

Application of natural killer cells in pancreatic cancer (Review)

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Abstract. Pancreatic cancer, a highly malignant disease, is characterized by rapid progression and early metastasis. Although the integrative treatment of pancreatic cancer has made great progress, the prognosis of patients with advanced pancreatic cancer remains extremely poor. In recent years, with the advancements in tumor immunology, immunotherapy has become a promising remedy for pancreatic cancer. Natural killer (NK) cells are the key lymphocytes in the innate immune system. NK cell function does not require antigen pre-sensitization and is not major histocompatibility complex restricted. By targeting tumors or virus-infected cells, the cells play a key role in immune surveillance. Although several questions about NK cells in pancreatic cancer still need to be further studied, there are extensive theories supporting the clinical application prospects of NK cell immunotherapy in pancreatic cancer. Since very few studies have evaluated the role of NK cells in pancreatic cancer, this review provides a comprehensive update of the role of NK cells in pancreatic cancer immunotherapy.

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1. Introduction

Pancreatic cancer is a highly malignant disease associated with rapid progression and early metastasis, with >200,000 associated deaths per year worldwide (1). In the United States, pancreatic cancer ranks fourth in terms of tumor-related mortality. In 2017, the number of new pancreatic cancer cases in the United States reached 53,670, and the number of deaths due to pancreatic cancer was ~43,090. Furthermore, pancreatic cancer is estimated to become the second-most common cause of cancer-related death by 2030 (2,3).

Natural killer (NK) cells are a group of innate lymphocytes, discovered >40 years ago, which can eliminate target cells infected by viruses or undergo malignant transformation (4). Derived from bone marrow lymphoid stem cells, the differentiation and development of NK cells depend on the microenvironments of the bone marrow or thymus. NK cells are located mainly within the peripheral blood and spleen, where they account for 10-20% of all peripheral blood lymphocytes. The cells do not express specific antigen-recognition receptors. NK cells are the third type of lymphocytes, differing from T lymphocytes and B lymphocytes (5). NK cells do not require antigen pre-sensitization and major histocompatibility complex restriction, and by targeting tumors, or virus-infected cells, they play a key role in immune surveillance (6,7). NK cells not only kill target cells directly, but also mobilize the entire immune system by producing several cytokines (8). The activity of NK cells is regulated by a variety of regulatory receptors on their surface; when NK cells encounter target cells, they can secrete perforin to destroy cell membranes and release granzymes for a lytic killing effect, acting through the Fas/FasL pathway or the TNF- α /TNFR-1 pathway to induce apoptosis (9,10). Moreover, activated NK cells can also secrete cytokines, such as TNF- α and IFN- γ , to exert antibacterial and antitumor effects. These cytokines can directly act on target cells or attack target cells by activating other types of immune cells (11).

The number and activity of NK cells is closely associated with pancreatic cancer. Lee *et al* (12) found that NK cell activity decreased as the disease progressed in patients with pancreatic cancer, which was associated with a poor clinical outcome. Marcon *et al* (13) analyzed the phenotype and function of NK cells in the blood and tumor tissues of patients with pancreatic ductal adenocarcinoma (PDAC), and found that NK cell quantity remained quite normal in the peripheral blood, but displayed the CD16^{hi}CD57^{hi} phenotype, with marked downregulation of NK group 2D (NKG2D). Notably, these cells demonstrate decreased

the cytotoxic activity and low IFN- γ expression levels, instead producing high intracellular levels of the immunoregulatory cytokine IL-10, which is found at increased levels in the blood of patients with PDAC. Masuyama *et al* (14) used antibodies against CD3 and CD52 to expand the number of NK cells *in vitro* and found that the number, cytotoxicity and antitumor activity of NK cells were significantly increased in the peripheral blood of a mouse model of pancreatic cancer. One patient diagnosed with pancreatic cancer was found to achieve greater survival benefits than expected, as well as the shrinkage of metastatic lesions (a metastatic peritoneal lesion disappeared and hepatic lesions shrunk on the CT scan); the patient eventually died 13 months after NK cell therapy, suggesting that the persistence of adoptively transferred NK cells can prolong overall survival (OS) time. Lin *et al* (15) used IL-2 and TKD to expand the number of NK cells *in vitro* in a mouse model and found that after adoptive treatment for primary and metastatic pancreatic cancer, both primary and metastatic lesions were decreased in size. The current study reviews the progress in the application of NK cells in the treatment of pancreatic cancer, hoping to provide new insights for improving the therapeutic effect of pancreatic cancer.

