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Review Article

Cell Types and Optimization Strategies in Tumor Immunotherapy

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Abstract

Adoptive immune cell therapy (ACT) utilizes a patient's own immune cells to target and eliminate cancer cells. This article explores the application of various cell types in ACT, including T cells, NK cells, macrophages, and dendritic cells. Each cell type offers unique advantages in combating cancer. The article highlights the importance of metabolic regulation in optimizing the anti-tumor function of immune cells. It then explores the immunosuppressive nature of the tumor microenvironment and the role of immune checkpoint molecules in this process. The article concludes by discussing the potential of gene editing tools to improve the efficacy and safety of ACT, while acknowledging the challenges associated with current ACT therapies and outlining future research directions. This approach of harnessing a patient's own immune system holds promise for a more personalized and effective approach to cancer treatment.

INTRODUCTION

The concept of utilizing cells as therapeutic agents for cancer treatment dates back to the early 20th century. In the 1970s, Rosenberg made the groundbreaking discovery that tumor-infiltrating lymphocytes (TILs) could be expanded in vitro and reinfused into patients to suppress tumor growth [1]. As understanding of the immune system deepened, the idea of harnessing different types of a patient's own immune cells to combat cancer emerged. One such representative therapy is chimeric antigen receptor T-cell (CAR-T) therapy.CAR-T therapy relies heavily on the function of the chimeric antigen receptor (CAR) structure on the cell surface. This structure is primarily composed of four domains: the single-chain variable fragment (scFv) that targets tumor antigens, the hinge region, the transmembrane domain, and the intracellular signaling domain that activates T cells, such as CD3ζ, CD28, and 4-1BB [2]. The tumor antigen-targeting scFv endows the engineered CAR-T cells with the ability to specifically recognize tumors, enabling them to precisely identify and eliminate tumor cells [3]. Multiple iterations of CAR structure design have led to the incorporation of cytokine secretion capabilities, such as IL2 and IL12, which enhance T-cell killing function and persistence in vivo [4]. In clinical trials, CD19-targeted CAR-T therapy, such as Kymriah, has demonstrated remarkable breakthroughs in treating refractory/relapsed acute B-cell lymphoblastic leukemia (B-ALL) and diffuse large B-cell lymphoma (DLBCL), achieving overall response rates of 82% and 41-59.5%, respectively [5].

Despite the remarkable efficacy of CAR-T cells in B-ALL, several limitations remain in clinical applications. Among patients treated with Kymriah, the rate of resistance and relapse is as high as 30%-70%, and most relapses occur within 12 months of treatment [2]. Moreover, this resistance is not limited to the CD19 target; studies on other targets, such as CD22 and BCMA, have also confirmed that resistance and relapse pose significant challenges for CAR-T cell therapy [6,7]. The primary mechanisms underlying CAR-T resistance and relapse include antigen escape, impaired CAR-T cell activity, and the suppressive effects of the tumor microenvironment on CAR-T cells [8]. For instance, tumor cells may evade CAR-T cell attack through gene mutations, antigen downregulation, or antigen loss. Furthermore, if T cells cannot maintain optimal activation, it can also compromise CAR-T cell persistence and tumor killing ability, leading to resistance [9]. For solid tumors, CAR-T cell therapy also faces the challenge of overcoming the immunosuppressive tumor microenvironment, where regulatory T cells, myeloid-derived suppressor cells, and tumor-associated macrophages can reduce the infiltration of cytotoxic T cells, thereby suppressing the antitumor immune response [2,10,11]. To address the suboptimal outcomes observed after CAR-T therapy, several research efforts are focused on identifying novel strategies to overcome the inherent defects of immune cells during treatment.

