Enhancing the therapeutic efficacy of NK cells in the treatment of ovarian cancer (Review)

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Abstract. Ovarian cancer is a prevalent gynecological malignancy associated with a high mortality rate and a low 5-year survival rate. Typically, >70% of patients present with an advanced stage of the disease, resulting in a high number of ovarian cancer-associated deaths worldwide. Over the past decade, adoptive cellular immunotherapy has been investigated in clinical trials, and the results have led to the increased use in cancer treatment. Natural killer (NK) cells are cytotoxic lymphoid cells that recognize and lyse transformed cells, thereby impeding tumor growth. Thus, NK cells exhibit potential as a form of immunotherapy in the treatment of cancer. However, some patients with ovarian cancer treated with NK cells have experienced unsatisfactory outcomes. Therefore, further optimization of NK cells is required to increase the number of patients achieving long-term remission. In the present review article, studies focusing on improving NK cell function were systematically summarized, and innovative strategies that augment the anticancer properties of NK cells were proposed.

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Abbreviations: NK cells, natural killer cells; IL-2, interleukin 2; IL-15, interleukin 15; IFNγ, interferon-γ; TME, tumor microenvironment; PBMC, peripheral blood mononuclear cells; CARs, Chimeric antigen receptors; CAR-NK, CAR-engineered NK; MSLN, mesothelin; αFR, folate receptor alpha; HLA-G, human leukocyte antigen G; GPC3, glypican-3; iPSC, induced pluripotent stem cell; NKG2DL, NKG2D ligands; miRNAs, microRNAs; OV, oncolytic viruses; mAbs, monoclonal antibodies; ADCC, antibody-dependent cellular cytotoxicity; siRNA, small interfering RNA; EXO, exosomes

Key words: ovarian cancer, NK cells, adoptive cell therapy, gene editing, cytokines

Contents

1. Introduction

Ovarian cancer is the leading cause of mortality among females diagnosed with gynecological cancer, ranking as the fifth most common cause of death in females overall. The majority of cases are detected at advanced stages of disease, resulting in unfavorable disease outcomes. Among all gynecological cancers, ovarian cancer exhibits the highest mortality rate, with a 5-year survival rate of <50% (1,2). The latest statistical study indicated that the number of patients with cancer in China exceeds 57,000, and ~27,000 new cancer-associated deaths (3). By 2023, ovarian cancer is projected to be ranked as the fifth leading cause of cancer-related mortality among females in the United States, accounting for 5% (equivalent to a total of 13,270) of all female cancer fatalities (4). In addition, results of a statistical analysis demonstrated that ~40,000 ovarian cancer-related deaths occur in females worldwide each year (5). Therefore, the development of novel innovative therapeutic strategies is required for the treatment of ovarian cancer.

Over the last two decades, adoptive immune cell therapies have been used in the clinical treatment of cancer. NK cells are cytotoxic lymphocytes of the innate immune system that eliminate cancerous cells. The application of immunotherapy mediated by NK cells has emerged as a safe and effective therapeutic approach for cancers (6). Thus, research has focused on the use of NK cells in immunotherapy. However, based on observed clinical treatment outcomes, further refinement of NK therapy is required. The development of therapies targeting NK cells generally focuses on two main aspects: i) Optimizing the quality of therapeutic NK cells through culture prescription optimization and enhancing NK cell cytotoxicity, and ii) gene editing.

The present review article highlighted the diverse approaches to enhancing NK cell properties and augmenting the corresponding antitumor efficacy. Strategies for enhancing the functionality of NK cells include cytokine-based culture prescriptions, immune-checkpoint inhibitors and gene editing techniques. In conclusion, further investigations and clinical evaluations are required to optimize the use of NK cells in cancer immunotherapy.

2. Additives induce the antitumor activity of NK cells

Results of previous studies demonstrated that NK cells may inhibit numerous types of tumors. To further enhance the antitumor efficacy of therapeutic NK cells, the corresponding cytotoxicity and persistence must be optimized *in vivo*. The use of cytokine-based agents and other drugs may provide a more stringent method for augmenting the cytotoxicity and longevity of NK cells.

