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Immunotherapy of cancer in single-cell RNA sequencing era: A precision medicine perspective

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ABSTRACT

Immunotherapy has revolutionized cancer treatment and brought new aspects into tumor immunology. Effective immunotherapy will require using the suitable target antigens, optimizing the interaction between the antigenic peptide, the APC, and the T cell, and the simultaneous inhibitor of the negative regulatory process that inhibits immunotherapeutic effects and develop resistance. Tumor heterogeneity and its microenvironment is the leading cause of resistance in patients. Recently by emerging the single-cell RNA sequencing technology and its combination with immunotherapy, now we can specifically evaluate the mechanism of tumors in the face of immunotherapy agents at the single-cell resolution by detecting the transcriptional activity of immune checkpoints, screening neoantigens with high transcription levels, identifying rare cells, and other important processes. This review focuses on scRNA-seq, particularly on its application in cancer immunotherapy.

1. Introduction

Cancer remains a serious problem despite recent breakthroughs in many fields of medicine, as it is the second leading cause of death in developed countries. For the past 50 years, cancer specialists have relied mainly on three treatment strategies: surgery, radiation therapy, and chemotherapy. Meanwhile, tremendous strides have recently been achieved in our understanding of cancer biology. Immune-based cancer treatments, which were just introduced within the last five years, fundamentally revolutionize therapeutic modalities [1]. Cancer immunotherapy includes targeted immune-based approaches that unleash the immune system for fighting cancer. Continuous research and clinical trials have been conducted to create novel therapeutic methods that lead to even further breakthroughs [2]. Despite these promising discoveries, most patients who get new immunotherapies do not respond or relapse [3,4]. According to recent research, only a few patients potentially respond to immune checkpoint inhibitors (ICIs) therapy [5]. Also, a more considerable proportion of patients who undergo CAR-T treatment, especially for solid tumors, may relapse because immunotherapies work better for some types of cancers, such as hematological cancers [6–8]. Tumor heterogeneity is the most significant obstacle for effective cancer therapy, which plays an essential role as a primary cause of acquired resistance against all treatments. Immunotherapy can generate selective pressure towards antigen-negative cells due to the immune system's reaction to particular tumor antigens, a major cause of relapse in the clinic. As a result, there is a pressing need to improve these immunotherapy approaches to overcome obstacles. This effectiveness is based on a better knowledge of cancer cell activity and the tumor

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microenvironment (TME) [9,10]. Fortunately, it is now possible to examine the gene expression profile of tumor cells at the single-cell level with the recent introduction of scRNA-seq technology, allowing for remarkable high-resolution insights into the genetic makeup of the TME and immune system [11]. Furthermore, scRNA-seq analyses have described special transcriptional programs in the TME, allowing for unprecedented high-resolution knowledge and insight into the TME's unique transcriptional programs and the discovery of new cellular subsets; it can also highlight the degree of "inter and intra tumoral heterogeneity [12].

2. Strategies for cancer immunotherapy

In today's immune-based cancer treatments, different immunotherapeutic techniques have shown significant clinical usefulness to many patients with various advanced cancers, which we summarized in the following subtitles:

2.1. Monoclonal antibodies

Kohler and Milstein proposed producing mAbs as therapies in 1975, using hybridomas generated from immortal myeloma cells and murine lymphocytes to produce antibodies against a single epitope of an antigen [13]. This crucial breakthrough paved the way for the efficient production of antibodies for a wide range of therapeutic applications, which are now regarded as desirable molecules for the diagnosis and/or therapy of a wide range of disorders, including cancer [14]. In clinical practice, mAbs are the main widely applied and approved cancer immunotherapy technique. Antibody-based immunotherapy is a particular treatment approach based on the variable fragment's (Fv) affinity for antibody targeting and the constant fragment (Fc) region's capacity to interact in host immune system components [15]. The effectiveness of mAbs in cancer immunotherapy is dependent on three main methods. These mechanisms include the following:

(i) Antibody binding inhibits factors and receptors which initiate signal pathways employed by cancer cells in division and angiogenesis.
(ii) The antibody-dependent cellular cytotoxicity (ADCC) is made up of target monoclonal antibodies formed from either chimeric or fully human antibody components that bind to specific tumor-associated antigens (TAAs).
(iii) Complement-dependent cytotoxicity (CDC) by complement activation [16,17].

Even though mAbs have distinct modes of action, they have become routine treatment in conjunction with chemotherapy and/or radiation [18]. However, uncontrolled and widespread immune system activation, known as cytokine release syndrome, can be life-threatening and also critical in immunotoxicological research on mAbs [19]. In the past few years, the number of approved or in-development mAbs for cancer treatment has increased. Although cancer immunotherapy research is ongoing, practical applications are still limited. As cancer biology becomes more well-understood, the number of hypotheses and research projects in this field grows [20].

2.2. Immune checkpoint inhibitors

The advent of ICIs is a revolutionary milestone in cancer immunotherapy. Tumor cells escape immune detection and develop many ways to escape, such as activation of immune checkpoint pathways that inhibit antitumor immune responses. ICIs are monoclonal antibodies that disrupt immunological inhibitory pathways, allowing cancerous cells to be eliminated by the immune system [3]. PD-1/PD-L1 and CTLA-4 inhibitors are among the ICIs that have demonstrated remarkable clinical efficacy in various forms of cancer and are quickly revolutionizing medical oncology. Some have been approved for specific cancer therapies, whereas others are still in clinical studies [10,21,22]. The balance between autoimmunity and immunological tolerance will be altered if immune checkpoints are blocked. Overall, anti-PD-1/PD-L1 therapy used for a prolonged time induces significant immune reactions. Hepatic enzyme abnormalities (AST and ALT) in serum levels have also been described as a side effect of anti-PD-1 treatment [23]. As a result, despite the effectiveness of anti-CTLA-4 and anti-PD-1/PD-L1 therapies, a small percentage of patients do not respond to them. Because anti-tumor immunity is controlled by a complex set of components in the TME, It can trigger various immune responses [24]. On the other hand, numerous issues about the best dose and timing for PD-1/PD-L1 checkpoint blockers remain unresolved. As a result, an especially sensitive test for identifying the expression of biomarker in a patient group is required to evaluate the efficacy of ICIs therapy.