Cell Types in Adoptive Immune Cell Therapy (ACT)

Adoptive immune cell therapy (ACT) utilizes ex vivo

techniques to enhance the effective number and natural immune function of immune cells for cancer treatment. Since CD8+ T cells are the primary effector cells of the antitumor immune response, previous clinical trials and products have largely focused on this cell type [12]. However, in clinical applications, T cell exhaustion and adverse reactions such as cytokine release syndrome (CRS) have become major limitations of this therapy [9,13]. In addition to T cells, the immune system consists of various immune cells including B cells, NK cells, APCs, and macrophages, each with unique characteristics that collectively maintain normal immune function and exert antitumor effects [14].

a. T Cells

CD4+ T cells are a subtype of T cells that, after activation by MHC-II molecule-presented peptides, can differentiate into different subsets such as Th1, Th2, Treg, and CD4+ cytotoxic T lymphocytes (CD4+ CTLs), and enhance the activity of CD8+ T cells by secreting cytokines [15]. As previously mentioned, most previous studies have considered CD8+ T cells to be the primary cytotoxic T cell subset in the immune response, while CD4+ T cells can lead to CRS due to cytokine release [16]. However, in patients with long-term remission after CAR-T therapy, it was found that the surviving CAR-positive T cells in the body were CD4+ T cells. Further in vivo experiments revealed that CD8+ CAR-T cells undergo exhaustion and apoptosis after multiple stimuli from TCR receptors and CAR signals on the surface, while CD4+ CAR-T cells retain their in vivo function [17,18]. Both in vivo creation of CD4+ CAR-T cells and equal-proportion infusion of CD4+ CAR-T and CD8+ CAR-T cells have achieved better treatment outcomes and lower incidence of adverse events [12,19]. This suggests that CD4+ T cells and CD8+ T cells have different functions in T cell-mediated antitumor immune responses, and adjusting the proportion of these two types of cells in the cell product can achieve better therapeutic effects.

Another prominent cell type in CAR-T therapy is memory T cells, which are characterized by lower levels of exhaustion markers such as PD-1 and TIGIT and higher levels of lymph node homing receptors CD62L and chemokine receptor 7 (CCR7), and can persist in the body for a long time [20]. In preclinical models, adoptive therapy with memory stem cell T cells (TSCM) or central memory (TCM) CAR-T cells can enhance the therapeutic response [21]. Additionally, the frequency of memory T cells is increased in patients treated with CD19 CAR-T cells [22]. These observations may be due to the memory phenotype of CAR-T cells, leading to prolonged survival of the cells in the body. γδ-T cells are another cell subset distinct from αβ-T cells, primarily distinguished by the different composition of their TCR chains. Commonly used CAR-Ts are primarily αβ-T cells, but γδ T cells possess both αβ T cell and natural killer cell antitumor mechanisms, mediated not only by T cell receptor (TCR) activation but also by NK cell receptor activation, making them another cell choice for CAR-T therapy [23].

b. NK Cells

In addition to T cells, NK cells are another immune cell type, primarily distributed in peripheral blood, accounting for 15% of lymphocytes [24]. Based on their functional status, they can be divided into a relatively immature precursor state (CD56bright/CD16-, 10%) and a relatively mature state with stronger cytotoxicity (CD56dim/CD16+, 90%) [25]. Compared to T cells, NK cells have advantages such as MHC molecule-independent activation, lower risk of graft-versus-host disease, and tumor cytotoxicity independent of cytokines [26]. Additionally, the expression of CD16 molecules allows NK cells to exert part of their adaptive immune function through antibody-dependent cell-mediated cytotoxicity (ADCC), and some studies have reported that the efficacy of monoclonal antibodies is related to the ADCC function of NK cells in the body [27]. These characteristics make NK cells a promising cell type for ACT therapy besides T cells. Another subtype of NK cells, NKT cells, can express αβ-TCR receptors to recognize antigens, and studies have shown that these cells have a memory-like phenotype and can persist in the body for a long time [28,29]. These features make NKT cells a useful target for immunotherapy and enable the activation of their effective antitumor mechanisms.