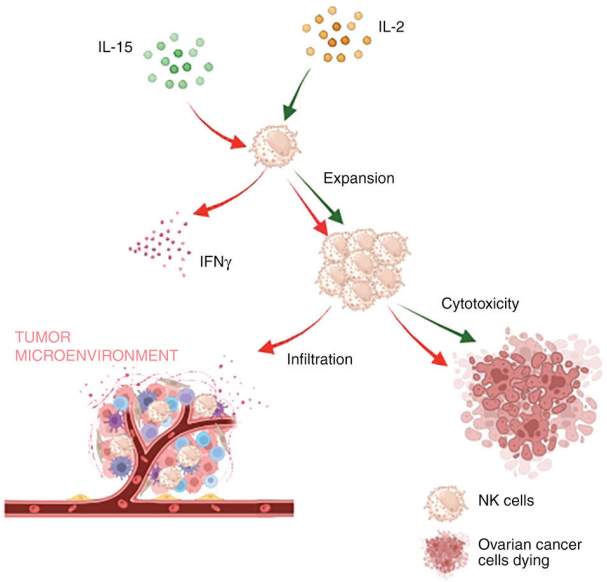

Figure 1. Regulation of the antitumor properties of NK cells via representative cytokines. This figure was created in Biorender.com. NK, natural killer. IL, interleukin; IFN, interferon.

Adding cytokines. Interleukin (IL)-2 has emerged as a pivotal catalyst in cancer immunotherapy, facilitating the expansion of purified NK cells (7) and activated NK cells (8). Results of a previous study revealed that interleukin-2 (IL-2) effectively augments the cytotoxicity of NK cells in the peripheral blood (9). Long-term culture with IL-2 results in a high number of functional NK cells through upregulation of NKp30 and DNAM-1 receptors on the cell (10). Furthermore, IL-2 treatment resulted in a significant expansion of NK cells and the complete regression of ovarian tumors in mice (11). Collectively, these studies highlighted the pivotal role of IL-2 in promoting the expansion of NK cells and enhancing their cytotoxic response against tumors.

Interleukin 15 (IL-15), a cytokine belonging to the common γ-chain family, exerts regulatory control over various aspects of NK cell-mediated immunity (12). Hoogstad-van *et al* (13) reported that the generation of NK cells in the presence of IL-15 may exert efficient cytotoxicity and interferon-γ (IFNγ) secretion towards ovarian cancer cells. In addition, NK cells actively migrate, infiltrate and execute tumor cell apoptosis within a three-dimensional multicellular ovarian cancer spheroid, thereby significantly inhibiting the progression of ovarian carcinoma *in vivo* (13). Moreover, IL-15 also enhances the survival of NK cells through sequestering the pro-apoptotic transcription factor FOXO3 in the cytoplasm (14). Incubation of NK cells with IL-12, IL-15 and IL-18 generates cytokine-induced memory-like NK cells. Treatment with IL-12, IL-15 and IL-18 promotes activation and proliferation of NK cells, while enhancing IFNγ production and the potent NK cell-induced inhibition of ovarian cancer (15).

Moreover, the functionality of NK cells against ovarian cancer cells was significantly enhanced following treatment with IL-15 super-agonist complexes, such as N-803 or ALT-803, which effectively promoted the proliferation of NK cells, and augmented the secretion of IFNγ, CXCL10, CD107a and TNFα (16-18). These findings demonstrated that IL-15 or IL-15 super-agonist complexes may enhance the functionality of NK cells against ovarian cancer (Fig. 1).

Adding molecule compounds. Results of previous studies demonstrated that certain small molecule compounds exhibit potential in enhancing the antitumor properties of NK cells. For example, cam1615B7H3 effectively promoted NK cell expansion, enhanced the antitumor activity against B7-H3^{+} carcinomas and inhibited the growth of aggressive ovarian cancer *in vivo* (19). The oncolytic therapy with HSV-1716 may enhance the antitumor immune response through promoting the recruitment of NK cells, and upregulating the expression of IFNγ, MIG and IP-10 within tumors (20). The expression of CD69 and NKG2D, as well as the secretion of IFNγ, perforin and granzyme B may be modulated by Vitamin C to enhance the properties of NK cells (21). Moreover, the streptococcal preparation, OK432, enhances the cytotoxic activity of NK cells against ovarian tumors (22). Treatment with the combination of CpG oligodeoxynucleotides and LL-37 enhanced the proliferation and activation of NK cells (23).

