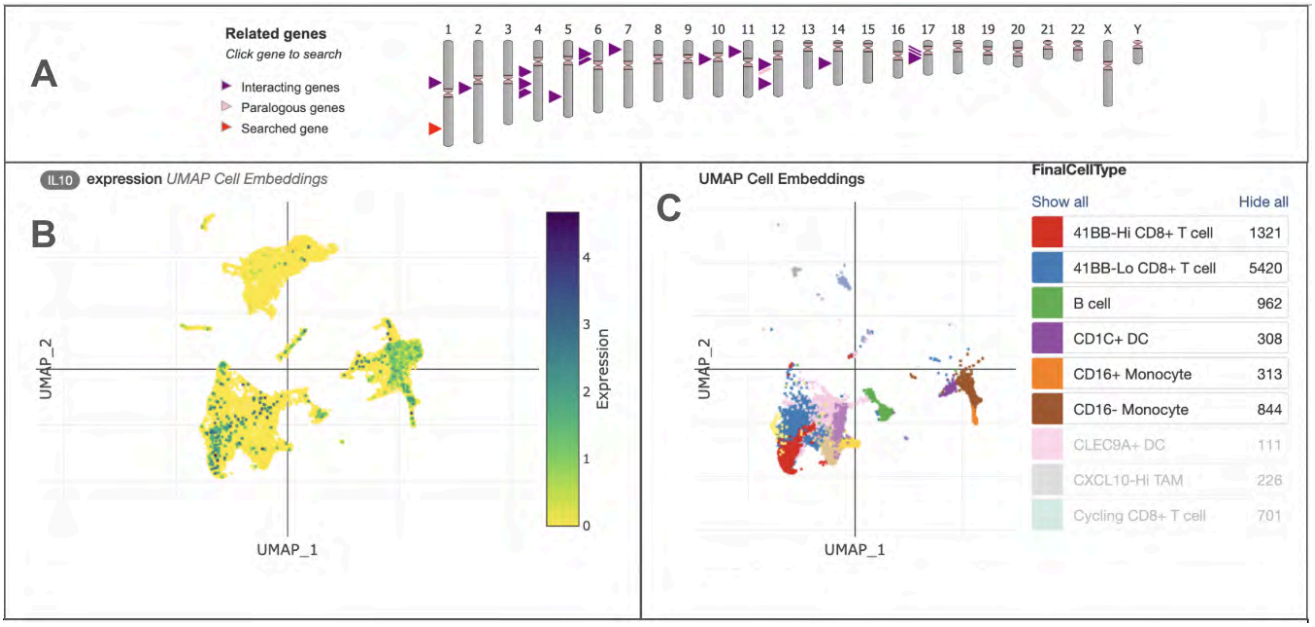

Figure 6. Visualization and UMAP plots of malignant and nonmalignant cells captured across all lesions colored by broad cell type generated using the Human Cell Atlas. Granular cell types and states were discerned through iterative reprojection and unsupervised clustering of lymphoid myeloid and tumor compartments and merged into broader cell type categories for visualization of expression of genes of interest **(6A).** Quantitative UMAP plot showing expression levels of IL-10 across different cell clusters within the tumor microenvironment. Each point represents an individual cell, colored with the according magnitude of expression. High IL-10 levels mark higher immunosuppressive areas that can dampen NK cell activity and cytotoxicity as illustrated in **6B**. UMAP plot illustrating the diversity of immune and stromal cell types present in the tumor microenvironment. Each color corresponds to a different cell type (CD8+T cells, B cells, dendritic cells, and various monocyte populates) as shown in the chart. This highlights the complexity in cytokine dynamics and their impact on NK cell states and tumor evasion **(6C)**.

IL-6 Family Cytokine Signaling Cascade

This figure provides a graphic of the IL-6 family cytokine signaling cascade in lung cancer. This includes several cytokines, IL-6, IL-11, IL-27, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin 1 and 2 (CT-1 and CT-2), and cardiotrophin-like cytokine (CLC). These drive pro-inflammatory and immunosuppressive signaling in the tumor microenvironment. This process details immune cell signaling, reduction of NK cell cytotoxicity, and immune evasion. While the data features lung cancer, similar pathways and cytokine-driven immune suppression are evident in breast, colon, and ovarian cancers as well.