2. Effect of surgery

The pancreas is located behind the peritoneum, surrounded by other organs. As a result, when pancreatic tumors occur, the symptoms are atypical and difficult to detect in the early stage. In total, 80-85% of patients are at a locally advanced or metastatic stage at the time of diagnosis and have missed the optimal treatment time, leading to the extremely poor effect of pancreatic cancer treatments. Studies have shown that the surgical resection of pancreatic cancer affects the number of NK cells in the peripheral blood of patients with pancreatic cancer. Some patients experience an increase in the number of NK cells after surgery. The number of NK cells is positively correlated with the survival rate of patients, and NK cells can decrease the recurrence rate of pancreatic cancer and decrease the occurrence of minimal residual disease (16,17). These findings lay a theoretical foundation for surgery combined with NK cell immunotherapy for pancreatic cancer.

3. Effect of chemotherapy

Chemotherapy is currently the cornerstone of pancreatic cancer treatment. The main chemotherapy drugs for PDAC are gemcitabine, albumin-bound paclitaxel, irinotecan, oxaliplatin and fluorouracil. For patients with untreated advanced disease, there are two options for the first-line standard chemotherapy regimen: A gemcitabine combined with albumin-bound paclitaxel regimen (18), which is relatively well tolerated and confers a median survival time of 8.7 months, and the 5-fluorouracil, leucovorin, irinotecan and oxaliplatin regimen (19), which has a median survival of 11.1 months, but significant toxicity. As patients with pancreatic cancer cannot tolerate long-term systemic drug treatment, the efficacy is difficult to guarantee. Furthermore, due to the heterogeneity of pancreatic cancer, there is no chemotherapeutic drug that can specifically kill pancreatic cancer cells to improve the survival rate of patients (20).

Mota Reyes *et al* (21) found that neoadjuvant therapy reshapes the microenvironment of pancreatic cancer by decreasing the number of primary immune cells. Survival analysis showed that intratumoral infiltration of CD4⁺ T cells and NK cells was an independent prognostic factor for the neoadjuvant treatment of pancreatic cancer. Hane *et al* (22) studied the altered expression of immune-related genes in the tumor microenvironment of pancreatic cancer after neoadjuvant chemotherapy, and observed that expression of 11 genes (LY86, SH2D1A, CD247, TIGIT, CR2, CD83, LAMP3, CXCR4, DUSP4, SELL and IL2RA) was significantly down-regulated in the neoadjuvant chemotherapy group compared with that in the surgery group. Gene expression analysis showed that the function of regulatory T cells, B cells and NK CD56^{dim} cells in the neoadjuvant chemotherapy group was significantly decreased. Gürlevik *et al* (23) found that the number of NK cells at the margin of tumor resection increased significantly after tumor resection in orthotopic pancreatic cancer mice and adjuvant gemcitabine treatment, suggesting that NK cells play an important role in adjuvant gemcitabine therapy. Miyashita *et al* (24) found that gemcitabine chemotherapy can increase the expression of MHC class I chain-related protein A and B (MICA/B) in patients with pancreatic cancer, thereby promoting the killing effect of NK cells on pancreatic cancer cells. In addition, gemcitabine also inhibits the shedding of human UL-16 binding protein-2 by mediating the expression of polymer metalloproteinase 10 (ADAM10), thereby enhancing the killing effect of NK cells on pancreatic cancer cells (25). Therefore, gemcitabine may exert a positive effect on NK cell activation in pancreatic cancer. When combining chemotherapy and NK cell immunotherapy to treat pancreatic cancer, the interaction between them must be fully understood to ensure that the patient can obtain the best treatment effect.

