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Advancements in Breast Cancer Treatment: The Role of Tumor-Infiltrating Lymphocytes

Zhouchang Shu

School of Basic Medical Sciences, Guangzhou Medical University, Guangzhou, China
ZC_Shu0607@outlook.com

Abstract: Breast cancer is one of the most common malignant tumors among women worldwide, and its treatment faces many challenges. Conventional treatments have limitations in improving patient survival and quality of life, making the development of new therapeutic approaches a top priority. Tumor-infiltrating lymphocyte (TIL) therapy, as an emerging immunotherapeutic approach, shows great potential by harnessing the anti-tumor capacity of patients' own immune cells. This article discusses the application of TIL therapy in breast cancer and analyzes the current challenges as well as future prospects. Studies indicate that high TIL infiltration levels in triple-negative breast cancer (TNBC) and HER2-positive subtypes are significantly associated with improved response rates to neoadjuvant chemotherapy and reduced risk of recurrence. Technological innovations such as the G-REX culture system to shorten the amplification cycle and gene editing to enhance homing efficiency have driven clinical translation. In addition, through automated preparation, liquid biopsy technology and multicenter data platforms, TIL therapy is expected to become an important tool for precision treatment of breast cancer and improve patient prognosis.

Keywords: tumor-infiltrating lymphocytes, immunotherapy, breast cancer, tumor microenvironment

1. Introduction

With the increasing incidence of tumors, traditional therapies (e.g., radiotherapy, chemotherapy) are facing many challenges. Recently, adoptive cell therapy (ACT), a form of "live cell" therapy, has become a new hotspot in tumor treatment. ACT is based on the isolation and identification of autologous or allogeneic lymphocytes, which can be expanded or genetically edited in vitro, and then infused back into the patient to play a tumor-killing role. They mainly include T cell receptor (TCR)-T cells, chimeric antigen receptor (CAR)-T cells, and tumor- infiltrating lymphocytes (TILs) [1].

Breast cancer is the most common malignant tumor among women worldwide. According to the World Health Organization data for 2025, breast cancer is firmly at the top of the global female cancer list with 2.3 million new cases (25% of female cancers) and 670,000 deaths (15.5%) [2]. High-income countries (e.g., North America, Western Europe) have the highest incidence rates (ASIR >80/100,000) but benefit from universal mammography screening (coverage >70%) and standardized treatment, with early diagnosis rates (stage I-II) of 60-77% and 5-year survival rates of more than 80%. In contrast, low- and middle-income countries (e.g., sub-Saharan Africa, South Asia),

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experience lower incidence rates (ASIR 30-50/100,000) but significantly higher mortality rate with 5-year survival rate generally below 50% due to limited screening (coverage <25%), delayed diagnosis (70%-80% of late-stage cases) and inadequate treatment resources. In China, a typical middle- and high-income transition country, there were 410,000 new cases (17.8% of the world) and 110,000 deaths (16.4% of the world) in 2022, with a concerning trend toward younger incidence (20% of cases in women under 50) [3]. Approximately 5% to 10% of women with breast cancer carry mutations in BRCA1 or BRCA2, with an 80-year risk of 72% and 69%, respectively, for developing breast cancer. Women with BRCA1 mutation also face a 44% lifetime risk of ovarian cancer, while those with BRCA2 mutations have a 17% risk. [4].

Recent studies have identified significant TIL presence in triple-negative and HER2-overexpressing breast cancers, with TIL abundance positively correlated with the response to immunotherapy. This provides a therapeutic breakthrough for traditionally "cold tumors". TIL expansion and infusion can reshape the anti-tumor immune microenvironment, presenting a new strategy for precision therapy.

This paper aims to investigate the application of TIL therapy in solid tumors (breast cancer), to promote its clinical application and achieve durable objective remission. The significance of the study is to provide new treatment options for breast cancer patients, improve their prognosis, and promote the development of individualized medicine.