c. Macrophages

Macrophages have the ability to engulf and digest foreign substances and present antigens, making them more effective than other immune cells in adoptive immune cell therapy. Under the influence of cytokines produced by T cells and the surrounding environment, they can be activated and differentiated into two different subtypes, M1 (promoting inflammatory response) and M2 (promoting wound healing) [30]. M1 macrophages, which exhibit significant tumor killing activity, have been recognized as an effective weapon in cellular immunotherapy [31]. Additionally, macrophages can be adjusted to an anti-inflammatory phenotype, such as eM2-Mφs, which have a stronger ability to cross tumor vessels and localize to glioblastoma (GBM) compared to M1 macrophages [32]. Early research in tumor immunotherapy has also made progress in understanding the critical role of tumor-associated macrophages (TAMs) in the success of T cell-directed checkpoint blockade therapy [33]. Overall, the unique characteristics of macrophages, including their plasticity and functional diversity, make them valuable candidates for adoptive immune cell therapy in cancer and infection treatment.

d. Dendritic Cells (DCs)

Dendritic cells (DCs) are used in adoptive immune cell therapy due to their ability to guide naïve T cells and induce antitumor immune responses [34]. By utilizing DCs as vaccine carriers or antigen-presenting cells (APCs), cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells can be activated to achieve tumor treatment [35]. However, DC vaccines have not shown sustained clinical benefit and have some limitations in clinical applications, such as tumor-mediated immunosuppression and the functional limitations of commonly used monocyte-derived DCs (MoDCs) [36]. To overcome these limitations, new methods for DC isolation, expansion, and stimulation of cytotoxic activity are needed. Additionally, DC-derived exosomes (DC exos) have attracted

attention due to their resistance to tumor-mediated suppression. Peripheral blood DCs, such as plasmacytoid DCs (pDCs), can generate antitumor CD8 T cell immunity by transferring antigens to conventional DCs (cDCs) through pDC-derived exosomes (pDCexos), and have shown promising therapeutic effects in some clinical trials [37]. Other subtypes, such as type 2 conventional dendritic cells (cDC2s), can also promote antitumor immunity by promoting cytotoxic T cell responses and T helper cell differentiation [38]. These characteristics and advantages make DCs a valuable tool in adoptive immune cell therapy for cancer treatment.

e. Metabolic Regulation of Immunity

Alterations in tumor cell metabolism not only affect the tumor itself but can also suppress anti-tumor immune responses by competing for and consuming essential nutrients or impairing the metabolic adaptation of tumor-infiltrating immune cells through other means [39,40]. Previous studies have suggested that T cell activation from a resting state requires three signals: MHC molecule-presented antigenic peptides activating the TCR signal, costimulatory factors such as CD3/28, and cytokines. Building on these stimulatory signals, scientists like Chi have identified nutrients as a fourth essential signal for T cell activation, proposing that the transport, uptake, and sensing of substances like glucose and amino acids drive T cell activity [41].

f. Metabolic Regulation

As previously mentioned, the longevity of immune cells infused for therapy and their differentiation into memory cells are key aspects of adoptive cellular therapy [21]. Numerous studies have shown that the differentiation of T cells into memory cells largely depends on specific metabolic pathways. IL-15 promotes mitochondrial biogenesis and the expression of carnitine palmitoyltransferase 1A (CPT1A), an enzyme involved in fatty acid oxidation. In memory T cells, CPT1A supports the shift from glycolysis to oxidative metabolism [42]. Therefore, it is possible to alter the metabolic profile of memory T cells through changes in in vitro culture conditions or genetic modifications to adapt to the tumor microenvironment.

Some studies have shown that inhibiting glycolysis with glycolysis inhibitors and culturing cells under conditions that reduce mitochondrial reactive oxygen species generation can support the survival of stem cell-like T cells and enhance their anti-tumor activity [43]. Other research has explored the different energy metabolism of memory T cells. CAR T cells with a memory phenotype primarily rely on oxidative phosphorylation for energy supply [21]. By using combinations of cytokines such as IL-2, IL-7, and IL-15, or supplements like L-arginine and rapamycin to enhance oxidative phosphorylation, promote oxidative metabolism, and improve mitochondrial quality and function, the expansion efficiency in vitro can be improved [21,44-46]. These methods enhance the longevity of T cell products and the expansion and cytokine production of T cells upon re-stimulation with tumor antigens. Moreover, this memory phenotype can also be achieved by editing the mitochondrial metabolism of CAR-T cells genetically. Research has shown that replacing the intracellular signaling domain of the chimeric receptor with the signaling domain of the co-stimulatory molecule 4-1BB can increase mitochondrial biogenesis, respiratory reserve capacity, and fatty acid utilization. Compared to CD28-based CAR T cells, these cells exhibit better longevity after infusion into the body [47]. Therefore, modifying T cell metabolism through CAR design or treating cells with metabolic modulators during in vitro expansion may help stabilize the memory-like phenotype, thereby enhancing the longevity and efficacy of T cells.