2.3. Cytokines

Cytokines are molecules that stimulate immune cell intercommunication, and they are first utilized as an immunotherapeutic strategy [25]. Several cytokines inhibit tumor cell development either directly (anti-proliferative or pro-apoptotic) or indirectly (stimulating immune cell cytotoxicity against tumor cells) [26]. The FDA has approved two cytokines as cancer therapeutics: IL-2 for metastatic melanoma and kidney malignancy and IFN-α for stage III melanoma adjuvant treatment [27]. IFNs-α belongs to the type I IFN family of pleiotropic cytokines. These cytokines have a lengthy history of therapeutic usage in patients with cancer and viral infections. IFN-α may also have various biological effects, including inducing/promoting apoptosis and inhibiting cell proliferation. Notably, endogenous IFN-α has been proposed to mediate some autoimmune diseases, which are frequently found in IFN-treated individuals [28], even in pegylated form and at low doses [29]. IL-2 is an influential immune growth factor with pleiotropic effects on the immune system that aids in the maintenance of T cell responses. Because of the ability of IL-2 in increasing T cells without causing them to lose functioning, it was used in cancer immunotherapy early on. Although IL-2 has been shown to cause full and long-lasting regressions in cancer patients, it has also been linked to immune-related adverse effects (irAE) [30]. The short half-life of most cytokines and restricted therapeutic windows just with moderate anti-tumor effectiveness, at least as monotherapies, are some of the limitations of utilizing cytokines [26].

However, due to the low response rate and severe toxicity related to high IL-2 and IFN- treatment, these cytokines have been pushed to the sideline in clinical practice in favor of targeted therapy and ICIs [31] and look for predictive biomarkers to help choose the people who are most likely to respond. Cytokines have been shown to be beneficial in cancer treatment. There is little question that they will continue to significantly impact the growth of cancer immunotherapy in the future [32].

2.4. Cancer vaccines

Clinical trials have been conducted on several cancer vaccine therapies since the FDA approved the first therapeutic cancer vaccine, primarily by inducing cellular (T-cell-mediated) immune responses [33]. Choosing antigens is crucial in the development of cancer vaccines. Because the antigen should typically be produced only by tumor cells, present on all of them, essential for tumor cell maintenance (so that tumor cells cannot evade immune response through downregulation of the antigen) [34]. Limited, whether any, antigens satisfy all of these characteristics; however, there have been numerous classes of antigens that are used in cancer vaccines. In particular, cancer vaccine targets may be divided into two categories: TAAs and TSAs [35]. Antigens produced through oncoviruses and neoantigens encoded by cancer mutations make up TSAs. As a result, high-affinity T lymphocytes could be expressed and significantly activated by these antigens. Individual oncovirus antigens are produced in specific cancer types (for example,

the HPV E6 and E7 antigens in cervical cancer); however, this happens in a large number of patients [36].

Furthermore, shared neoantigens are antigens encoded by oncogenic driver mutations seen in various patients and tumor types. The vast majority of neoantigens (private neoantigens) are specific to individual patients' malignancies, necessitating the development of personalized treatment [37,38].

Regarding vaccine platforms, for cancer therapy, three types are being developed: cellular vaccines, virus vector vaccines, and molecular vaccines made up of peptides, DNA, or RNA. These technologies provide benefits and drawbacks, and they are currently in development [37]. Building on the enormous amount of information that has been created in immunology over the last 50 years and using a rational, evidence-based approach, we believe that cancer vaccines will emerge as an essential tool for cancer management in the future years.

2.5. Cell-based immunotherapy

Cell-based immunotherapy applies mechanisms for helping immune cells by identifying tumor antigens and then destroying the cancer cells [39]. Immune cells are used as a therapeutic agent in cell-based immunotherapy. By this means, the cells are taken out of the body, activated or changed, expanded, and then re-infused back into the patient. These are just some of the techniques that are presently being researched:

(i) **Adoptive T cell treatment: This kind of cell therapy aims to increase the number of tumor-specific T cells. This method has been utilized with a variety of T cell types from different sources: Ex vivo genetic engineering of peripheral blood T cells or ex vivo expansion of tumor-infiltrating lymphocytes (TILs) to express a tumor-specific T cell receptor (TCR) or a chimeric antigen receptor (CAR; CAR-T cells) versus a tumor epitope** [40,41].

(ii) **Natural killer cell therapy: NK therapy stimulates the body's immune system. Adoptive transfer of ex vivo expanded NK cells has emerged as a novel strategy in treating solid malignancies. NK cells differ from cytotoxic T lymphocytes in that they attack cancer cells that escape from the immune detection of the host by the down-regulation of the self-antigen presentation. According to new research, NK cells can also target tumor progenitor cells** [42].

(iii) **Dendritic cell therapy: Professional APCs, such as DCs, stimulate the immune system. To increase the presence of a tumor-specific antigen, DCs are pulsed ex vivo with tumor lysate or even a specific tumor antigen or genetically modified to display the antigen. T cells stimulate the adaptive immune response when the APCs are re-infused into the patient. Combining DC vaccines with conventional cancer medicines such as chemotherapy and mAbs, on the other hand, might result in effective cancer medications** [43].

(iv) **Macrophage-based therapy: Macrophages are powerful immune effector cells whose functional flexibility allows them to serve as both antitumor and protumor cells in various situations. This flexibility has resulted in significant attempts to deplete or repolarize tumor-associated macrophages. Furthermore, following ex vivo genetic alteration, macrophages could be adoptively transferred. In general, there are two types of macrophage states: M1 that has been activated in the traditional way or M2 that have alternatively activated** [44]. M1 macrophages increase the number of Th1 cells in the inflammatory region by secreting cytokines that induce a pro-inflammatory Th1 response. M1 also up-regulates antigen processing and presentation genes and also co-stimulatory molecules to boost T-cell reactions. These functions are necessary for antitumor immunity to work. In comparison, M2 macrophages have a significant role in normal immunological performance and homeostasis, including promoting Th2 responses and immunoregulation. Particular M2 macrophage subsets potentially have a key function in cancer development [45]. Whereas anti- or pro-inflammatory responses are necessary, therapy techniques aim to transition from M1 to M2 or the other way around. Thus, reducing anti-inflammatory macrophages while increasing pro-inflammatory (antitumor) macrophages is a major objective of macrophage-based cancer therapy [46]. However, Recently, in contrast to traditional M1 and M2 cancer polarization frameworks, researchers demonstrated that macrophage activation in some malignancies, such as NPC and PC, has an M1–M2 coupled pattern, wherein M1 and M2 are not separate states but rather coupled programs [47,48]. It is important to notice that new treatments such as Monoclonal Antibodies, cell-based immunotherapies, cancer vaccines, cytokines, and checkpoint therapies are not without adverse effects; novel immunotherapies that are personalized to the genetic profile of patients, have longer responses, and don't show any immune resistance. Even existing methods for non-responding patients, such as those targeting checkpoint molecules, tumor ligands, and gene-related treatments, are challenging to utilize. Immune resistance is most commonly caused by tumor heterogeneity [10].