In addition to the aforementioned natural compounds, Choi *et al* (24) reported that NK cells chemically primed with 25 kDa branched polyethylenimine (25Kb PEI) exhibit increased expression of activating, adhesion and chemokine receptors. Chemically primed NK cells also promote perforin accumulation, and the subsequent migration and antitumor activity is enhanced (24). SN-38 or metformin activates NK cells to infiltrate the tumor microenvironment (TME), and secrete IFNγ and granzyme B, resulting in the elimination of cancer cells (25). Moreover, results of a previous study demonstrated that cimetidine enhanced the activity of NK cells in patients. The augmentation of NK cell function following cimetidine treatment was more pronounced in patients with a large residual tumor, compared with those without any remaining tumor (26).

In conclusion, molecular compounds play a pivotal role in augmenting the antitumor properties of NK cells, and exhibit potential as novel strategies for enhancing cancer immunotherapies (Fig. 2).

Adding natural extracts. Natural plant extracts exhibit potential in the treatment of cancer. Results of a previous study

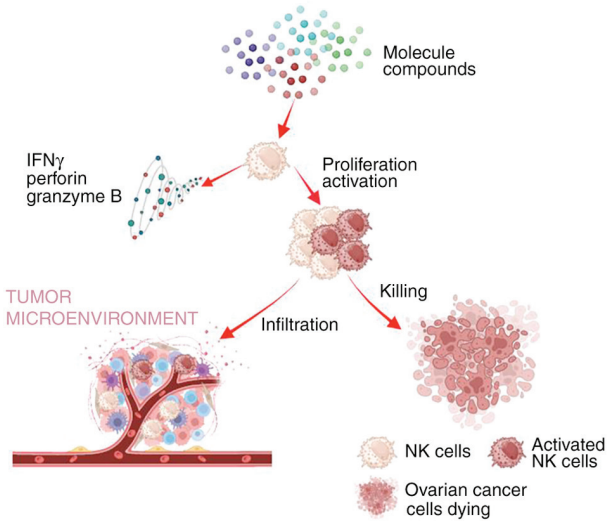

Figure 2. Molecule compounds improve the antitumor properties of NK cells. This figure was created in Biorender.com. NK, natural killer.

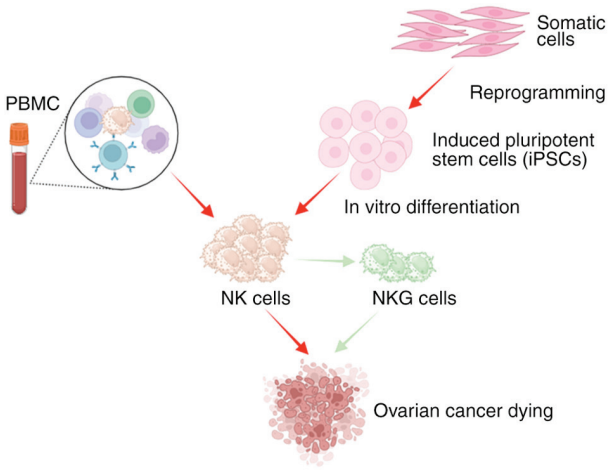

Figure 3. NK cells derived from diverse origins inhibit cancer cells. This figure was created in Biorender.com. NK, natural killer; PBMC, peripheral blood mononuclear cells.

demonstrated that Ashwagandha significantly augments the population of NK cells both in stromal and intra-tumoral compartments. In addition, Ashwagandha enhances the anti-tumor activity of NK cells in patients with ovarian cancer (27). Moreover, the leaf extracts of *L. indica* and its phytoconstituent methyl gallate exhibited an augmented cytotoxic effect on ovarian tumor cells through enhancing the activity of NK cells (28). These studies may provide a basis for further clinical investigations aimed at assessing the impact of natural extracts on the immune function of NK cells in patients with cancer.

Optimizing the cell source. The proportion of functional NK cells was significantly reduced in patients with cancer (29). Thus, optimizing the source of NK cells is a pivotal determinant for the success or failure of NK cell therapy.

