Research Progress of Cellular Immunotherapy in Breast Cancer

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Abstract: In the global female population, breast cancer is common cancer with high morbidity and high mortality, and the number of patients is increasing year by year. The current treatment is still based on resection, chemotherapy and other programs. However, these methods can have considerable side effects or altogether remove breast cancer lesions and lead to recurrence and further development. Immunotherapy refers to a treatment that artificially enhances or suppresses the patient's immune function when the immune function of the patient is in a low or hyperactive state to activate the patient's immune system and rely on the autoimmune mechanism to attack cancer cells. More and more trials have proved that immunotherapy has great potential in treating breast cancer in recent years. Adjuvant treatments can improve the survival rate of patients and are better than conventional programs such as chemotherapy. However, due to technical barriers, the development of breast cancer immunotherapy is still immature. This article will describe four kinds of standard cellular immunotherapy: TILs therapy, TAR-T therapy, CAR-T therapy and NK cell therapy and their latest research progress in breast cancer treatment.

1. Introduction

Breast cancer is one of the most common malignant tomours in women all over the world. According to the latest cancer burden data released by the World Health Organization in 2020: 2.26 million new breast cancers accounted for 11.7%, surpassing other cancers to become the global incidence for the first time. The highest cancer rate, its incidence (24.5%) and mortality (15.5%) are both the first among new cancers and cancer deaths in women. Due to the biological characteristics of breast cancer, such as complex subtypes and high heterogeneity, not all patients can benefit from traditional treatment methods such as radiotherapy and chemotherapy, especially the most aggressive triple-negative breast cancer (Triple-negative breast cancer, TNBC) and human epidermal growth factor receptor 2 positive breast cancer (human epidermal growth factor receptor 2, HER2). Traditional medical programs have enormous side effects and drug resistance, which affect the anti-tomour response. Therefore, the emergence of cellular immunotherapy provides researchers with a new direction for breast cancer treatment.

Immunotherapy mainly uses the immune effect of the autologous or allogeneic immune cells in the patient to improve the patient's symptoms, prolong the patient's survival period, and improve the prognosis. In recent years, breast cancer immunotherapy has become a new treatment method, and its effectiveness and safety have gradually been confirmed. However, the efficacy of cellular immunotherapy in solid tomours such as breast cancer is not as good as that of blood cancer. One of the main reasons is an immunosuppressive tumour microenvironment (TME) around the solid tumour. TME is highly dynamic. The environment includes cellular and non-cellular components, such as tomour cells, stroboscopic cells, blood vessels, immune cells (macrophages, lymphocytes, NK cells), extracellular matrix (ECM), Tomour metabolites and cytokines [1]. That is a close correlation between

cellular and non-cellular components. Various components in TME have essential significance in the treatment and prognosis of breast cancer.

The TME components of different subtypes of breast cancer are quite different. A subset of immune cells has anti-tomour or pro-tomour properties related to tomour aggressiveness and poor prognosis [1]. The components of TME include transformed ECM, soluble factors, immunosuppressive cells, epigenetic modification and reprogrammed fibroblasts. That can collectively hinder the anti-tomour response and lead to the progression and metastasis of TNBCs [2].

This article focuses on the four traditional immunotherapy TILs therapies, TCR-T therapy, CAR-T therapy, and NK cell therapy, summarising and explaining their research progress in breast cancer treatment.

2. TILs immunotherapy

2.1 The mechanism of TILs immunotherapy

Tumour infiltrating lymphocytes (TILs) are special lymphocytes existing in the tumour microenvironment. In the process of tumour treatment, TILs located around tumour tissue can penetrate the tumour to kill tumours and reduce tumour metastasis. According to the location, TILs can be divided into intraepithelial tumour infiltrating lymphocytes (iTILs) in direct contact with cancer cells and stromal tumour infiltrating lymphocytes (sTILs) located in the tumour stroma without direct contact with invasive cancer cells [3]. Based on the biological characteristics of TILs, researchers try to use TILs to treat breast cancer patients.