Tumor structure is incredibly complicated, including various tumor cell types with an extensive range of genetic and epigenetic variations. This heterogeneity is a continual challenge to immunotherapies because subclones with acquired resistance mechanisms and self-renewing characteristics often survive therapy and stay quiescent until the selection pressure is gone. ScRNA-seq is a powerful method for deconvolution heterogeneous cell populations and decoding the gene expression profiles of individual cells that gain a deeper understanding of the tumor composition and microenvironment [49].

3. ScRNA-seq technologies

The bulk RNA-seq methods have been commonly applied for transcriptome studies, the patterns of splicing, and determining the level of genes and transcripts expression. despite its popularity, this approach does not enable researchers to investigate cell heterogeneity in transcriptome dynamics. scRNA-seq offered, investigating gene expression patterns at the single-cell level have, and the expression profiles of the vast majority of numerous issues have been deciphered [50]. It allows for a much greater resolution measurement of intra-population heterogeneity, possibly identifying dynamics in different cell populations and complex tissues. Because of these improvements, scRNA-seq can be employed in various medicinal purposes, such as cancer immunotherapy [51] (Fig. 1).

3.1. Tissue dissociation and single-cell suspension

The starting material in all single-cell procedures is a cell suspension consisting of fully dissociated, intact, and live cells. RNA integrity should be preserved entirely [52]. Different techniques can be employed to obtain an even cell suspension. Mechanical methods as mashing, dicing, or slicing might be employed to disseminate tissues. Enzymatic digestion is recruited to remove collagen and other structural components found in the extracellular matrix. This step is necessary when the cells in the tissue are embedded in a high densely packed matrix. The enzyme used for each tissue will be determined by the extracellular matrix composition, including molecules like fibronectin and various kinds of collagen [53]. Cold-active protease is a Recently developed protocol in which tissue dissociation is performed on ice and consequently eliminates extracellular matrix [54]. Experiments with intricate designs in which specimens cannot be handled quickly exert significant

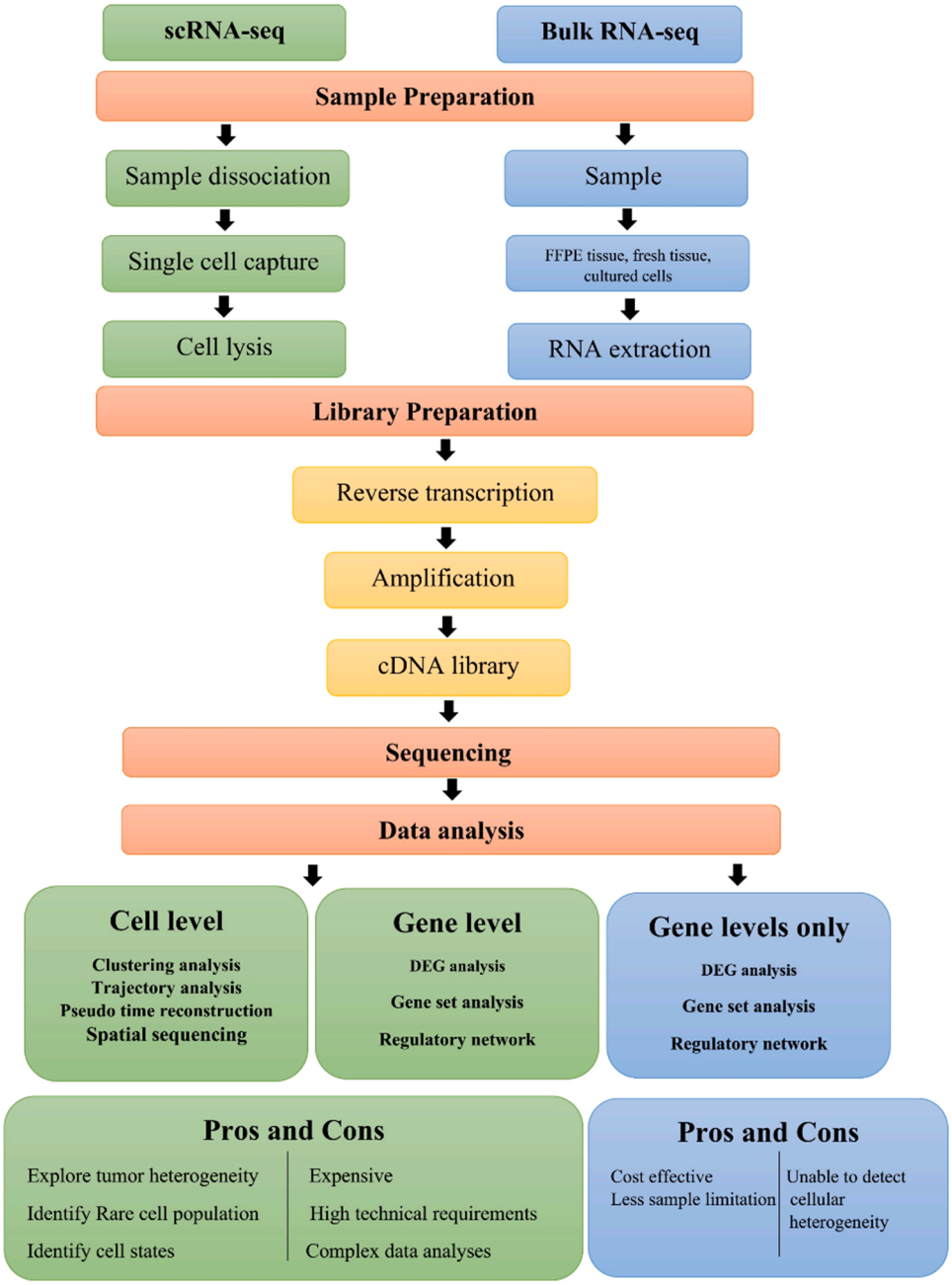

Fig. 1. Workflow of a typical scRNA-seq experiment vs. traditional bulk RNA sequencing.

constraints and obstacles. In this condition, samples must be maintained either as intact tissue or as a dissociated single-cell suspension. Some biases are created depending on the procedure for generating cell suspension that must be addressed when designing and processing data from a single-cell experiment; nevertheless, they are yet poorly understood [52]. When a suspension of cells is prepared, now it's time for single-cell isolating. Current cell separation approaches may be divided into two groups depending on the different concepts employed. The first group includes physical characteristics such as size, density, electric changes, and deformability.