In patients with ovarian cancer, the cytotoxicity of NK cells in tumor-infiltrating lymphocytes was significantly lower, compared with peripheral blood mononuclear cells (PBMCs) or ascitic fluid (30). NK cells derived from PBMCs that are expanded using a feeder cell-free expansion system are referred to as eNKs, and these migrate to the tumor site while retaining cytotoxicity. Moreover, eNKs demonstrate robust proliferation capabilities, ensuring sustained high cell counts in cutaneous xenograft mice models. eNKs effectively suppress tumor growth in diverse ovarian cancer xenograft mouse models and mitigate ascites formation in peritoneal tumor models of ovarian cancer (31). Nham *et al* (32) used an artificial APC-based *ex vivo* expansion technique to produce cytotoxic, expanded NK cells from OCPs ascites-NK cells derived from patients with ovarian cancer. These NK cells exhibited increased expression of NKG2D, NKp30 and NKp44, produced increased amounts of antitumor cytokines in the presence of OC cells, and mediated direct tumor cytotoxicity against OC cells (32).

Moreover, Hermanson *et al* (33) revealed that induced pluripotent stem cell (iPSC)-derived NK cells demonstrate comparable anti-ovarian cancer efficacy to PBMC-NK cells, highlighting their potential as a valuable resource for ovarian cancer immunotherapy. Generating large quantities of well-characterized iPSC-derived NK cells that can be stored in biobanks may be useful in the treatment of a large group of patients (33). NKG, a novel human NK cell line, exhibited robust expression of an array of adhesive molecules, activating receptors, and cytolysis-related receptors and molecules. Irradiated NKG cells demonstrated potent cytotoxicity against ovarian cancer cells *in vitro*. In addition, these cells effectively suppressed human ovarian cancer growth while exhibiting a suitable safety profile *in vivo*; however, they did not exhibit increased proliferation (34).

These findings demonstrated the potential of NK cells in the treatment of ovarian cancers when derived from various sources (Fig. 3).

3. Improving the anticancer efficacy of NK cells using genetic editing

Due to the optimal recognition of cancer cells, NK cells serve as potent effector cells for adoptive cellular therapy in patients with cancer. However, the clinical application of NK cells has been significantly limited by factors such as the TME. Genetically engineered NK cells effectively address these limitations and further augment the antitumor properties of NK cells. The adoptive transfer of genetically modified NK cells for the treatment of ovarian cancer is an emerging and rapidly advancing field that has demonstrated a high level of potential.

Chimeric antigen receptor (CAR)-NK cells. CARs markedly enhance the antitumor efficacy of immune effector cells. As CAR-engineered T (CAR-T) cells have demonstrated a high level of effectiveness in cancer treatment, research has focused on developing CAR-NK cells for solid tumor therapy, and diverse CAR constructs are being devised to augment NK cell-mediated cytotoxicity (Fig. 4).

CD44 is a widely expressed marker on ovarian cancer cells that is associated with properties of ovarian cancer stem cells and intraperitoneal tumor spread. CD44 demonstrated potent and specific cytotoxic activity against both CD44-positive ovarian cancer cell lines and primary ovarian