TILs therapy refers to separating TILs from the resected tomour tissues, enriching and culturing them in vitro, and reinfusing them into the patient's body, as shown in Figure 1. Breast cancer patients can remove the tomour tissue from the body or isolate the required TILs from the metastatic lymphocytes; since the effector cells that exert the immune function are derived from the body, they can kill the target cells specifically and reduce the tomour lesions, Maximize the breast preservation rate. At present, the collagenase-free mechanical method is used to isolate TILs with solid targeting and amplification capabilities to increase the recognition of breast cancer tomours. In the early stage of TIL, it is divided into isolation, amplification and reinfusion. With the in-depth research, a targeted screening step was added before expansion only to expand immune cells that can recognize tomour cells, which improves the effectiveness of TIL; in addition, breast cancer patients generally need to undergo chemotherapy before receiving cell reinfusion to improve the patients' Cure rate and long-term survival rate. However, whether the therapy can smoothly separate lymphocytes in breast cancer tissues; whether the separated lymphocytes have sufficient activity is still a blind spot. In TME, when the body detects and kills tomour tissue through its immune system, the surface of tomour tissue can also express and produce inhibitory molecules and secrete cytokines to form an inhibitory environment, thereby causing immune escape. Therefore, after reinfusion, whether the function and activity of lymphocytes can relieve the inhibition of the tomour microenvironment and successfully reach the tomour tissue to play an immune role is a technical barrier for TILs therapy in the treatment of breast cancer.

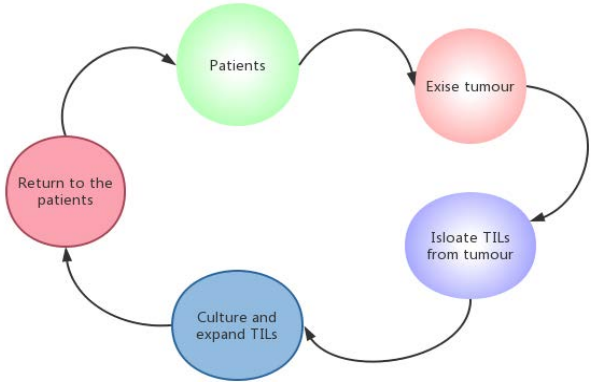

Figure 1. TILs immunotherapy treatment process

2.2 TILs and the prognosis of breast cancer

For breast cancer patients to choose the best option among a wide range of treatments, biomarkers are needed to assess each patient's condition. Several experiments have found that the number of TILs in breast cancer is relatively large, easy to observe and stable. Lymphocytes can infiltrate up to 75% of tumours, and high-density TILs can be observed in 20% of tumours. In addition, TILs can quickly respond to the state of tumour cells in the microenvironment. Hence, TILs are a prognostic indicator with research value, particularly in TNBC and HER2-positive breast cancer.

Some experiments illustrated that high TILs was associated with a good prognosis. In the collected samples of TNBC patients, when the TIL content in the tumour stroma of lymph node-negative patients is ≧30%, the three-year aggressive disease-free survival rate of the patients is 92%, the survival without distal disease is 97%, and the overall survival rate is 99% [4]. In 2020, researchers such as Gao divided the infiltration degree of TILs in HER2-positive breast cancer into high-TILs and low-TILs.The trial showed that the 10-year overall survival rate of HER2-positive breast cancer patients in the high-TILs group was 78% higher than that in the low-TILs group (57%) [5].