2. Basic principles of TIL immunotherapy

Early TIL preparation consisted of only three stages: isolation, amplification and transfusion. As research progressed, a screening step was added before expansion, greatly improving the effectiveness of TIL. The general process begins with obtaining fresh tumor tissues by surgery, ensuring the sample is larger than 1 cm^3 and processed promptly. Then different types of T-lymphocytes are cloned and cultured with interleukin 2. The T-lymphocytes proliferate and form cell populations stimulated by interleukin 2. The proliferating T-lymphocytes react with tumor cells and retain the positive cells. Tumor-specific TILs are then expanded. Patients receive chemotherapy before receiving cell transfusions. Chemotherapy enhances the immunogenicity of the tumor, which enhances immunotherapy and improves the cure rate and long-term survival of patients.

Isolation of TIL from tumors is the first step, typically obtained by surgical resection of solid tumors, cancerous pleural effusions and metastatic lymph nodes, followed by the preparation of single-cell suspensions via mechanical or enzymatic digestion. Traditional methods often resulted in poorly functional TILs. Rosenberg et al. pointed out that it was easier to isolate tumor-specific TIL in PD1-positive CD8 + TIL than in PD1-negative CD8 + TIL, which solved the problem of screening hundreds of T-lymphocyte clones to obtain tumor-specific TILs [5].

The screening of specific effector cells has a direct impact on the clinical efficacy of TIL therapy in the critical preparation process. Some researchers have established a screening system based on tumor neoantigensinvolving whole-exome sequencing to identify mutations, predicting potential immunogenic epitopes, and validating their ability to activate TILs, as shown in Figure 1.

Enhancing the TIL therapeutic efficacy lies in two core problems: rapidly obtaining sufficient highly active TILs and ensuring accurate targeting to tumor tissues. Traditional expansion techniques are time-consuming, often taking more than a month. Innovations like the gas permeable culture system (G-REX flasks) have boosted TIL yields and shortened the preparation cycle [6]. A research team even achieved rapid enrichment of CD8-positive killer T cells in melanoma therapy by introducing CD137 (4-1BB) co-stimulatory signaling in conjunction with IL-2 stimulation, which allowed cell preparation in only 3 weeks, which is particularly important for advanced patients requiring urgent intervention [7].

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In terms of cell quality control, modern testing technology has been upgraded and iterated, with flow cytometry providing more accurate analysis of TIL subpopulations compared to traditional immunohistochemistry [8]. However, even high-quality TILs face challenges with tumor homing efficacy. Studies have revealed that the interaction between CXCL1/CXCL8 chemokines and CXCR1 receptors on the surface of T cells in the tumor microenvironment is a key factor affecting TIL migration. Modification of TIL to highly express CXCR1 receptor by gene technology can significantly enhance cellular responsiveness to tumor chemotactic signals, presenting a promising strategy for therapies targeting low immune-infiltrating tumors such as breast cancer [9].

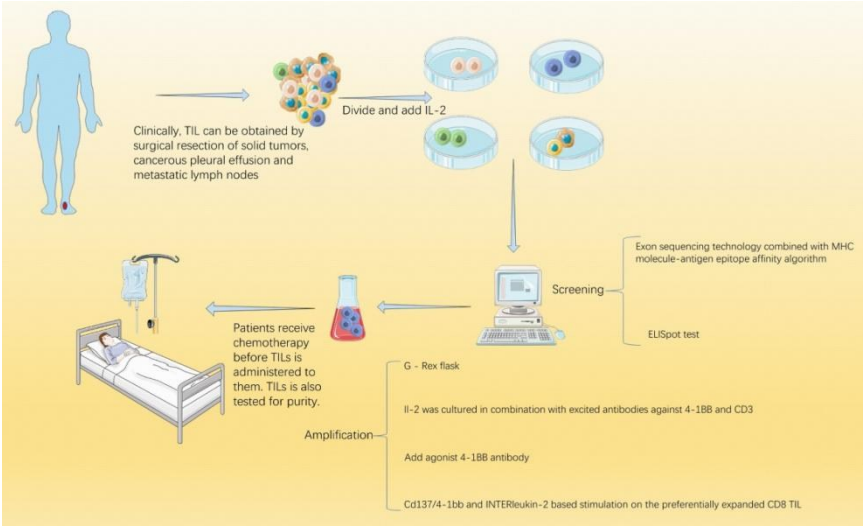


Figure 1: Preparation and treatment process of TIL [10]