g. Nutrient Supply

Due to the glycolytic metabolism of tumor cells, which can affect the glucose supply to T cells, there is a reduction in T cell infiltration into tumor tissues and cytotoxicity against tumor cells. Consequently, tumor cells relying primarily on glycolysis are more likely to develop resistance to adoptive cellular therapy [48]. Modifying immune cells can overcome glucose deficiency in the tumor microenvironment, thereby enhancing the therapeutic effect against tumors.

The low glucose environment caused by tumor cell glycolysis can reduce pyruvate (a key product in aerobic glucose oxidation) levels and T cell receptor signaling. Counteracting this effect by increasing pyruvate levels can improve the efficacy of adoptive cellular therapy. Forced expression of phosphoenolpyruvate carboxykinase 1 (PCK1) (which converts oxaloacetate to pyruvate) in transferred T cells can increase intracellular pyruvate levels, support T cell survival, and better control tumor growth [49]. Inhibition of pyruvate dehydrogenase (PDH) also leads to the diversion of pyruvate to lactate instead of entering the mitochondria for the TCA cycle during aerobic glycolysis [50]. By inhibiting pyruvate dehydrogenase kinase 1 (PDHK1) with dichloroacetate (DCA), glycolysis can be restored to baseline levels, reducing mitochondrial stress, increasing mitochondrial biogenesis, and restoring the energy metabolism of therapeutic T cells. These findings suggest that reducing T cell dependency on glycolysis can improve its anti-tumor function.

Lymphocytes infiltrating tumors often quickly develop stress-related metabolic defects, including reduced glucose uptake capacity and loss of functional mitochondria. Some studies suggest that this reduction in mitochondria is mediated by the inhibition of PGC1a (a transcriptional coactivator for mitochondrial biogenesis). Based on this evidence, Delgoffe and colleagues overexpressed PGC1a in TCR-engineered or CAR-T cells and found that it significantly improved T cell responses and outcomes [51]. Furthermore, expression of FOXP3 can diversify the metabolic pathways of CD8+ T cells, manifesting as enhanced glucose uptake. Under limited nutrient supply conditions, these cells can utilize fatty acids to drive oxidative phosphorylation (OXPHOS) to meet energy demands [52]. This might be another way to improve T cell nutrient supply in CAR-T therapy, aside from PGC1a.

h. Hypoxia

Low oxygen levels are a key factor causing the dysfunction

of T cells in the tumor microenvironment. Low oxygen levels in the tumor microenvironment lead to changes in proliferation, metabolism, and other vital cellular mechanisms to protect cells from damage and apoptosis [53]. This is particularly important for solid tumors, where oxygen levels can drop as low as 0.3% [54]. Deletion of HIF-1α in therapeutic T cells before transferring them into mouse lung (LLC) or melanoma (B16F10) models resulted in reduced tumor-infiltrating T cells and increased tumor burden, indicating the importance of adapting to low oxygen levels for T cell responses [55]. Expanding therapeutic T cells in a culture medium with low oxygen levels to adapt them to hypoxia before using them for patient treatment can increase their anti-tumor activity [56]. Additionally, it has been shown that metformin can improve the adaptability of CD8+ T cells to hypoxic conditions [57]. Therefore, combining metformin with CAR-T therapy might be another approach to overcome hypoxic conditions, aside from HIF-1α. Overall, these data indicate the importance of T cell adaptability to hypoxia for anti-tumor activity, and modifying T cells in different ways can improve the efficacy of tumor treatment.