Due to TNBC and HER2-positive breast cancer have high invasiveness and risk, and neoadjuvant chemotherapy is increasingly used in breast cancer treatment to reduce tumour stage or make the best choice from drugs. Postoperative pathologic complete response (pCR) represents a better long-term prognosis and is the basis for formulating chemotherapy regimens. In 2010, Denket et al. found that high TILs in breast cancer before treatment were associated with the clinical response of anthracycline-based neoadjuvant therapy; the total pCR rate of patients was 12.8%, and the pCR rate of breast cancer patients with intratomoural content greater than 10% was 31%. In LPBC (lymphocyte-predominant breast cancer), lymphocyte-predominant breast cancer with TILs or intratomoural TILs greater than 60%, the pCR rate is 41.7%, and the pCR rate increases with the increase of TILs [6]. Some pieces of evidence have shown that TILS is related to the response of HER2-positive breast cancer after targeted drugs combined with neoadjuvant therapy. HER2-positive breast cancer patients receive trastuzumab targeted therapy. For every 10% increase in interstitial TILs, the patient will have a better effect on trastuzumab combined with neoadjuvant therapy. In addition, there is evidence that, to a certain extent, the increase of interstitial TILs can increase the sensitivity of highly aggressive breast cancer to drug therapy. In the GeparSixto experiment, after carboplatin was added to the combination of anthracycline and paclitaxel, the PCR rate of non-LPBC patients increased by 1.01 times, while that of LPBC patients increased by 3.71 times. Results have proved that in the use of targeted drugs combined with neoadjuvant chemotherapy, the TILs of HER2-positive breast cancer patients are related to the pCR rate [6]. According to the currently existing research, it can be proved that the effect of HER2-negative breast cancer after neoadjuvant chemotherapy is also related to TILs. The West trial showed that among ER-breast cancer patients treated with anthracycline neoadjuvant therapy, 74% of patients with high TILs achieved pCR, while only 31% of patients with low TILs achieved pCR. However, there are few related studies on ER-negative breast cancer. Whether TILs can be used as a prognostic factor for HER2-negative breast cancer needs further verification.

3. TCR-T therapy

3.1 The mechanism of TCR-T therapy

With the development of genetic engineering, researchers have developed more targeted immunotherapy T cell receptor-gene engineered T cell (TCR-T) therapy based on TILs. TCR is a heterodimer located on the surface of T cells that can specifically recognize antigens and participate in immune responses [7]. The antigen specificity of T lymphocytes stems from the particular structure of the TCR polypeptide chain, two TCR chains consisting of α, β, γ, and δ, αβTCR and YδTCR. TCR-T therapy screens out TCR α and β chains that can recognize tumour-specific antigenicity from patients, transfect them into T cells, enrich and culture them in vitro and return them to patients. The modified T lymphocytes can recognize the complexity of human leukocyte antigen (HLA) and peptide on the tumour surface, and phosphorylate the immune tyrosine-based activation motif (immune

tyrosine-based activation motif, ITAM) transmits antigen stimulation signals to trigger T cell immune effects, thereby killing tumour cells (Figure 2) [8].

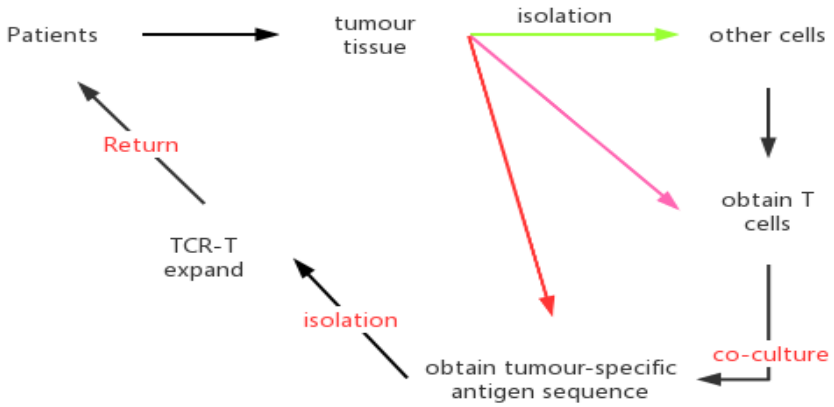

Figure 2. The process of TCR-T

3.2 Application of TCR-T therapy in breast cancer

Preclinical trials have proved the feasibility of TCR-T therapy in the treatment of breast cancer. Based on a nude mouse subcutaneous human breast cancer xenograft model, the researchers used infected CD8+T (NC-T) as the control cell to determine the placenta-specific gene (PLACI) specific TCR-T and NC-T against breast cancer tumours. Effectively, TCR-T has a strong killing effect on breast cancer cells [9]. In the completed TCR-T clinical trial, the researchers used HLA-DPB1*0401 restricted TCR adoptive CD4+ T cell therapy and evaluated its safety and effectiveness. They found that breast cancer patients increased T cells were no deaths. Lu believes that the trial proves the feasibility of the therapy for the treatment of breast cancer [10]. At the same time, Lu et al. proposed that this therapy combined with other related operations may improve the efficacy. For example, in TCR-T therapy, modification and manufacture of T cells that are less differentiated or Th17 polarized during cell production can be combined with immune checkpoint blocking to prevent T cell failure [10]. However, some experiments show that the safety and effectiveness of TCR-T therapy in breast cancer treatment still need to be further confirmed. Melanoma antigen gene A3 (MAGE-A3) is a common therapeutic target in TCR-T therapy. In NCT02153905, retroviruses were used to deliver anti-MAGE-A3 cells to 3 breast cancer patients expressing MAGE-A3 molecules. Once the cells grew, two cases appeared severe adverse reactions after the patients received chemotherapy, anti-MAGE-A3 cells and aldosterone treatment [7]. Besides, whether the therapy can reduce the tumour size and whether the TCR-T therapy effectively treats breast cancer should be more proven.