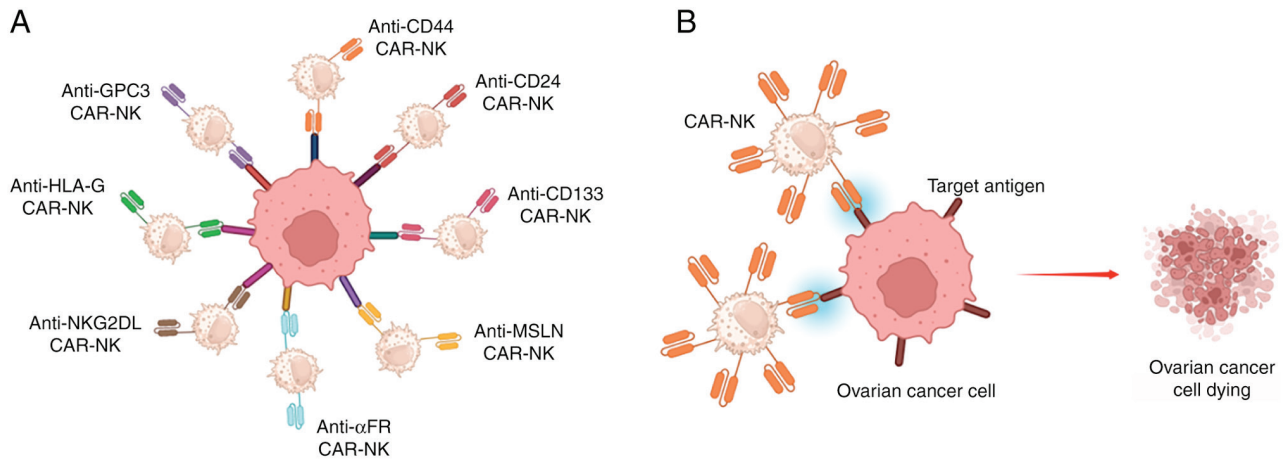

Figure 4. A schematic representation of CAR-NK cells. (A) Distinct targets of CAR-NK cells. (B) CAR-NK cells recognize antigens on the surface of ovarian cancer cells and effectively trigger the inhibition of cancer cells. This figure was created in Biorender.com. CAR, chimeric antigen receptor; NK, natural killer; HLA-G, human leukocyte antigen G; GPC3, glypican-3; NKG2DL, NKG2D ligand; MSLN, mesothelin; αFR, folate receptor alpha.

cancer cells, when targeted by anti-CD44-CAR-NK. Notably, the concurrent administration of anti-CD44-CAR-NK and cisplatin exhibited enhanced antitumor efficacy compared with sequential treatment (35). Moreover, a third-generation anti-CD133-CAR-NK exhibited specific cytotoxicity against CD133-positive ovarian cancer cell lines and primary ovarian cancer cells. The targeted elimination of ovarian cancer stem cells by anti-CD133-CAR-NK exhibits potential for future clinical trials (36). Furthermore, anti-CD24-CAR-NK demonstrated potent cytotoxicity against CD24-positive ovarian cancer cell lines, and high levels of efficacy against patient-derived primary ovarian cancer cells (37).

Mesothelin (MSLN) is overexpressed in ovarian cancer, and therefore exhibits potential as a target for immunotherapy. MSLN-CAR-NK cells exhibited specific cytotoxicity against MSLN-positive ovarian cancer cells, accompanied by enhanced cytokine secretion. Moreover, *in vivo* studies demonstrated the effective eradication of ovarian cancer cells by MSLN-CAR-NK cells, leading to significantly prolonged survival of tumor-bearing mice (38). Folate receptor alpha (αFR) is overexpressed in 90% of ovarian cancers. Anti-αFR-CAR-NK cells exhibit specific cytotoxicity against αFR-positive ovarian cancer cells. NK cells expressing αFR-28BBζ demonstrate enhanced antigen-specific cytotoxicity, proliferation, degranulation and cytokine secretion, and reduced antigen-induced apoptosis. Moreover, anti-αFR CAR-NK cells effectively eradicate ovarian cancer cells *in vivo*, and significantly prolong the survival of tumor-bearing mice (39). The immune checkpoint protein, human leukocyte antigen G (HLA-G), is expressed in the majority of tumor cells as a mechanism to evade immune surveillance. Results of a previous *in vitro* study demonstrated that anti-HLA-G-CAR-NK cells exhibit potent cytolytic activity against ovarian cancer cells and significantly suppress xenograft tumor growth, leading to prolonged survival (40).

iPSC is a high-quality source of engineered NK cells. Li *et al* (41) constructed anti-hMesothelin-CAR-iPSC-NK cells, which contain the transmembrane domain of NKG2D, the 2B4 co-stimulatory domain and the CD3ζ signaling domain to mediate strong antigen-specific NK cell signaling. These cells significantly prevented ovarian cancer growth and prolonged the survival of mice with low levels of toxicity (41). In addition, anti-glypican (GPC3)-3-CAR-iPSC-NK cells exhibited consistent effector functions against GPC3-expressing tumor cells, in terms of cytotoxicity and IFN-γ production. Notably, these cells significantly prolonged the survival of mice bearing GPC3-positive ovarian-tumors (42).

Ng *et al* (43) constructed anti-NKG2D ligand (NKG2DL)-CAR-NK cells that expressed chemokine receptor CXCR1. Enhanced CXCR1 expression in NK cells promoted tumor trafficking and exhibited significantly augmented anti-tumor responses in a murine model of ovarian cancer (43).

Collectively, these findings demonstrated that the genetic engineering of NK cells may lead to the increased targeting of diverse antigens, enhance proliferation, augment tumor infiltration and selectively eliminate malignant cells. These results provide a method for improving the therapeutic efficacy of NK cells against ovarian cancer, and provide a theoretical basis for future clinical investigations.

Requisite regulation of specific target genes. The antitumor properties of NK cells are regulated by multiple genes derived from both NK cells and tumor cells. Targeted regulation of these genes markedly enhances the antitumor properties of NK cells and sensitizes tumor cells to NK cell treatment.