The second group, which includes affinity techniques, is based on cellular biological features [55]. In this way, various isolation methods for separating the requested cells have been presented in recent years, including Manual Micromanipulation, Robotic Micromanipulation, Fluorescence-Activated Cell Sorting (FACS), Immuno-panning, Magnet-Activated Cell Sorting (MACS), Laser Microdissection, and Microfluidics [56] (Table 1). Their selection, however, is influenced by cell type, cost information, and single-cell production. Microfluidics technology is acknowledged as a significant facilitator for single-cell sorting among them [55]. Unlike FACS and MACS, which are dependent on the labeling strategies, microfluidics avoids this technique [57]. Recently several microfluidic systems for single-cell gene expression studies have been created. For single-cell transcriptomics, many droplet microfluidics methods, like Drop-Seq, Microwell-seq, and Indexing droplet RNA sequencing (inDrop), have been reported [58]. Although significant development has been achieved in single-cell isolation in recent years, we still require to design more effective techniques in this area to solve the enigma of cellular heterogeneity completely.

3.2. *ScRNA-seq platforms*

ScRNA-seq technology, which is quickly improving, provides

Table 1
Progress in single-cell capturing approaches.

Capturing method	Number of cells	Operation times	Equipment	Application
Micromanipulation (mouth pipetting)	Rare samples (~ 100)	Time-consuming	No	Tang-seq, Smart-seq/Smart-seq2
Laser capture microdissection	Rare samples (~ 100)	Time-consuming	Yes	Smart-seq/Smart-seq2
Flow cytometry	Hundreds of cells	Fast	Yes	Smart-seq/Smart-seq2, CEL-seq/CEL-seq2, MARS-seq, STRT-seq
Integrated microfluidic circuits	Hundreds of cells	Fast	Yes	Smart-seq/Smart-seq2, CEL-seq/CEL-seq2, STRT-seq
Microwell platform	Thousands of cells	Fast	No	Cyto-seq, Seq-well, Microwell-seq
Microdroplet platform	Thousands of cells	Fast	Yes	Drop-seq, inDrop
In-situ barcoding	Tens of thousands	Fast	No	SPLit-seq, Sci-RNA-seq

remarkable accuracy in designing single-cell sequencing platforms, particularly in the RNA sequencing field [59]. However, there are concerns about the difference between the accuracy and repeatability of platforms. The primary platforms have relied on low-throughput methods that took much longer to capture cells of interest and had problems with transfer effectiveness (the percentage of cells effectively moved from the medium to the collector); the cost per cell was quite high, considering whole-genome amplification (WGA) and whole transcriptome amplification (WTA), which are both expensive. The important benefit of these approaches was that they could select just relevant cells for investigation based on morphology or antibody staining, which could then be confirmed visually using a microscope. With the introduction of high-throughput methods, it is now feasible to produce a massive number of cells in a short time while also reducing the per-cell analysis cost. They are notably appropriate for the identification of rare cells. However, these methods generally need a greater number of cells, to begin with, do not permit for visual observation, and the ability to choose cells is restricted [59]. Some platforms, like the Chromium System (10 × Genomics), the Nadia or RNA-Seq System (Dolomite Bio), the InDrop System (1CellBio), the Single-Cell Sequencing Solution (Illumina, Bio-Rad), the Tapestri Platform (MissionBio), and the Rhapsody Single-Cell Analysis System/Resolve (BD), are based on high-throughput approaches. Fluidigm C1, Clontech iCell8 (previously Wafergen), and 10x Genomics Chromium are three of the most successful and prominent commercial scRNA-seq platforms. The sensitivity, specificity, throughput, and other analytical characteristics of these platforms are different [60]. For example, the integrated microfluidic chip (IFC) is used for storage in the Fluidigm C1 platform to collect, image, and do cell lysis, reverse transcription (RT), and first PCR reactions for single cells. IFCs are divided into two types: 96 and 800 HT, which could capture 96 and 800 cells, respectively.

Furthermore, each form of IFC makes it easier to capture cells with diameters ranging 5–25 μm [61]. Clontech's iCell8 technology is built on an alloy nanogrid wafer with 5184 nano wells. Oligonucleotides comprising poly d(T), unique molecular identifiers (UMIs), and unique good barcodes are preprinted within each nano-well. An automated system distributes cells from the main 384-well plate into each nano-well. According to the Poisson distribution, on average, ~1800 single cells can be collected optimally. The automated imaging system can detect and pick single living cells. The iCell8 system can handle cells with a diameter of up to 100 μm [61]. 10 × Genomics Chromium is the third platform, a droplet-based technology that can characterize and profile thousands to millions of cells [59]. It is the GemCode system for barcoding (Gel bead in Emulsion) that permits capturing input cells [62]. The gel beads are covered with oligonucleotides, including RT primers, UMIs, and cell barcodes, mixed with RT reagents and cells in an oil environment to form droplets in which cDNA is synthesized. The droplets are then collected, dissolved, and a UMI-containing cDNA library is created. With a very low doublet rate, the technique can achieve a cell capture efficiency of 65% [59]. The Chromium scRNA-seq chemistry has been provided in two versions (V1 and V2), with the final RNA-seq library configuration differing between the two versions [63]. This platform suggests processing cells with a diameter of less than 50 μm [64]. The three single-cell systems have different productivity, single-cell track-ability, and ultimate single-cell libraries due to their different designs, indicating that scRNA-seq technologies have not yet reached complete development [65]. So, efforts must be continuous to produce new instruments to overcome these barriers.

3.3. ScRNA-seq downstream data analysis

Thanks to the scRNA-seq, researchers can address new biological questions at the single-cell level; however, it represents a unique and distinct set of challenges. Due to the limited amount of material available per cell, observations are fraught with uncertainty. Amplification adds technical noise to the resulting data when it is used to generate more material. Furthermore, any increase in resolution leads to a rapid increase in dimension in data matrices. As a result, there is a significant need for analysis tools to better utilize these relatively small amounts of data. Normalization, batch effect correction, drop out and dimensionality reduction, and cell clustering and annotation are all part of the overall pipeline for scRNA-seq data analysis. Nonetheless, clarification for this aspect of the study is beyond the scope of this review, and readers should refer to related papers for more information (Table 2).

Here we briefly review the most recent scRNA-seq analysis method, including cell-cell communications, lineage reconstruction, and prediction of intercellular communication.

Table 2
Packages for single-cell analysis.