4. CAR-T therapy in breast cancer

4.1 The principle and the basic process of CAR-T therapy

CAR-T therapy, the chimeric antigen receptor T cell technology, is a new cell immunotherapy for tomour, which activate T cells by genetic engineering technology, combined with tomour chimeric antigen receptor, the T cells are engineered to CAR-T cells which could express the tomour surface associated antigen and directly targeted to identify tomour cells, not through the pathway of traditional main histocompatibility complex immune activation, hence the MHC limitation avoided, so as to achieve the purpose of killing tomour cells efficiently.

When CAR-T therapy is specifically implemented, we should filter the peripheral blood from patients or healthy humans, then separate T cells and other leukocytes, mix the collected T cells with the virus who has lost its pathogenic power. Let T cells grow out the genetic material of an artificial receptor which is chimeric antigen receptor (CAR), then the CAR can track cancer cells and attack them. Millions of modified cells were cultured in the laboratory and then injected to the patient.

So far, CAR-T therapy mainly used for the cure of lymphatic and hematopoietic system malignancies and is effective for a variety of hematological malignancies [11]. Breast cancer is a kind of solid tomour with low immunogenicity. At present, CAR-T therapy has a good effect on treating solid tomours, but it is not as effective as in non-solid tomours such as leukemia. The main reason is the limitation or even lack of clear targets provided by solid tomours to CAR-T cells, furthermore immunosuppressive tomour microenvironment (TME) around solid tomours is hypoxia and low nutrition, which makes it difficult for CAR-T cells to survive and expand [12].

4.2 Four generations structure of the CAR protein and the improvement of functions between each two generations

The main construction of CAR involves single chain antibody variable fragments (scFvs) which is used to identify antigen, hinge or spacer, transmembrane region, the T cell receptor β chain cd3ζ (the first signaling of T cell activation) and synergistic domain for stimulating signal (the second signaling of T cell activation), via constructing different scFv binding-specific antigens. CAR-T can be used in multiple tomour diseases [13]. Four generations of CAR-T cells have been developed successfully (table 1).

Table.1. Four generations structure of the CAR protein and the improvement of functions between each two generations

CAR-T cells	Changes in structure	Improvement of functions
1st	Only included CD3ζ[14]	Unable to antagonize tomour effectively[14]
2nd	Added one costimulatory domain from CD28 or 4-1BB in the intracellular region of CAR to the first generation[14]	Increase the intensity of signal transduction, the production of cytokine, improve the proliferation and life time of CAR-T cells[15]
3rd	Added two costimulatory domains from CD28 or 4-1BB in the intracellular region of CAR to the first generation[14]	
4th	Added IL-12 to the bases of the second generation which contributed to T cells redirected for universal cytokine-mediated killing, TRUCKs[14]	High levels of IL-12 could activate natural immune cells to antagonize tomour cells and activate Treg and MDSCs[15]

4.3 Some latest progresses of CAR-T therapy in breast cancer

4.3.1 Combined soluble tomour virus OV19t, CD19 - CAR – T

On account of that the low immunogenicity of breast cancer, lack of clear target spot and the presence of immunosuppressive tomour microenvironment, Park's team designed the oncolytic virus OV19t to reverse the immunosuppressive tomour microenvironment. OV19t is able to enter tomour cells and replicate autonomously, enabling tomour cells to express a shortened version of CD19 on the surface, which is CD19t, providing a target for CD19-CAR-T to kill tomour cells.