i. Other Metabolites

Certain metabolites present in the tumor microenvironment can inhibit anti-tumor immune responses. CD39 and CD73 are ectoenzymes expressed by Treg cells, B cells, tumor-associated macrophages, tumor cells, and endothelial cells of the tumor vasculature, which convert ATP to adenosine. While ATP has an immune-stimulatory effect, adenosine suppresses the effector functions of immune cells [58]. Studies have shown that antagonizing adenosine receptors in mice can improve the effector functions of CD8+ T cells. Combining adenosine receptor targeting with CAR-T cell therapy can enhance therapeutic outcomes in a breast cancer mouse model [59].

Furthermore, ion imbalances of Ca2+, K+, and Cl- due to low pH, increased activity of ion channels, and release of ions from necrotic tumors in the tumor microenvironment can affect T cell function [60]. This is because ion-gated Ca2+ signaling is crucial for T cell survival and function [61]. Overexpressing K+ efflux pumps in T cells to improve excessive K+ efflux can increase interferon-γ production, tumor clearance, and survival rates in a mouse model post T cell transfer [62]. This suggests that ion imbalances in the TME may disrupt T cell signaling and lead to T cell dysfunction. Modifying cells to function better under these conditions might improve adoptive cellular therapy for solid tumors.

Low arginine levels can also affect T cell proliferation. In pediatric patients with acute myeloid leukemia, arginine levels in peripheral blood were found to be lower than in healthy controls; this might impair the proliferation and maintenance of CAR-T cells post-transplant. Compared to CAR-T cells without these enzymes, CAR-T cells engineered to express argininosuccinate synthase (ASS) and ornithine transcarbamylase (OTC) exhibited better proliferation in arginine-depleted culture medium and improved survival rates in mice [63].

j. Signaling Regulation Mechanisms of Tumor Microenvironment

Adoptive cellular therapy often requires cells to be rapidly activated and expanded in vitro under optimal conditions. However, within tumor tissues, due to factors leading to immune evasion by tumor cells, these cells may experience varying degrees of impairment in initiation, expansion, adhesion, and recognition capabilities [14]. This immune evasion typically involves the loss of stimulatory molecules and/or an increase in inhibitory molecules, such as downregulation of HLA molecules, loss of stimulatory cytokines, and upregulation of inhibitory receptors like PD-1 and CTLA-4[64]. Currently, monoclonal antibodies targeting inhibitory receptors like PD-1 and CTLA-4 have shown mixed results in tumor therapy, with an overall response rate not exceeding 20~30% [65]. However, in immune cell therapies where CAR-T cell expression of PD-1 and CTLA-4 is interfered with using gene editing tools like transcription activator-like effector nucleases (TALENs) and CRISPR, different therapeutic outcomes have been observed [66].

k. LAG-3

LAG-3 is an important inhibitory receptor expressed on activated CD4 and CD8 T cells, regulatory T cells (Tregs), natural killer cells (NK), B cells, and plasmacytoid dendritic cells. Although structurally homologous to CD4, LAG-3 has a higher affinity for MHC class II molecules than CD4 [67]. Additionally, LAG-3 interacts with the DC-SIGN family surface lectin LSECT, which is expressed on dendritic cells and tumor tissues, promoting tumor progression by inhibiting T cell anti-tumor responses [68].

l. TIM-3

TIM-3 is expressed on T cells (excluding Th2 cells), NK cells, macrophages, and dendritic cells (DCs). When TIM-3 binds to its ligand, immune cell maturation and activation are attenuated, thereby favoring tumor cell proliferation and survival [69].

m. TIGIT and CD96

TIGIT is typically expressed in activated αβ T cells but is also found in memory T cells, NKT cells, and NK cells, and is upregulated in the tumor microenvironment [70]. By competing with CD226 for binding to CD155, TIGIT inhibits the activating role of CD226 on immune cells, thereby suppressing T/NK cell activation [71]. Moreover, TIGIT can promote Treg immunosuppressive function and induce T/NK cell suppression through Fap2, leading to tumor immune evasion [71]. CD96, similar to TIGIT, is a member of the same immunoglobulin superfamily and has a similar inhibitory role but lower affinity for its ligand CD155 [72]. Blocking CD96 can inhibit the growth of primary tumors in mouse models, dependent on CD8+ T cells, and combination therapy with anti-CD96 and anti-PD-1 can increase the percentage of IFNγ-expressing CD8+ T lymphocytes [73].