3. Immunotherapy in breast cancer

3.1. Pathologic features of breast cancer

Breast cancer is characterized by the uncontrolled growth of abnormal breast cells, primarily manifesting as epithelial tumors (carcinomas) that develop from the overlying cells of the ducts or lobules. These are categorized as carcinomas in situ , such as ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS), and invasive carcinomas, predominantly invasive ductal carcinoma (75%) and invasive lobular (10%). Epithelial carcinomas may express hormone receptors (mesenchymal tumors do not express hormone receptors, such as lobular tumors). About 80% of postmenopausal and 20% of premenopausal cancer patients have estrogen receptor-positive (ER+) tumors; about 70% of breast cancers are progesterone receptor-positive. Human epidermal growth factor receptor 2 (HER2) is overexpressed in about 15% of breast cancer patients and is associated with poorer prognosis. The majority of breast cancers are hormone receptor-positive and HER2-negative (about 70%) while about 12% are triple-negative (hormone receptor-negative and HER2-negative).

Recent studies have have highlighted the immune environment in breast cancer as a prognostic biomarker and target for immunotherapy, particularly in highly proliferative subtypes like triple-negative and HER2-overexpressing breast cancers, making TIL technology attract widespread attention.

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3.2. Clinical application of TIL in breast cancer treatment

In triple-negative breast cancer and HER2-positive subtypes, the clinical value of tumor-infiltrating lymphocytes (TIL) has gradually emerged. Studies indicate that higher TIL density correlates with significantly improved responses to chemotherapy and immunotherapies, such as PD-1/PD-L1 inhibitors. For instance, patients with higher TIL levels receiving neoadjuvant chemotherapy may have more than double the pathologic complete remission rate compared to those with lower TILs, with a 60% reduction in recurrence risk within five years. This phenomenon may be related to the "immune activation-suppression balance" created by the high expression of PD-L1 molecules in the TIL-enriched tumor microenvironment.

Based on this, researchers developed a therapeutic regimen of in vitro expansion of autologous TIL followed by back infusion, and the results of laboratory studies showed that about 70% of PD-L1-positive tumor models showed significant regression when combining expanded TIL with PD-1 inhibitors, and the mechanism of action may involve the reprogramming of T-cell function (e.g., decreased expression of depletion markers, increased secretion of killing factors, etc.), which provides a new breakthrough for specific breast cancer subtypes providing a new breakthrough for precision immunotherapy [11].

In 2014, some important breakthroughs were made in the assessment of the immune microenvironment of breast cancer, and the research team innovatively established a quantitative assessment system for lymphocyte infiltration (TIL) based on routine pathology H&E stained sections [12]. This method has successfully achieved a clinically scalable quantitative analysis of the immune microenvironment by standardizing the process of calculating the percentage of lymphocyte infiltration area. A review in Ann Oncol noted that for every 10% elevation in TIL levels, the risk of invasive recurrence/death, the risk of distant metastasis, and the risk of all-cause mortality, corrected by multifactorial correction, were reduced by 13%, 17%, and 16%, respectively [12]. This finding is based on a meta-analysis of 2148 patients, which established for the first time TIL as an independent prognostic indicator for triple-negative breast cancer (TNBC), and its predictive efficacy can help the clinic to make precise staging adjustments and provide a threshold reference for therapeutic decisions.

In 2019, the Park team further validated the predictive value of TIL with an important clinical study. In a cohort of stage I TNBC patients not receiving adjuvant chemotherapy, the high TIL group (≥30%) demonstrated excellent 5-year distant metastasis-free survival (97%) and overall survival (98%) [13]. This finding has driven international guidelines to recommend the inclusion of TIL in the design of early TNBC clinical trials, especially more instructive in the era of immune checkpoint inhibitor therapy. Notably, TIL assessment does not require additional PD-L1 immunohistochemistry testing, and information on the baseline immune status of the patient can be obtained only from routine pathology sections, a feature that makes it valuable for clinical practice in advanced TNBC and HER2-positive breast cancer.