In vitro, OV19t can efficiently deliver CD19t to a variety of solid tomour cells, and express a new CD19t protein on the surface of tomour cell before oncolytic virus-mediated tomour lysis, allowing CD19-CAR-T cells to extensively kill tomour cells. In the mice tomour model, combining OV19t with CAR-T cells built a synergistic effect, the killed tomour cells cleave and release oncolytic virus copies, prompting adjacent tomour cells to express the CD19, the cascade reaction makes CAR-T cells more potent, resulting immune memory to prevent tomour recurrence. According to Park's examination, about 60% of mice tomours are relieved, whereas only 22% of mice which receiving OV19t treatment alone resolved tomours completely. After reimplanted tomour cells into recovered mice no new tomour formation was detected, indicating that oncolytic viral OV19t and CD19-CAR-T combination therapy could establish tomour-specific immune memory and thus prevent tomour recurrence [16].

4.3.2 EGFR CAR-T therapy

The epidermal growth factor EGFR is a receptor tyrosine kinase that was highly expressed in triple-negative breast cancer (TNBC)[17], thus it can be served as a specific target for CAR-T therapy.

EGFR-CAR-T cells can activate specific signaling pathways to promote the killing effect on tomour cells, and show higher killing activity against primary TNBC cells which express high EGFR than those express low EGFR. Results of animal tests show that the best curative effect cannot be achieved with either over low or high injection of EGFR-CAR-T cells. Three generations of EGFR-CAR-T cells have been developed successfully so far, The third-generation EGFR - CAR - T cells, the antigen recognition part of the CAR is designed according to EGFR antibody heavy chain variable region and light chain sequences which connected with one joint, producing recombinant antibody against single variable fragment scFv of EGFR, and IL-2 allows the antibody to acquire antigen specificity, the spacer in the traditional CAR could increase the flexibility of identifying antigens, the IgG1 hinge area was used in designing the third generation of EGFR-CAR, and the structure of CAR includes CD28 transmembrane domain and CD28, 4-1BBand CD3+ intracellular signal domain [18]. The third generation of EGFR-CAR-T cells could enhance cytokine secretion and enhanced cytolytic activity, specifically inhibit the growth of TNBC cells in vitro and in vivo model[17], including tomour xenografts derived from TNBC patients. TNBC has a tendency to metastasize to the lung, while EGFR-CAR-T cells could inhibit tomour metastasis [18].

4.3.3 AXL-CAR-T therapy

It was found that expression of AXL is significantly higher in TNBC tomour tissue than normal breast tissue, therefore AXL can be served as a specific target for CAR-T cells. In addition, AXL was found to be expressed on MDSC, which indicates that AXL-CAR-T cells may deplete TME MDSC, overcome immunosuppressive TME by inhibiting inhibitory cytokines from TAM to fundamentally change TME to a proinflammatory state, and polarize TME to a declarative state which benefits effective antitomour immune responses. Results of several trials indicate that AXL-CAR-T therapy has antitomour activity against AXL-positive TNBC cells [17].

Earlier studies showed that IL-7 can prolong the survival of tomour-specific T cells and IL-7 receptor can enhance the anti-tomour effect. To further improve the efficacy of AXL-CAR-T cells, the researchers constructed the CAR sequence targeting AXL and specifically activated IL-7 receptor C7R by replacing the extracellular domain and coexpressing C7R, extended the survival of AXL-CAR-T cells, and effectively enhanced its antitomour activity and cytotoxicity [19].

4.3.4 C-Met CAR-T therapy

Hepatocyte growth factor receptor (c-Met) is showed in all three subtypes in breast cancer, which are ER, PR and Her2[20], and mediates the proliferation, angiogenesis, migration, and metastasis of solid tomours by activating the signaling cascade[17].

c-Met CAR-T cells, that is, replaced the scFv fraction of CD19-CAR-T cell with onartuzumab, a novel antibody against c-Met. In vitro assays, compared with mRNA-CAR-T-CD19 cells, mRNA c-Met-CAR-T cells (mRNA transfected c-Met-CAR-T cells) showed significantly higher cell lytic activity in both breast cancer cell lines, BT20 (TNBC) and TB129 (Her2). Implanted a human ovarian cancer cell line labeled c-met with green luciferase into NOD/scid/γc (NSG) mice with mRNA-CAR-T-CD19 and mRNA c-Met-CAR-T cells injected respectively, the result shows that multiple injections of mRNA c-Met-CAR-T cells could control the tomour growth and allogeneic effects effectively compared to intertomoural injection of mRNA CAR-T-CD19 [20]. A phase I clinical trial from the University of Pennsylvania tested the safety of injecting c-Met CAR-T cells into TNBC patients, the result shows that c-Met-CAR-T cells were well tolerated by patients and caused inflammatory responses within TNBC tomours [20].