Step	Name	Ref.
Normalization	DCA	[66]
	SAUCIE	[67]
	Auto Impute	[68]
	Deep Impute	[69]
	DeepMc	[70]
	sc Scope	[71]
	scVI	[72]
Data correction	ResNets	[73]
	MNNs	[74]
	Scanorama	[75]
	BBKNN	[76]
	DESC	[77]
	Batch-Free Encoding	[78]
	BERMUDA	[79]
Drop out	DCA	[66]
	scScope	[71]
	TRANSLATE	[80]
Dimensionality reduction	scvis	[81]
	VASC	[82]
	scVI	[72]
	BasisVAE	[83]
	GOAE and GONN	[84]
	SAUCIE	[67]
Clustering and cell annotation	DESC	[77]
	scAnCluster	[85]
	scVAE.	[86]
	scDeepCluster.	[87]
	GOAE and GONN	[84]

3.3.1. Cell-cell communication

Cell-cell communication (CCC) is a basic method for studying cell interactions that involve the discovery and quantification of intercellular signaling pathways. The signaling pathways that lead to CCC are mainly regulated through protein interactions, including ligand-receptor, receptor–receptor, and extracellular matrix–receptor interactions. Receiver cells activate downstream signaling via cognate receptors, resulting in changes in transcription factor activity and gene expression in most cases. Then these cells with changed expression interact with their microenvironment. For understanding the 'landscape' of biological systems, we need to decipher the CCCs at every stage of development and in any multicellular community by using gene expression data. Previously, CCC could only be examined via in vitro studies using one or two cell types and a few genes. But CCC affects a wide range of cell types and activates a large number of genes, and these studies remain incomplete [88,89]. Fortunately, with the emergence of scRNA-seq technology, the analysis of single-cell transcriptomics has begun to transition from " just " focusing on what cells are present to also focusing on what interactions between cells are present. Now researchers can quantitatively infer and investigate intercellular communication networks from scRNA-seq data and predict main signaling inputs and outputs for cells as well as how those cells and signals coordinate for functions through applying network analysis and pattern recognition methods [90]. The number of techniques has rapidly expanded in recent years, but additional unique approaches are needed to move this fast-expanding field ahead in important ways.

3.3.2. Lineage reconstruction

Single-cell lineage reconstruction effectively identifies the cellular basis of development, regeneration, and disease. It means that this ability of scRNA-seq provides critical information on the fates of single cells to researchers. Tracing cell lineages has previously been accomplished via low-throughput and invasive procedures like tagging cells by dyes or radioactive tracers or inserting genetic elements. Recent developments in scRNA-seq have made it possible to track genome-wide omics data from thousands of individual cells and predict the direction of these cells along developmental paths computationally. In this way, Over 70 trajectory inference tools have already been created to analyze extremely vast and intricate single-cell datasets and rebuild cell-state transitional trajectories. Most of the time, the resulting trajectories are linear, bifurcating, or tree-shaped, but more modern approaches can also detect more intricate topologies, such as cyclic or disconnected networks. Despite these advances, we still have a long way to go in comprehending cellular differentiation as well as cell fate decisions. Hence there is a great need to introduce more integrative approaches to address long-standing concerns about cell fate decisions and lineage specification [91,92].

3.3.3. Intercellular communication

scRNA-seq is a promising tool for investigating intercellular communication within tissues. Extracellular signals created by cells in their microenvironment could affect cells and are crucial for organizing a broad range of biological processes, such as development, differentiation, and inflammation. Profiling gene expression in interacting cells is one way to investigate these intricate communications. Many recent findings have shown that single-cell transcriptomics can be used to examine intercellular communication and assess the physical cell-cell interaction network. To carry out these analyzes, several methods have been developed. They all start with a database of interacting molecular partners (such as ligand and receptor pairs) then use their expression patterns to anticipate a list of possible signaling pathways across cell types. These research findings may be beneficial in understanding the mechanics of different tissues made up of a range of cell types [93,94].

4. ScRNA-seq applications in cancer immunotherapy

During the last decade, precision medicine and immunotherapeutic methods have grown increasingly prominent in oncology. The development of scRNA-seq has recently improved the potency to explore the immune system and broken the immunological constraint. The ability to divide subpopulations in the ecosystem defines developing hierarchies, finds new drivers, rate possible therapeutic responses, and identify advances in scRNA-seq technology that have provided infiltrating-immune surveillance/evasion relevant to cancer. Although early clinical studies yielded encouraging findings, response rates in phase III trials have not been very optimal. Following translational studies have revealed the efficacy of targeting the TME in the fight against immunotherapy resistance. It is critical to address both inter-and intra-tumoral heterogeneity in this era of precision medicine. Single-cell analysis is a cutting-edge technique for better defining the tumor cell population and identifying new immunotherapy or combination treatment targets. Modern cancer therapy is shifting from a "one drug fits all" strategy toward a personalized approach based on biomarkers, mutational studies, and examination of the TME, especially immune cell type. Tumors are composed of complicated admixtures of diverse cell populations that interact with non-malignant cells in the surrounding area.

Regarding their approval for melanoma and non-small-cell lung cancer (NSCLC), there is increasing attention in utilizing ICIs to treat additional solid tumors like HCC. But, only a small number of people benefit from immunotherapy, and scientists don't know why the majority of them are resistant. ScRNA-seq can give a more specialized look at the immunological component in the tumor, allowing researchers to examine the precise molecular mechanisms needed to overcome the failure of therapy or find novel therapies. ScRNA-seq has also discovered novel critical components and cellular subpopulations that either drive tumor development or make malignancies immune-resistant [47,95].

4.1. Determining the dominant drivers of cancer immunity

The heterogeneity of cancer cells, their immunogenicity, and their interaction with their environment and immune system are at the center of the difficulties in developing a long-lasting therapy. Cytotoxic cells are the primary drivers of the immune response to the tumor. Cytotoxic T cell infiltration or elevated PD-L1 expression by tumor cells are both signs of inflamed tumors. Chemoimmunotherapy (CIT) response is linked to inflamed or "hot" tumors. TGF-β signaling in the stroma, myeloid inflammation, and angiogenesis are all hallmarks of immune-excluded tumors, which also respond weakly to CIT. However, there is some indication that using anti-TGF-β and anti-PD-L1 together can make these tumors "hot" and sensitive. Immune desert tumors have a high rate of tumor cell growth, are metabolically active, and are stressful, making them good candidates for pathway-specific treatments that block the metabolic pathways that these cancer cells rely on. Tumor antigenicity is another driver of cancer immunity: antigenicity for tumor cells is created by genomic instability characterized by MSI or high tumor mutational burden (TMB), and biomarkers such as IFNg signatures and B cells reflect the presence of antitumor immunity. CIT is generally effective against inflamed tumors with a high antigenicity [96]. Signaling and epigenetic components are other important drivers of immune activities, and they are probably to be druggable, making them potential therapeutic targets. Most of these drivers are difficult to investigate, so they are named 'hidden drivers' because they are changed by post-translational changes (PTMs; e.g., phosphorylation) or other processes rather than being genetically altered or differently expressed at the mRNA or protein level.