CA125, a fragment of mucin 16 shed from ovarian cancer tumors, exerts significant protective effects on ovarian tumors through evading recognition by NK cells, inhibiting the activation and cytotoxicity of NK cells, and further impairing the corresponding antitumor activity. Therefore, targeted knockdown of mucin 16 may restore the antitumor function of NK cells (44,45). In addition, the downregulation of IDO and PP4 significantly enhanced the infiltrative capacity and cytotoxicity of NK cells, thereby augmenting the anti-ovarian cancer properties (46,47). Inhibition of CD9, TIGIT, GSK3, PI3K/AKT signaling and HLA-G expression restored the cytotoxicity of NK cells against ovarian cancer (48-52). Notably, increasing the expression of NKG2DL, ULBP1, inhibited DOT1L-mediated ovarian cancer eradication, and increased the antitumor functionality of NK cells (53). Knockdown of SESN2 and SESN3

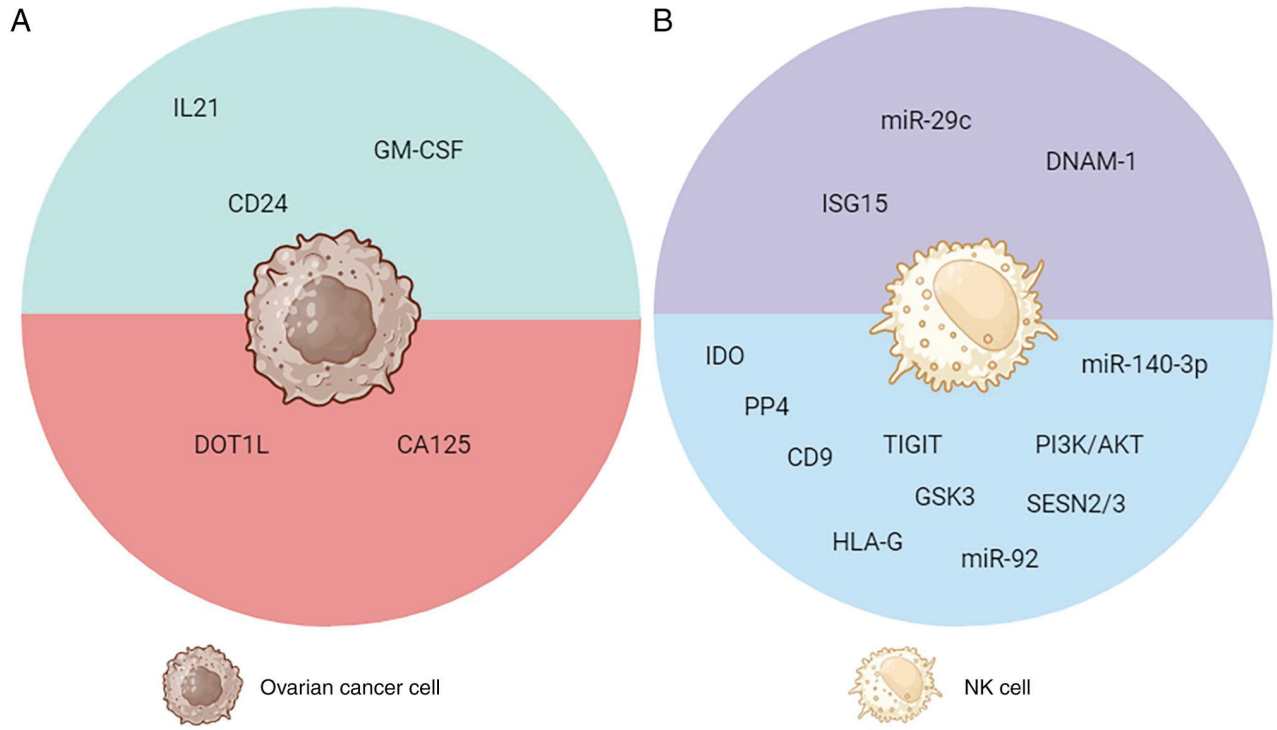

Figure 5. Targeted gene regulation enhances the antitumor activity of NK cells. (A) Targeted genes derived from ovarian cancer cells. (B) Targeted genes derived from NK cells. Upregulated genes in the upper half of the circle enhance the antitumor activity of NK cells. Downregulated genes in the lower half of the circle improve the antitumor property of NK cells. This figure was created in Biorender.com. NK, natural killer; miR, microRNA; HLA-G, human leukocyte antigen G; IL, interleukin; ISG15, interferon-stimulated gene 15.

restored the tumoricidal effects of NK cells both *in vitro* and *in vivo* (54). These genes may exhibit potential as novel therapeutic targets in NK cell immunotherapy.