4.4 The main negative effects of CAR-T therapy in breast cancer

4.4.1 Cytokine release syndrome, CRS

Cytokine release syndrome (CRS), a cytokine storm, immune activation after cell infusion induces substantial release of inflammatory cytokines including TNF-α, IFN-γ, IL-6 and IL-10[21], the main symptoms includes high fever, fatigue, myalgia, hypotension, tachycardia, cardiac insufficiency, renal damage, liver failure and disseminated intravascular coagulation. The severity of CRS was correlative with the tomour load when the patient received CAR-T therapy, the greater the tomour load was, the

more intense the CRS would be. It can be alleviated by adding controllable suicide genes to the CAR during the designing process [22], such as iCasp9, HSV-TK, CD20 and EGFRt safety switches, etc [22]. Probably side effects associated with abnormal cytokine levels also includes neurological toxicity [21]. Since the α isotope of folate acid receptor (FR) is expressed in tomours such as breast cancer but extremely low in normal tissue, the tartaric acid-FITC double-specific T cell Participator can modulate CAR liveness, target folate acid receptor-positive tomour tissue and reduce CAR-T cytotoxicity [22].

4.4.2 On-target/off-tomour

On-target/off-tomour effect is currently the major obstacle to the application of CAR-T in solid tomours[23] which means the target lesions were identified but the tomour was missed, The reason is that the target tomour antigen of CAR-T is also expressed in normal human cells, To avoid this kind of effects, double specificity CAR-T cells were designed, which is AND-gate t cells (dual-receptor and-gate t cells), AND-gate t cells can be activated only when they recognize both epitopes while normal cells express only one of these antigens, in this way, CAR-T cells cannot be fully activated to avoid killing normal tissues. Procedural death receptor in another genetically modified inhibitory CAR-T cells or cytotoxic T cell-associated inhibitory signaling could attenuate T cell activation signals from CAR, resulting in genetically modified CAR-T cells could not be activated, thereby avoiding attacking normal cells and on-target/off-tomour effect. Kosti et al. have demonstrated that the on-target/off-tomour effect can also be prevented by designing a CAR which controlled by oxygen levels in the tomour environment and using a stringent dioxygen sensing system [24].

5. CAR-NK therapy in breast cancer

5.1 NK cells against cancer

In addition to B cells and T cells, natural killer cell also plays an important role in fighting against cancer. NK cells can kill cancerous cells in multiple ways: (1) Antibody de-pendent cell mediated cytotoxicity; (2) degranulation (perforin and granase B); (3) Apoptosis inducing ligand (Fas/FasL, tomour necrosis factor-α). Besides, NK cells can activate immune cells and enhance the adaptive immune response. Meanwhile, activated NK cells also have a certain antigen-presenting function. NK cell's activation-inhibitory balance determines whether it performs its function (Table 2).

Table.2. NK cell receptors

Activated receptor	Inhibitory receptor	Cytokine receptor
NKp30 NKp44 NKp46 SLAM6 SLAM7 2B4 NKG2D NKG2C CD16 ULBPs KIR2DS KIR3DS DNAM1 CD226	NKG2A NKG2B TIGIT TIM-3 LAG3 ILT-2 KIR-2DL KIR-3DL	IL-2R IL-12R IL-15R IL-18R IL-21R IL-5R IL-6R IL-10R TGF-βR

NK cells have some special advantages in cancer therapy. NK cells are antigen-non-specific and are not limited by the recognition of human leukocyte antigen (HLA) molecules [25]. It is worth noting that the KIR of NK cell is an inhibitory receptor which recognizes MHC-I. In order to avoid cytotoxic T lymphocyte detection, tomour cells tend to lower HLA expression, while NK cells are more sensitive to low HLA expression cells. It is a result of coevolution. Meanwhile, allogeneic NK cells do not cause graft-versus-host disease (GVHD), which represents that the source of the cells can be healthy human peripheral blood, cord blood, or differentiation [26, 27].