Furthermore, both intracellular gene networks and cross-talk among various kinds of immune cells in particular tissues and their microenvironment are involved in immune responses, and their dysfunction leads to diseases such as malignancy and inflammatory conditions. Overall, molecular and cellular networks in cancer immunity and their

drivers and 'hidden' drivers are difficult to identify using traditional techniques. They must be analyzed using higher-resolution techniques like scRNA-seq to create effective and curative cancer immunotherapies [97]. With the help of scATAC-seq, today, researchers can study epigenetics or post-translational changes to profile the chromatin accessibility environment at a single cell level and highlight cell-to-cell diversity in gene regulation. scATAC works by inserting sequencing primers into open chromatin areas with the help of a transposase. At single-cell resolution, this technique, like classical ATAC-Seq, generates profiles of open and accessible chromatin regions indicative of active regulatory areas. In a study by Zhang et al., they searched for a potential cell of origin for renal cell carcinoma (RCC) subtypes through scRNA seq. Finally, they discovered that tumor epithelial cells in clear cell renal cell carcinoma (ccRCC) are the reasons which actively promote immune infiltration; eventually, they emphasized the TME's critical involvement in ccRCC biology and treatment response [98]. Zhao et al. performed a study on the nasopharyngeal carcinoma from three NPC cancerous tissue using scRNA-seq. They proved that in NPC tumors, immune cells have a diverse makeup and the various active states of T cells. Their findings shed light on the processes by which immune cells in the microenvironment remove tumors, which would promote the development of targeted and immunological treatments for NPC [99].

In another study by scRNA-seq, Chung et al. categorized non-tumor cells into three types of immune cells: T cells, B cells, and macrophages, each having activating and suppressive gene expression patterns. They discovered numerous T cells with significant cytokine and chemokine expression in immune cell infiltrates predominantly collected from triple-negative breast cancer (TNBC) tumors, indicating continuing immunological responses [100]. Altogether, these findings show the potential effect of intra-tumoral heterogeneity and indicate that single-cell transcriptome profiling might uncover and define clinically relevant subpopulations that may be the drivers of cancer immunity, making them excellent options for targeted therapies.

4.2. Pathways targeted through immunotherapy

In diverse kinds of cancer, novel therapies based on the usage of ICIs have shown to be highly effective. Unfortunately, most patients do not respond or have long-term responses, and the reasons for this remain unknown. Over the last two decades, an urgent need to fully characterize either the TME or the cells that control the immune response has motivated researchers to combine data from conventional techniques with data from newer approaches, such as single-cell methods [101]. The advent and application of scRNA seq helped researchers understand the cells and pathways better than immunotherapy drugs target, resulting in a higher success rate in clinical trials. A significant step forward can be accomplished if immunotherapy targets cells and pathways are identified at high resolution before and after treatment in clinical cohorts and model systems [102].

In this way, Kim et al. employed scRNA seq of $CD45^+$ TILs from untreated controls, AB680-treated, and PD-1-blockade-treated murine CRC in vivo models to better realize the mechanism of AB680 compared with that of a neutralizing antibody against murine PD-1 using it as a PD-1 blocker. They discovered that the expressions of Nt5e (a CD73 gene) and Entpd1 (a CD39 gene) influence TCR diversity and transcriptional profiles of T cells, indicating that these genes are important in T cell exhaustion within tumors. The TCR heterogeneity of Entpd1-negative T cells and Pdcd1-positive T cells was dramatically enhanced by PD-1 inhibition. AB680 also enhanced the anti-cancer activities of immunosuppressed cells like Treg and exhausted T cells, whereas the PD-1 blocker decreased Malat1high Treg and M2 macrophages quantitatively [103].

Yu et al., by using scRNA-seq of mouse bone marrow progenitors, discovered innate lymphoid cells (ILC) precursor subsets, defined particular ILC progress steps and pathways, and reported that significant expression of programmed death 1 (PD-1hi) characterized a committed ILC progenitor which was similar to an innate lymphoid cell progenitor. They established PD-1hiIL-25Rhi as an early checkpoint in ILC2 formation that was disrupted by a Bcl11b defect but restored by IL-25R overexpression. PD-1 was up-regulated on activated ILCs in the same way it was on T cells. In a mice influenza infection model, a PD-1 antibody decreased PD-1hi ILCs and lowered cytokine levels, as well as blocking papain-induced acute lung inflammation. Their findings suggest that PD-1 and its ligand (PD-L1) might be used in immunotherapy to effectively manipulate the immune system for disease control and treatment [104].

Based on scRNA-seq research, these findings will open up more significant and novel therapeutic options for immunotherapy drug discovery pipelines in the future, allowing for the quick development of successful immunotherapies and data-driven design.

4.3. Molecular mechanisms of immunotherapy resistance

As mentioned, immunotherapy has brought a new era in cancer treatment, yet despite these promising outcomes, most of the patients who are treated with innovative immunotherapies don't react or relapse. According to recent research, only 10% of patients may respond to ICB treatment, and 30–60% of patients may relapse. So, the development of resistant cancer cells is a significant obstacle to full cures. Secondary mutations can induce resistance; however, there are cases in which there is no apparent genetic reason, increasing the potential of non-genetic rare cell heterogeneity [105,106]. Studying the activity of cancer cells and the TME before, during, and after immunotherapy will be critical in optimizing these new treatments to overcome resistance. ScRNA-seq is developing rapidly as a helpful tool for dissecting diverse cell populations, a complex network of proliferating malignant cells, immunological infiltrates, and tumor stroma [107]. In 2021 in a study by Sehgal et al., they used scRNA-seq to discover a distinct subpopulation of immunotherapy persister cells (IPCs) that resisted $CD8^+$ T cell-mediated death in murine organotypic tumor spheroids following PD-1 inhibition. In mice, combining PD-1 inhibition with Birc2/3 antagonism decreased IPCs and increased cancer cell death in vivo, resulting in a long-lasting response that matched TNF cytotoxicity thresholds in vitro. These results indicate how high-resolution functional ex vivo analysis can reveal underlying mechanisms of immune escape from long-lasting anti-PD-1 responses while also recognizing IPCs as a tumor cell subpopulation that can be targeted by particular treatment combinations [108].

In that year, Jiang et al. used scRNA-seq data from patients before and after anti-PD-1 therapy to evaluate resistance to anti-PD-1. They discovered a cluster of T cells with a unique ligand/receptor expression pattern utilizing ligand/receptor gene analysis of tumor-specific exhausted CD8 T cells. After anti-PD-1 treatment, these cells expressed more survival- and tissue-residence-related genes, such as heat shock protein genes and the interleukin-7 receptor (IL-7R), CACYBP, and IFITM3 genes. These findings shed light on the processes behind anti-PD-1 treatment response and provide a wealth of information for future immunotherapy methods [109]. Before and after treatment with tipifarnib, Lee et al. used scRNA-seq to evaluate the matching patient-derived xenograft (PDX). Tipifarnib had a lot of anti-cancer actions, but it couldn't get a complete response. The characteristics of tipifarnib-refractory cancer cells and the tumor-supporting microenvironment were identified by a comparative scRNA-seq study of pre-and post-tipifarnib-treated PDX. A PD-L1 inhibitor, atezolizumab, was clinically used dependent on upregulation of programmed death-ligand 1 (PD-L1) in surviving cancer cells and the formation of multiple immune-suppressive subsets from post-tipifarnib-treated PDX; This resulted in a positive reaction from the patient who had developed tipifarnib resistance [110].