n. BTLA

B and T lymphocyte attenuator (BTLA) belongs to the CD28

immunoglobulin superfamily and is widely expressed in the immune system. BTLA mainly negatively regulates immune responses through the recruitment of phosphatases SHP-1 and SHP-2 via two tyrosine-based immune receptor inhibitory motifs (ITIMs) [74].

o. NKG2A and CD94

NKG2A and CD94 are mainly expressed in NK cells and some T cell subsets. HLA-E, a non-classical MHC molecule overexpressed in various tumors, is the main ligand for NKG2A and CD94. By binding to HLA-E, they can inhibit the function of cytotoxic lymphocytes, including NK cells [75]. Monotherapy with the humanized anti-NKG2A antibody monalizumab showed limited clinical activity in gynecologic cancer patients, but combined blockade of PD-L1 and NKG2A led to tumor suppression in colorectal cancer patients resistant to PD-L1 antibody therapy [76].

p. CD200R

The role of CD200R in tumor immunotherapy is complex. On one hand, in certain tumor cells like pancreatic ductal carcinoma epithelial cells and stromal cells, CD200R promotes the expansion of myeloid-derived suppressor cells and Tregs, leading to the suppression of NK cell and cytotoxic T cell functions [77]. On the other hand, CD200 is widely expressed in the body, and blockade of the CD200/CD200R pathway may lead to excessive inflammation, exacerbating symptoms in cancer patients undergoing immunotherapy [78]. Currently, clinical trials targeting CD200R are limited, and no immunotherapy targets this pathway, but antibodies and cell therapies designed against CD200R hold potential as another immunotherapeutic option.

q. B7-H3 and B7-H4

B7-H3 and B7-H4 are another class of important immune checkpoint molecules. B7-H3 is overexpressed in various cancers and is associated with disease progression and poor prognosis. Due to its low expression in normal tissues, it has become an attractive target for cancer therapy. However, the exact receptors mediating the tumor immune suppression by B7-H3 are unknown, with potential receptors identified including TLT-2, IL20RA, and PLA2R1 [79]. B7-H4 expression is increased in several cancers, especially in gynecologic tumors, renal cancer, and lung cancer. High expression of B7-H4 is associated with cancer progression, metastasis, and poor prognosis. Therefore, B7-H4 is another potential target for tumor immune cell therapy [80].

2. Conclusion and Future Perspectives

The use of cells as "living drugs" in CAR-T therapies for cancer treatment offers advantages that traditional drugs cannot provide. The remarkable efficacy of CAR-T therapies in hematological malignancies such as B-cell ALL and refractory or relapsed B-cell lymphomas makes them a promising approach in cancer treatment. However, current immune cell therapies still face challenges, including tumor resistance and relapse, poor efficacy against solid tumors, and severe adverse reactions. These issues may be related to factors such as immune escape by tumors, exhaustion of therapeutic cell products, and the absence of tumor antigen epitopes.

To address these challenges, advancements in gene editing tools, including lentiviruses, Sleeping Beauty transposons, zinc-finger nucleases, and CRISPR, have led to various genetic engineering strategies. Logical CAR circuits, "armored" CARs carrying cytokines or therapeutic antibodies, and inducible gene switch CARs can improve non-specific killing by immune cells and address issues like poor tumor treatment efficacy. However, the addition of target gene length may pose difficulties in immune cell engineering approaches, treatment cycle, and cost, along with concerns about the safety of gene editing.

As our understanding of tumor immunity deepens, future strategies may involve designing novel immune cell therapies by leveraging the metabolism of tumor-infiltrating immune cells, the characteristics of different immune cells, and tumor immune suppression regulatory signals. These approaches could potentially offer more optimal strategies for cancer treatment.

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