5.2 Some recently identified targets of CAR-NK therapy in breast cancer and the corresponding clinical studies

Chimeric antigen receptor (CAR) is a synthetic transmembrane protein. The development of CAR-NK inherits the ideas of classic CAR-T development. The target specificity of CAR-NK is conferred

by the extracellular antigen binding region, and the extracellular antigen binding region is responsible for tomour-specific or tomour-associated antigens [28].

There have been 3 generations of CAR-NK cells(Figure 3) [29]. First-generation cars contain only CD3ζ or DAP12 or DAP10 as a signaling domain. Second-generation cars bind CD3ζ to express a second signal domain. Third-generation cars contains two costimulatory signal domains.

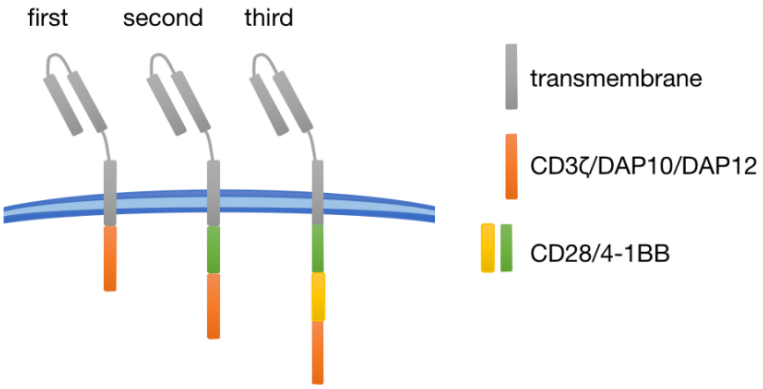

Figure 3. The construction of CAR-NK

The NK-92 cell line has been well studied and proved to be an important candidate for immunotherapy of adoptive cancer as a clonal, molecular and functional means of continuous expansion of cells [30].

More and more potential targets of breast cancer have been discovered and verified, which lays a foundation for the construction of CAR-NK for precise treatment of breast cancer.

5.2.1 GD2

GD2 has been identified as a marker antigen associated with the phenotype of breast cancer stem cell-like cells (BCSC), which has a good therapeutic effect on curing inhibiting tomour metastasis. Seitz's team completed the evaluation of GD2 as a bCSC-specific immunotherapy target antigen [31], confirming the effectiveness of this target in triple negative breast cancer.

5.2.2 HER2

Human epidermal growth factor receptor 2 (HER2) has been shown to be an important car-NK target for HER2-positive breast cancer. In breast cancer, overexpression of HER2 is associated with aggressiveness and poor prognosis of malignancies. Breast cancer with HER2 amplification is more aggressive and has a higher risk of central nervous system metastasis. Experiments[30, 32] have shown that, HER2 antigen specific CAR has a significant killing effect on HER2-positive breast cancer cells, and specifically enhances cell death of HUMAN breast cancer cell lines MDA-MB-453 and SKBr3 expressing HER2. Moreover, CAR-NK-92 cells can effectively dissolve all tomour cell lines and primary cancer cells that are not sensitive to NK-92-mediated killing but express HER2. Even at a low reactant/target ratio, CAR-NK-92 cells can rapidly induce apoptosis of her2-expressing target cells and completely eliminate tomours after a long period of culture.

5.2.3 ERGF

EPGF has a high positive rate in triple negative breast cancer. Tyrosine kinase plays an important role in cell reproduction and differentiation, which may lead to chemotherapy resistance and poor prognosis. It may be a potential therapeutic target [33].

5.2.4 EpCam

EpCam is highly expressed in triple negative breast cancer and is associated with promoting cancer and metastasis, Zhang's experiment [34] mentioned that the combination therapy of regolafenib and CAR-NK-92 cells had a good inhibitory effect on the growth of established EPCAM-positive xenograft tomours, and the cytokine release test proved that CAR-NK-92 cells could specifically recognize and be activated by EpCam-positive cells.

5.2.5 NTRK

NTRK is one of the most popular anti-cancer drug targets for widespread cancer, and its positive rate varies greatly among different cancer types, while the positive rate of NTRK can reach more than 90% in secretory breast cancer.