Kieffer et al. discovered eight tumor-associated fibroblasts (FAP^+/CAF-S1) clusters that promote immunosuppression in breast tumors using scRNA-seq. These myofibroblasts are a sign of initial

immunotherapy resistance, although their diversity and influence on immunotherapy response are unclear. ECM-myCAF and TGF-myCAF, two of the five most prevalent clusters, are mainly linked with an immunosuppressive surrounding, as their frequency corresponds with that of PD-1$^+$ and/or CTLA4$^+$ CD4$^+$ T cells. These findings indicate an intriguing reciprocal cross-talk between particular ecm-myCAF and TGF-myCAF with CD4$^+$ CD25$^+$ T cells, which might increase immunosuppression and be implicated in immunotherapy resistance [111]. Ma et al. found that IFN-γ signaling pathway genes are highly heterogeneous expressed and coregulated with other genes in single tumor cells, included MHC II genes, using primary lung adenocarcinoma cells and cell lines. An acquired resistance phenotype is associated with the downregulation of genes in IFN-γ signaling pathways in cell lines. They recommended using multiantigen combination treatments for inhibiting cancer escape and set the foundation for future prognostic measures depending on intratumor heterogeneity [112].

Wang et al. published a study in 2019 on mice with breast cancers that developed resistance to a combination treatment that included the CDK4/6 inhibitor Palbociclib. Surprisingly, scRNA-seq showed significant numbers of immunosuppressive immature myeloid cells in CDK4/6-resistant cancers, which up-regulated Kit and Met's oncogenic drivers. In mice, utilizing cabozantinib in conjunction with ICB to target Kit and Met resulted in substantial breast tumor reduction and considerably increased survival time [113]. Jerby-Arnon et al. by using clinical scRNA-seq data and different patient cohorts, mapped tumor cell states related to ICI resistance indicating a cohesive program that might be therapeutically targeted. They showed that a CDK4/6 inhibitor reverses the resistant cell condition, activates components of SASP (Senescence-Associated Secretory Phenotype: suppressed component in the resistance pathway), and enhances ICI, and improves responses to ICI in vivo. According to a recent finding, CDK4/6 inhibitors given in a phased fashion might possibly relieve ICI resistance in certain melanoma patients. In general, the program's in vitro suppression may be used to search for other compounds that make melanoma cancers more sensitive to ICI [114].

Ho et al., by studying human melanoma cells and looking for drug-resistant cellular populations that react to targeted BRAF inhibitors (BRAFi), discovered both new and recognized resistance markers. They found that BRAFi resistance markers overlap with previous investigations in various melanoma cell lines, and they also confirmed one new resistance marker gene, DCT, which has failed to recognize by bulk RNA-seq studies due to its high expression in the majority of BRAFi-resistant cells and permit expression in 99% of the parental population. High AXL, JUN, and NRG1 expression were also linked to a rare cancer cell population that was in a "pre-resistant" state [115]. Such findings have proven the practical guidance provided by scRNA-seq in the creation of novel immunotherapeutic regimens and would provide a wealth of insights for future immunotherapy methods, as well as allow strategic choices to avoid treatment failure for many malignancies.

4.4. Finding novel immunotherapy targets

Inhibition of the PD1/PDL1 pathway has resulted in significant clinical success for immunotherapy in some individuals, while it has little or no effect in others. To improve immunotherapy efficacy, it is important to screen out new targets in order to produce successful therapies or for use in combination treatments, but this requires a greater understanding of tumors and their microenvironments. Intratumoral and TME heterogeneity has recently been discovered through scRNA-seq analyses of human tumors, which is important for deeply studying tumor-related processes and revealing the critical elements engaged in the vulnerability of tumor-induced immunological changes, which can be used to advance novel immunotherapy techniques. As a result, it's critical to look into new targets in order to produce successful therapies or to employ them in joint treatments.

Deng et al. identified seven main CD8$^+$ T cell subpopulations in melanoma TME, each of which, and the decrease or increase of the proportion between them perform distinct roles in prognosis, progression of the disease, and response to immunotherapy. They discovered that three overexpressed genes, *PMEL*, *TYRP1*, and *EDNRB*, which are just expressed in exhausted CD8$^+$ T cell subpopulation 2, are noticeably linked to poor prognoses and possess the highest expression in melanoma in comparison to other cancers. Still, these three genes, along with immunosuppressive checkpoint genes, can be used as potential targets for melanoma treatment [116] (Fig. 2).

About Papillary thyroid carcinoma (PTC), Wang et al. discovered that inhibitory checkpoints TIGIT and CD96 are more suitable targets for immune treatment than PD-1 in PTC patients with LN metastases. PD-1, TIGIT, and CD96, on the other hand, might be effective immunotherapy targets for PTC patients without Lymph node metastases [117].

Pancreatic ductal adenocarcinoma (PDAC) has imperfect management for immunotherapy, in this way, Wang et al. found a new subtype of cancer-associated fibroblasts (CAFs) with a highly activated metabolic state (meCAFs) in loose-type PDAC, especially in comparison to dense-type PDAC, which is essential for PDAC progression, and discovered that patients with abundant meCAFs had an increased risk of metastasis and decreased prognosis, but interestingly, had a significantly stronger answer to PD-1 blockade treatment [118].

Li et al. identified sialic acid-binding Ig-like lectins (Siglecs) as new immunotherapy targets, with the ability to improve the efficacy of current ICIs in glioma immunotherapy. Because they displayed distinct expression patterns in gliomas and performed various roles in the immunosuppression process, interacting with various immune checkpoints, a combination of Siglec inhibitors and immune checkpoint inhibitors has a high capability to solve the current immunotherapy issue in glioma [119].