4. Effect of targeted therapy

Although a clinical trial of erlotinib in advanced pancreatic cancer achieved positive results, the OS time of patients was only extended by 0.33 months, with a limited clinical application value (26). The POLO study (27) was the first randomized phase III study of pancreatic cancer that had undergone biomarker screening. Patients who achieved stable disease after at least 4 months of platinum therapy were randomized to receive olaparib. These patients demonstrated a longer median PFS time than those who underwent placebo maintenance therapy ($P < 0.004$), indicating that some patients with germline BRCA mutation benefited from olaparib maintenance treatment. However, there was no significant difference in OS time, and only 4-7% of patients had germline or somatic cells with BRCA1/2 mutation (28-31).

The alternative strategy of monoclonal antibodies to block EGFR is essential to improve the therapeutic effect of patients with locally advanced or metastatic pancreatic cancer. McMichael *et al* (32) found that cetuximab combined with IL-21 treatment had a significant effect on the activation of NK cells in patients with EGFR-positive pancreatic cancer, regardless of the KRAS mutation status, and that this may be a potential treatment strategy. A polysaccharide isolated from hard straight hypocotyl eggs (SEP) has immunological activity in the body. Xie *et al* (33) found that SEP enhances NK cell

toxicity mainly through the TLR4/MAPK/NF- κ B signaling pathway, effectively inhibiting pancreatic cancer, and that it is a potential target for pancreatic cancer immunotherapy.

5. Effect of cytokine therapy

Cytokines regulate immune function by regulating the dynamic balance of NK cells in the body. Different cytokines exert different effects on NK cells. Iannone *et al* (17) studied the effects of IL-2, -10, -12, -15, -18, -21, -22 and -23, as well as CCL-21, in patients with pancreatic cancer. IL-2 is the most commonly used cytokine, which can be used as an independent agent to increase the number of circulating NK cells in patients (17). IL-12 is a powerful activator of NK cells and can inhibit the growth of pancreatic cancer (34). In addition, IL-4 synergistically enhances both IL-2- and IL-12-induced IFN- γ expression in murine NK cells, which depend upon STAT6 (35). However, IL-4 could not promote the proliferation of the human NK cell line (NK-92) (36). IL-15 is also a strong activator of NK cells that can kill tumor cells *in vitro* and delay the infiltration and growth of NK cells *in vivo* (16). IL-15-activated NK cells kill pancreatic cancer cells and pancreatic stellate cells in a contact-dependent manner, and the improvement in killing of these cells depends, at least partly, on the upregulation of T-cell immunoglobulin and mucin domain-containing protein 3 and NKG2D induced by IL-15 (16). The immunostimulatory factors IL-15 and ALT-803 can enhance the activity of NK cells *in vitro* (37,38), and can also protect NK cells from the immunosuppressive effect mediated by TGF- β 1 (39,40). Van Audenaerde *et al* (41) studied the combined effect of CD40 agonist and IL-15 in pancreatic cancer, and found that this combination enhanced the infiltration of T cells and NK cells to the tumors, as well as significantly increasing the ratio of CD8⁺ T cells to regulatory T cells. IL-21 has potential antitumor effects and can enhance the antibody-dependent cellular cytotoxicity (ADCC) effect of NK cells (32). However, not all cytokines have antitumor effects. For example, IL-22 has been shown to not only protect pancreatic cancer cells from being killed by NK cells, to promote tumor angiogenesis and to inhibit tumor cell apoptosis factors, but also to produce immunosuppressive factors, including IL-10 and TGF- β (32,37). Dhar and Wu (42) found that CCL-21 decreased NK cell-mediated tumor cells in local and remote locations in mice. IL-8 was upregulated in multiple types of cancer, where it promoted the gain of mesenchymal features, stemness, therapy resistance and recruitment of cells for immune suppression to the tumor site (43). Secreted by tumor cells, IL-8 impaired the activity and function of NK cells via STAT3 signaling (44). The different effects of cytokine therapy on NK cells is presented in Table I.