MicroRNAs (miRNAs) exhibit potential as therapeutic tools for restoring cellular functions. As potent genetic regulators, miRNAs effectively modulate cellular pathways through direct interactions with target genes (55). Deng *et al* (56) reported that miR-29c enhanced the antitumor efficacy of NK cells through directly targeting B7-H3, and mitigating NK-cell exhaustion. Conversely, treatment with miR-92 and miR-140-3p resulted in enhanced tumor growth through suppression of NK cell cytotoxicity towards ovarian cancer cells (57,58). These findings elucidated the regulatory role of miRNAs in modulating NK cell activity, and highlighted potential strategies to reactivate NK cells for ovarian cancer immunotherapy.

Dou *et al* (59) genetically engineered ovarian cancer cells to secrete IL-21 and GM-CSF, which enhanced NK cell cytotoxicity and elicited antitumor immunity. Moreover, interferon-stimulated gene 15 suppressed ovarian cancer progression through activation of NK cells (60). DNAM-1 signaling is essential for NK cells to recognize and target tumor cells (61). Notably, $CD24^+$ ovarian cancer cell lines are more susceptible to NK cell lysis (62). The aforementioned findings suggested that modulation of specific genes effectively impedes the progression of ovarian cancer through the activation of NK cells, thereby highlighting the potential therapeutic value of targeting these genes for the treatment of ovarian cancer (Fig. 5).

4. Improving the anticancer efficacy of NK cells using combination therapy

Combination therapy has the potential to overcome deficiencies in NK cells, increase the corresponding tumor properties, reverse immunosuppression, and enhance cancer cell susceptibility to NK cell-mediated cytotoxicity.

Oncolytic virus (OV). OVs elicit robust innate and adaptive immune responses, including contact-dependent activation of NK cells and augmentation of their cytotoxicity against adenovirus-infected ovarian cancer cells (63). *Parapoxvirus ovis* (Orf virus, OrfV) demonstrated significant efficacy as a monotherapy in an advanced-stage murine model of epithelial ovarian cancer. The therapeutic intervention of OrfV relied on the activation of NK cells, highlighting its potential as an immunotherapeutic agent for the treatment of advanced-stage ovarian cancer (64). These findings provided a theoretical basis for the potential clinical application of OVs in patients diagnosed with ovarian cancer (Fig. 6A).

Monoclonal antibodies (mAbs). The cytotoxicity of NK cells may be enhanced following the addition of therapeutic mAbs that mediate antibody-dependent cellular cytotoxicity (ADCC). Zhu *et al* (65) revealed that when combined with anti-HER2 mAb, hnCD16-iNK cells demonstrate enhanced survival outcomes in an ovarian cancer xenograft model. Ovarian cancer cells pretreated with anti-EGFR TKIs demonstrated increased sensitivity towards NK cell-mediated ADCC (66). In addition, the potency of ADCC was further

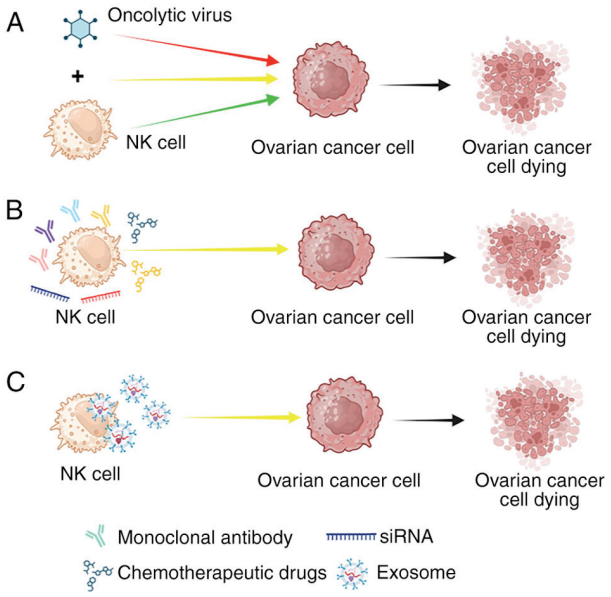

Figure 6. Improving the anticancer efficacy of NK cells using combination therapy. (A) Oncolytic viruses combined with NK cells inhibit ovarian cancer cells. (B) Monoclonal antibodies, siRNA and chemotherapeutic drugs enhance the antitumor activity of NK cells. (C) Exosomes derived from NK cells inhibit ovarian cancer cells. This figure was created in Biorender.com. NK, natural killer; siRNA, small interfering RNA.