4. Challenges and prospects

Tumor infiltrating lymphocyte (TIL) therapy has emerged in recent years as an important breakthrough in the treatment of solid tumors. Unlike CAR-T or TCR-T therapies, which require genetic modification, this therapy is unique in that it directly uses the patient's own immune cells to fight the tumor. It has been found that the T-cell population in TIL is diverse, not only recognizing common antigens expressed by tumor cells, but also specifically targeting de novo antigens generated due to genetic mutations [14-15]. This dual recognition capability gives TIL an advantage in dealing with tumor heterogeneity. In addition, effector memory T cells enriched in this cell class exhibit enhanced migratory capacity and are able to infiltrate tumor tissues more efficiently. Their safety has

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also been clinically validated as they are entirely derived from the patients themselves and do not require gene editing.

However, the dissemination of the therapy still faces a number of obstacles. The primary challenge lies in the prerequisite for treatment - sufficient numbers of functional T cells must be present in the tumor microenvironment, but clinical data show that more than 60% of patients with solid tumors suffer from insufficient immune infiltration. Although natural killer (NK) cells have more potential for in vitro expansion, their practical application is often limited by immunosuppressive factors in the microenvironment [16]. Researchers are currently exploring alternatives such as γδ T cells, which achieve non-specific killing by secreting cytokines such as interferon γ. However, how to improve their ability to target tumors still requires the use of bispecific antibodies or local drug delivery techniques.

At the technical level, the precise screening of TILs recognizing de novo antigens remains an unsolved problem. In contrast, TCR-T therapies gain the ability of T cells to recognize specific mutations through gene editing, e.g., TCR therapies developed to target mutations in the TP53 gene have progressed in patients with specific genetic backgrounds [15]. Whereas attempts to integrate CAR technology into TIL (e.g., anti-Her2 CAR-TIL) have enhanced targeting, the complex diversity of tumor antigens still constrains the development of general-purpose therapies.

Studies on TIL functional depletion have recently made important discoveries. Through single-cell sequencing technology, scientists have found that GATA-3 transcription factor plays a key role in CD8+ T-cell dysfunction, while the aggregation of PD-L1-positive depleted T-cells in the breast cancer microenvironment suggests new therapeutic targets [17-18]. More excitingly, French researchers found that specific structures in the tumor vasculature (TA-HEV) are closely related to TIL infiltration efficiency, which provides new ideas to improve the efficacy of cellular therapies.

Current research is focusing on three main directions, namely, developing more accurate T-cell depletion assays, optimizing in vitro expansion processes, and enhancing tumor homing through gene editing. With these technological advances, TIL therapy is expected to expand from melanoma to more solid tumor types, become one of the main treatments for breast cancer, and provide a scientific basis for combination immunotherapy.

5. Conclusion

Tumor infiltrating lymphocyte (TIL) therapy has emerged as a new direction in breast cancer treatment by extracting and expanding immune cells from patient tumor tissues to take advantage of the dual antigen recognition ability of their polyclonal T-cells (self-antigens and de novo antigens) and the migratory advantage of effector memory T-cells. Studies have shown that high TIL infiltration levels are significantly associated with improved response rates to neoadjuvant chemotherapy and reduced risk of recurrence in patients with triple-negative breast cancer (TNBC) and HER2-positive subtypes. On the technical level, TIL therapy has progressed in clinical translation through optimization of in vitro amplification processes (e.g., G-REX culture system to shorten cycle time) and gene editing to enhance homing ability (e.g., CXCR1 receptor overexpression). However, the therapy is still limited by challenges such as insufficient immune cells in the tumor microenvironment, functional depletion and difficulties in screening for de novo antigens.

However, there are some limitations to this paper. For example, although this paper provides insight into the potential of tumor-infiltrating lymphocyte (TIL) therapy in breast cancer treatment, it still lacks a detailed analysis of clinical trial data and empirical support for long-term efficacy, which may affect a comprehensive assessment of the effectiveness of its practical application.

Future research needs to focus on key issues of TIL therapy in breast cancer. The first is to improve the immunosuppressive properties of the tumor microenvironment by combining immune checkpoint

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inhibitors or gene editing techniques (e.g., modifying TIL to secrete CXCL10). The second is to combine single-cell multi-omics techniques to deeply resolve the mechanism of TIL functional depletion and to optimize cell expansion methods to retain highly active subpopulations. By integrating nanotechnology, artificial intelligence predictive modeling, and real-world data, TIL therapies will be driven from the lab to the clinic on a broad scale.

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