6. Effect of immune checkpoint inhibitor treatment

Immune checkpoint inhibitors targeting T cells have emerged as a new promising treatment for tumors. However, ipilimumab (CTLA-4 inhibitor) or programmed cell death protein 1/programmed death ligand 1 (PD-L1) inhibitor monoclonal antibodies are not effective in advanced pancreatic cancer (45-47). The resistance of pancreatic cancer to immunotherapy includes multiple factors. For example,

pancreatic cancer usually has a low tumor mutation burden (TMB) [1 mutation/megabase (mut/Mb)]. By contrast, the TMB of melanoma or lung cancer is ~10 mut/Mb. A high TMB is usually associated with a high response rate to immunotherapy. One of the characteristics of deficient mismatch repair (dMMR) tumors is a very high TMB (usually >10 mut/Mb), although the incidence of dMMR in pancreatic cancer is <1% (48,49). Kaur *et al* (50) demonstrated that NK cells kill stem cell-like/poorly differentiated tumors and highly differentiated tumors through direct cytotoxicity and ADCC. Differentiated tumor cells were more susceptible to NK cell-mediated ADCC in the presence of anti-EGFR and anti-PDL1 monoclonal antibodies compared with their stem-like/poorly differentiated counterparts. These results indicated that CD16 receptors may mediate direct cytotoxicity and ADCC, meaning that these receptors could be competitively applied in either direct killing or ADCC, dependent on the maturation and activation stage of the NK cells and the tumor cell differentiation status.

7. Influence of metabolism

Abnormal metabolic conditions, such as high sugar levels, may create an environment of cellular stress that induces immune dysfunction. Hyperglycemia is a common symptom in patients with pancreatic cancer and is associated with a poor prognosis (51,52). Duan *et al* (53) found that high levels of glucose can promote the immune escape of pancreatic cancer cells in the hyperglycemic tumor microenvironment. Constitutive AMPK-Bmi1-GATA2 axis activation mediates the inhibition of MICA/B, which may act as a therapeutic target to further interfere with the immune escape of pancreatic cancer. The histone deacetylase inhibitor valproic acid (VPA) exerts an anti-tumor effect by upregulating the expression of NKG2D ligands on tumor cells. Shi *et al* (54) found that VPA upregulates the expression of MICA and MICB via the PI3K/Akt signaling pathway, enhancing the sensitivity of pancreatic cancer cells to NK cell-mediated cytotoxicity *in vitro* and *in vivo*. The synthetic dsRNA polyinosine-polycytidylic acid (pIC) can act as an immune agonist of TLR3 and RLR, thereby activating dendritic and NK cells, which are able to kill tumor cells. pIC is additionally able to trigger cell apoptosis in PDAC. Bhoopathi *et al* (55) found that pIC stimulated PDAC cell apoptosis without affecting normal pancreatic epithelial cells. In terms of mechanism, pIC with polyethylenimine inhibits the expression of X-linked inhibitor of apoptosis protein and survivin, and activates the immune response by inducing melanoma differentiation associated gene-5, retinoic acid-inducible gene I and NADPH oxidase activator. The hypoxic microenvironment plays an important role in the distant metastasis of pancreatic cancer. A previous study (56) showed that hypoxia could induce the upregulation of hypoxia inducible factor 1 subunit α , ADAM10 and soluble MICA expression, resulting in a decreased level of NKG2D expression in NK cells, and tumor cells evading immune surveillance and NK cell-mediated lysis. Ou *et al* (56) found that hypoxia induced MICA shedding, while HIF1A mediated the immune escape of pancreatic cancer cells from NK cells. Fiala (57) found that curcumin and omega-3 fatty acids enhanced the apoptosis of pancreatic cancer cells induced by NK cells, while curcumin inhibited the production of γ -interferon.

Table I. Effects of different cytokines on NK cells.