enhanced following the pre-stimulation of NK cells with monocytes and the immunostimulatory mycobacterial protein, PstS-1 (67). The combined treatment with anti-PD-L1 significantly enhanced the antitumor efficacy of NK cells (68). These findings suggested that targeted antibody therapy may confer benefits against ovarian cells by augmenting the functional capacity of supplementary cytolytic NK cells (Fig. 6B).

Small interfering (si)RNA. siRNA is derived from the mechanism of post-transcriptional gene silencing, and it exhibits high specificity and efficacy in suppressing disease-associated genes. HER-2 siRNA-treated tumor cells were efficiently lysed by NK cells *in vitro*, leading to a significant inhibition of xenografted tumor growth. A combination of HER-2 siRNA with NK cell therapy may exhibit potential in the biological treatment of ovarian cancer with high HER-2 expression (Fig. 6B) (69).

Chemotherapeutic drugs. The combination of NK cell adoptive transfer and tumor-sensitizing chemotherapy may exhibit potential in the treatment of ovarian cancer. In combination, NK cells and gemcitabine exhibited an additive effect in suppressing tumor growth in mice with ovarian cancer. Enhanced cytotoxicity against ovarian cancer is achieved through a synergistic combination of NK cells and gemcitabine, both *in vitro* and *in vivo* (70). Oxaliplatin, an immunogenic cell death inducer, increased the cytolysis of ovarian cancer cells mediated by NK cells (71). A combination of cisplatin and NK cell-mediated immunotherapy may overcome the immunoresistance of chemoresistant ovarian cancers (72). Results of a recent study demonstrated that SN-38 and 5-FU acted synergistically to inhibit ovarian cancer cells, and promote the sensitivity of cancer stem cells to NK cells (73). Collectively, these findings presented the potent efficacy of chemotherapeutic agents in conjunction with NK cells against therapy-resistant ovarian cancer cells, thereby establishing the viability of novel combination therapeutic strategies in the treatment of ovarian cancer (Fig. 6B).

5. Exosomes (EXOs) derived from NK cells

EXOs are membranous vesicles derived from cells that play a crucial role in intercellular material transportation. Moreover, they exhibit potential as drug carriers for targeted delivery to specific cell types or tissues. In addition, immune cell-derived EXOs possess immunomodulatory properties (74). NK-EXOs exhibit potent antitumor activity against ovarian cancer. Moreover, NK-EXOs serve as effective carriers for cisplatin delivery, thereby enhancing the cytotoxic effects in drug-resistant ovarian cancer cells, and reversing the immunosuppressive state of NK cells (75). Collectively, these results highlighted the potential of NK-EXOs in the clinical treatment of ovarian cancer, while also establishing a basis for further investigations into the effects of NK-EXOs in other solid tumors (Fig. 6C).

6. Conclusions

Ovarian cancer is a prevalent malignancy affecting females, and a lack of effective treatment options contributes to a 5-year survival rate of ~45% (76). NK cells possess distinct cytotoxic properties against cancer cells, highlighting their potential in the treatment of ovarian cancer. However, the limited clinical efficacy of NK cells poses a challenge to their application in treating ovarian cancer. In the present review, innovative strategies that aim to enhance the cytotoxicity of NK cells against ovarian cancer cells were summarized, including the utilization of additives, gene editing techniques and combination therapies.

At present, novel strategies are employed to enhance the antitumor properties of NK cells. However, limitations remain, and it is crucial to develop specific approaches for expanding functional NK cells, achieve accurate delivery of siRNA into targeted cells, and mitigate the side effects associated with combined therapy. Notably, NK cell therapy exhibits potential in the treatment of ovarian cancer. Thus, further investigations into the widespread application of NK cell adoptive transfer are required for the treatment of various solid tumors.

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Authors' contributions

YH and XN conducted project administration and acquired funding. YH, XZ and XN prepared the original draft of the

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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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