Currently, CAR-NK treatment for breast cancer is still in the clinical trial stage (Table 3), and the main source of NK cells is the NK-92 cell line.

Table.3. CAR-NK's clinical-phase program

Target	Malignancy	CAR structure	Source of NK cells	References
EGFR	Breast cancer brain metastases	scFv+CD28+CD3ζ	NK-92 cells	[33]
HER2	Breast,ovarian,squamous cell carcinoma	scFv+CD3ζ	NK-92 cells	[32]
HER2	Glioblastoma or breast cancer	scFv+CD28+CD3ζ	NK-92 cells	[35]
EpCam	Breast carcinoma	scFv+CD28+CD3ζ+IL-15	NK-92 cells and NKL cells	[36]

5.3 The difficulties and forecasts of CAR-NK therapy in breast cancer

5.3.1 Viral vectors of CAR-NK still exist defects

Similar to CAR-T, CAR-NK mainly uses viral vectors such as lentiviruses and retroviruses to transfer CAR to effector cells, which is a problem. The high transfer efficiency of viral vectors is accompanied by some defects, such as limited vector capacity, potential toxicity and tomour-causing, and induction of immune responses [37]. Therefore, exploring and optimizing non-viral vectors and improving their transfection efficiency are effective ways to solve the above problems.

5.3.2 The curative effect of CAR-NK is influenced by immune inhibitory tomour microenvironment

CAR-NK cell therapy has been shown to be more successful in hematological tomours than in solid tomours [38]. Currently, there have been cases of complete cure in blood cancers, but there are still great challenges in solid tomours such as breast cancer, where CAR-NK has to face a tomour microenvironment characterized by oxidative stress, nutrient deficiency, acidic pH and hypoxia as well as many immunosuppressive soluble factors, cytokines and immunosuppressive immune cells. Therefore, in the treatment of breast cancer, the attempt to use combination therapy to reverse the immunosuppressive tomour microenvironment will significantly improve the efficacy of CAR-NK therapy. For example, KIR2DL4, which normally works in NK cells, can enhance the cytotoxicity of NK cells by increasing the production of IFN-γ. However, the immune tolerance molecule hLA-G expressed in breast cancer can bind to KIR2DL4 on NK cells and damage THE ADCC of NK cells, thus damaging the cytotoxicity of NK cells [39] and combination therapy with Herceptin can be performed.

5.3.3 The low dependence of CAR-NK on CAR antigens added to the immunotherapy library

Finally, if a tomour cell downregulates CAR antigen, CAR-T cells will become ineffective while CAR-NK cells still can recognize tomours through their germline encoded receptors, thereby reducing the chance of tomour escape via antigen-mediated escape.

6. Conclusion

TILs brings hope to breast cancer patients. Its treatment result could be affected by the tumour microenvironment, immune escape, and other factors. However, many experiments have proved the feasibility of TILs and neoadjuvant therapy and their value as biomarkers. If early staged patients can also benefit from TILs therapy, it may provide a new direction for locally advanced or metastatic breast cancer. As an extension of TILs, TCR-T therapy has solid antigen recognition ability and extended survival time. However, its efficacy in different subtypes of breast cancer remains to be further clinical verification. The future research focus of TCR-T therapy lies in overcoming the inhibition of the tumour microenvironment.

Major challenges of CAR-T cell therapy which applied in solid tomours include the lack of targeted tomour-specific surface antigens, heterogeneity of tumour cells, immunosuppressive tomour microenvironment, and side effects due to cytotoxicity. CAR-T combined with chemotherapy can improve T cell activity and alleviate side effects during treatment. Finding more new targets or designing new viruses and modifying the tomour microenvironment can still be the direction of future research.

CAR-NK is similar to CAR-T, but it does not cause GVHD and can be obtained from healthy people or through synthetic methods. Meanwhile, it is more sensitive to tomour cells that can evade cytotoxic T cells. Although CAR-NK has been shown to be a complete cure for blood cancer, it is not so effective in solid tomours, such as breast cancer, due to the presence of immunosuppressive microenvironment. Possible solutions are combination therapy with drugs that restore NK cell activity or finding better targets.

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