Figure 7. IL-6 Family Cytokine Signaling Pathway in Lung Cancer
Representation of the IL-6 family cytokine signaling cascade in lung cancer. This diagram follows tyrosine phosphorylation events from the IL-6 receptor and downstream activation of JAK and STAT pathways, involving IL6, IL11, LIF, OSM, CNTF, CT-1, CT-2, and CLC. This demonstrates how events like phosphorylation can translate cytokine signals into programs that mediate inflammation, immune suppression, and NK cell dysfunction. **From www.reactome.com**

ROS and NK Cell Function in Tumor Microenvironments

Reactive oxygen species (ROS) emerged as an important mediator of NK cell function, with effects that shape the tumor microenvironment. In experiments with analyzed tumor samples and model systems, ROS levels were found to be increased, particularly in areas of rapid growth and inflammation. In normal conditions, this ROS activity enhanced NK cell activation and supported the release of effector molecules, boosting anti-tumor responses. However, when ROS accumulation passed a certain threshold, NK cells became susceptible to damage. This led to the downregulation of activating receptors (e.g. NKG2D), lowered cytokine production, and ultimately apoptosis of NK cells themselves. In tumors with ROS suppression, both T cell and NK cell function were impaired, highlighting the importance of a finely tuned environment and state. In another example, in advanced colorectal and lung tumors, excessive ROS was associated with a decline in NK cell numbers and function, further supporting that fluctuating levels of ROS may lead to disruption in the immune system and tumor escape. Thus, the balance and levels of ROS within the tumor microenvironments not only dictate NK cell fate, but also overall immune pressure on cancer progression.

DISCUSSION

My findings reinforce that the function and makeup of NK cells within the tumor environment are important determinants of cancer cell death and survival outcomes. As shown in Figure 5, Table 1, and Table 3, higher E:T ratios of mature NK cells has led to greater tumor cell lysis, supporting their role as immune regulators. Their cytotoxic responses mirror the expected survival correlation of higher NK cell infiltration with improved clinical results in active tumors, such as lung and colorectal cancers. This also explains the variability in outcomes when NK cell maturity and other factors shift in microenvironments which are suppressive like breast or melanoma cancers.

Additionally, this data demonstrates that high NK cell presence does not necessarily translate to similar outcomes across all tissues; instead, the function and local environment dictate the outcome. As shown in Table 3, in lung cancer, the survival benefit was linked to mature, cytotoxic NK cell subsets, echoing findings from other trials that emphasize the importance of NK cell phenotype. In contrast, triple negative breast cancer features a high number of immature, cytokine-secreting NK cells which contributes to decreased immune regulation and less direct tumor lysis.

The analysis of cytokine and chemokine profiles further shows the interplay between immune activation and suppression in the tumor microenvironment. Pro-inflammatory cytokines, such as IL-15 and IFN-γ, correlate with robust NK cell function, driving cytotoxicity. In contrast, immunosuppressive cytokines like IL-10 and IL-6, as visualized in the Human Cell Atlas and Reactome figures, are predominantly expressed in immune and stromal cell populations, reinforcing suppression of NK cell activity and promoting NK exhaustion through STAT3 pathways. Figure 6 confirms that IL-10 is abundantly secreted by tumor and myeloid cells, with immunosuppressive signaling in critical niches, while Figure 6 illustrates how IL-6 members activate downstream JAK-STAT signaling, supporting immunoexpression. This evidence highlights the critical role of cytokines in shaping NK cell phenotype and effectiveness. Similarly, the role of ROS as a regulator and switch for immune activation, capable of both activating and inhibiting NK cell function depending on concentration and duration, further supports findings indicating that a balanced microenvironment may be a target for restoring NK cell activity in tumors. Together, these results show that this balance, with cytokines and controlled ROS levels, coincides with the understanding that modulating these factors is essential to the function of cytokine-based therapies.

These insights point towards the need to develop more refined therapeutic strategies. Further research should focus on not only increasing NK cell numbers, but also promoting maturation, persistence, and resistance to suppressive areas of the cancer microenvironment. These results suggest that cytokine based therapies, immune checkpoint blockade, and genetically engineered NK cell products appear to be promising tools for enhancing NK cell activity and tumor control, especially in varied microenvironments.

CONCLUSION

In summary, this research paper investigates how the tumor microenvironment, across Lung, Breast, Ovarian, Gastric, Renal, Colon & Colorectal cancers shapes NK cell presence, phenotype, and function. This paper discussed the roles of cytokines, chemokines, and ROS dynamics. Using quantitative analyses of functional assays and patient data, this study demonstrates that patient/ survival outcomes and tumor cell death are closely linked to the higher abundance and maturity of NK cells, with higher effector to target ratios and mature phenotypes correlating with improved outcomes. This paper highlights the functional role of cytokines; how immunosuppressive cytokines such as IL-10 and TGF-β, as well as elevated levels of ROS, impair NK cell cytotoxicity. Overall, this paper provides insight into the dynamics of NK cells and its relation with the tumor microenvironment, touching on the potential frameworks that are necessary for improving cancer immunotherapy outcomes.

ACKNOWLEDGEMENTS

Thank you to Luke Fisher and Alexandra Massa for their edits to this paper. I would also like to thank Jacqueline Howells for her support and mentorship. Lastly, thank you to my family for their support.

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