Cytokine	Effect	(Refs.)
IL-2	Promotes expansion of NK cells	(17)
IL-12	Promotes activation of NK cells	(34)
IL-15	Promotes activation of NK cells	(16,37-40)
IL-15 + CD40 agonist	Enhances the infiltration of NK cells and T cells to tumors	(41)
IL-21	Enhances the ADCC effect of NK cells	(32)
IL-22	Produces immunosuppressive factors, including IL-10 and TGF- β	(32,37)
CCL-21	Decreases the number of tumor cells mediated by NK cells in local and remote locations in mice	(42)
IL-4	Synergistically enhances both IL-2- and IL-12-induced IFN- γ expression in NK cells	(35,36)
IL-8	Impairs the function of NK cells via the STAT3 pathway	(43,44)

NK, natural killer; ADCC, antibody-dependent cellular cytotoxicity.

8. Clinical infusion of NK cells in pancreatic cancer

The clinical infusion of NK cells or its predecessors, lymphokine-activated killer cells, to treat metastatic tumors is a popular therapeutic direction (50,58,59). In a previous study, the NK-92 cell line was modified with a chimeric antigen receptor specific for PD-L1, ER-retained IL-2 and CD16 (60); in the study, preclinical data showed that these NK-92 cells could kill 15 tumor cell lines *in vitro*. These data were used in the phase I clinical trial that assessed the safety and preliminary efficacy of PD-L1 targeting high-affinity NK (t-haNK) in individuals diagnosed with locally advanced or metastatic solid cancer (ClinicalTrials.gov ID, NCT04050709). In a planned Phase II study that is currently actively recruiting, the combination of PD-L1 t-haNK with other agents is being assessed for safety and efficacy in those who have been diagnosed with locally advanced or metastatic pancreatic cancer (ClinicalTrials.gov ID, NCT04390399) (61). Long *et al* (62) presented the case of a patient with advanced pancreatic cancer treated with cell-based immunotherapy, using expanded activated allogeneic lymphocytes *in vitro*, with cytotoxic T lymphocytes positive for CD3 and CD8, and NKs negative for CD3 and CD56 as the major effector cells. This was combined with chemotherapy and targeted agents. The subsequent carbohydrate antigen 19-9 (CA19-9) levels were <37 U/ml (previously 136 U/ml) and marked regression of the lesions was noted.

9. Effect of other treatments

Lin *et al* (63) found that allogeneic NK cell therapy combined with percutaneous irreversible electroporation is effective in the treatment of metastatic pancreatic cancer, which significantly improves the prognosis of patients with pancreatic cancer. Pan *et al* (64) explored the efficacy of irreversible electroporation (IRE) ablation combined with NK cells in the treatment of locally advanced pancreatic cancer (LAPC), and found that IRE ablation combined with NK cells has a good therapeutic effect on LAPC, with results demonstrating a synergistic therapeutic effect, enhanced immune function and decreased CA19-9 expression.

10. Summary and prospects

NK cells are an important component of pancreatic cancer immunotherapy. The mechanism of inhibiting the growth of pancreatic cancer mainly includes upregulation of the expression of multiple receptors and ligands, and promotion of the release of NK cell-related cytokines. The secretion of cytokines, multifunctional antibody-mediated NK cell activation, regulation of the transmission of inhibitory and activating signals, immune drug stimulation and other ways to enhance the activity of NK cells are new strategies to enhance the tumor-killing effect of NK cells. In the future, more NK cell-related immunotherapies will be applied to the treatment of pancreatic cancer. However, many issues, such as the safety of clinical applications and potential complications, are still unclear, and further research is needed to explore them. Achieving satisfactory results using a single target to treat pancreatic cancer is difficult, and the use of multidisciplinary cooperation to screen specific targets for comprehensive treatment is a future research direction in the field of pancreatic cancer treatment. With a growing number of studies on the immunotherapy of pancreatic cancer, the status of NK cells in the immunotherapy of pancreatic cancer will be paid increasing attention.

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Availability of data and materials

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Authors' contributions

XP, LC, YJ and XZ conceived the present study. XP, YW and ZH performed the literature search and data analysis. The first draft of the manuscript was written by XP, LC, YJ and XZ. XZ, YW and ZH critically revised the work. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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