5. Single-cell TCR sequencing

T-cell receptors are heterodimers made up of two chains, TCRα and TCRβ, that cause peptides to be recognized in the context of MHC molecules. A variable region and a constant region make up each of the two chains. There are two constant region gene segments in the TCRβ chain, Cb1, and Cb2, which share certain sequences. Ca is the only constant region gene segment in the TCRα chain. The variable region of the b chain is made up of three gene segments: variable (V), diversity (D), and junctional (J); however, only the V and J sections make up a chain. There are three hypervariable areas, or complementarity-determining regions, within each V segment (CDR1, CDR2, and CDR3). The V segment encodes CDR1 and CDR2, whereas the CDR3 regions originate from the juxtaposition of the V, (D), and J segments. TCR's excellent structure enables a lot of variation, which is enhanced even more by the α and β chains' heterodimeric coupling. The whole number of potential combinations is believed to be more than 10^{18}. TCRs' high diversity is required for their specific capacity to identify antigenic targets, such as infections or tumor cells. TCR must contact the MHC molecule on the cell surface via antigen identification, which is done primarily through particular interactions with CDR1 and CDR2. The TCR interacts with the peptide given by the MHC molecule in a variety of ways, the most common of which is through a particular contact with the CDR3. T-cells also play a role in immune response and cancer treatment. Because the discovery of antigen-specific TCRs, as well as cancer antigens, is a prominent investigate subject. Despite the existence of tumor-specific T cells in cancer patients' TILs and peripheral blood, even after checkpoint immunotherapy, the existence of these cells is typically insufficient to cause cancer regression. In order to answer such concerns, a greater understanding of the biology of TCRs in cancer immunotherapy is required. Recent improvements in scRNA-seq methods have opened up new avenues for TCR research, allowing scientists to find novel biology in T-cell receptors at the single-cell level. This powerful tool couldn't just confirm earlier results made using traditional methods, but it can also open the door for novel discoveries,

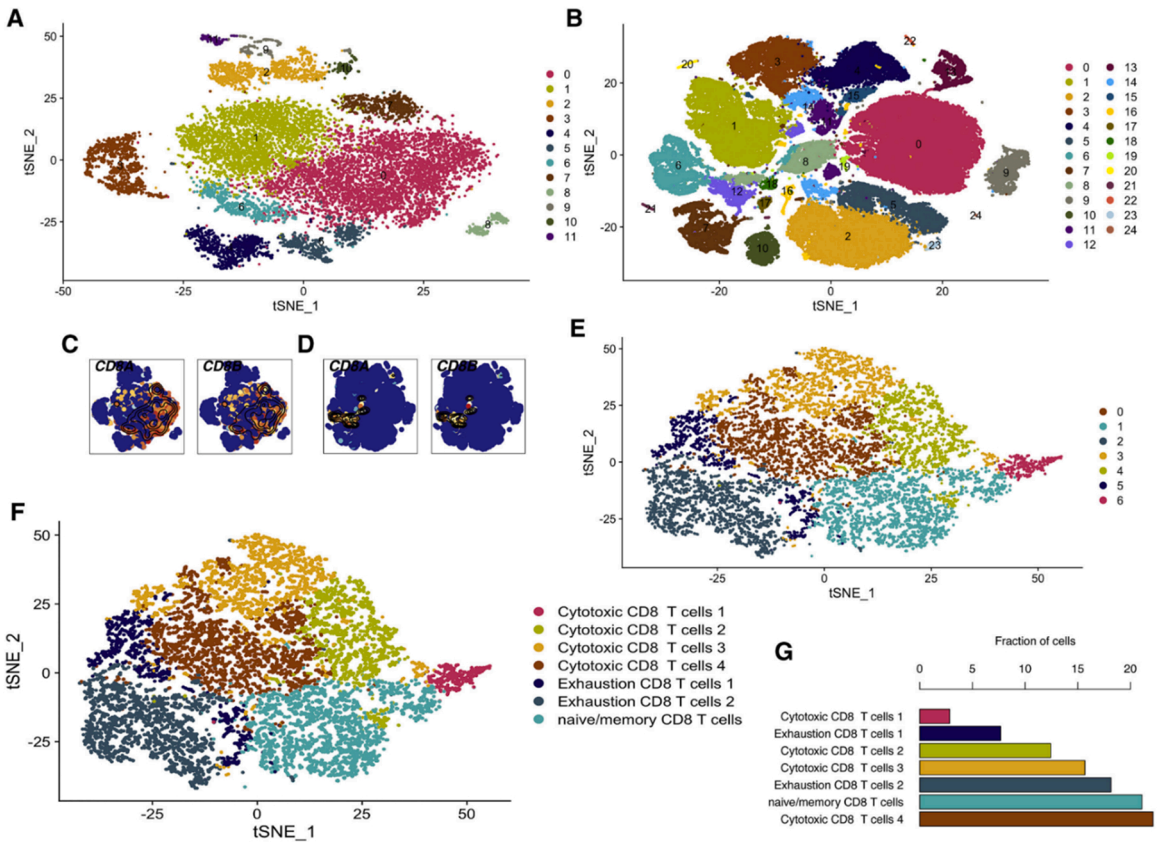

Fig. 2. CD8$^+$ T cell subpopulations in melanoma TME. Seven primary CD8$^+$ cells, including four cytotoxic subpopulations, two exhausted subpopulations, and one naive/memory subpopulation, were identified based on the Single-cell RNA-sequencing analyses [116].

like previously unknown T-cell subpopulations that may be involved in clinical consequences in patients [120].

Lu et al. used scRNAseq to examine T cell-mediated responses following immunotherapy using neoantigen-specific TCR obtained from three melanoma and three colorectal tumor tissues. They initially extracted tumor-infiltrating T cells from a tumor sample, then used neoantigen-loaded DCs to activate T cells, followed by sc-seq for TCR and T-cell activation markers, IFN-γ, and IL-2. Finally, they discovered that IFN-γ and IL-2 expression levels are two indicators for accurately identifying neoantigen-specific TCRs, and they discovered a total of 28 neoantigen-specific TCRs [121].

Wang et al. give important insights on exhausted T cell subsets in leukemia by analyzing functional T cell clusters, which may be used to design immunotherapy methods and predict clinical outcomes in leukemia. They used scRNA-seq to discover 13 T cell clusters in patients with B cell acute lymphoblastic leukemia (B-ALL) depending on the molecular characteristics in T cells separated from the peripheral blood of healthy people and patients with B-ALL. Patients with B-ALL have all 11 major T cell subsets present in healthy people, with the patients' counterparts displaying higher activated features across the board. In B-ALL patients, two exhausted T cell populations with ten sub-clusters have been identified [122].

Zheng et al. studied the full TCR sequences and transcriptomes of $>$ 5000 single T cells derived from HCC patients using scRNA-seq techniques. They discovered 11 different T cell subsets, each with its specific tissue distribution pattern. These subgroups' connection and probable developmental route were found using a combination of expression and TCR-based studies. They looked at signature genes for exhausted CD8$^+$ T cells and tumor-specific Tregs, such as LAYN. They discovered that overexpressing LAYN in primary CD8$^+$ T cells inhibited interferon (IFN) production implying that LAYN has a regulatory function. Their large-scale transcriptome data of T cells can be utilized to learn more about TILs' fundamental features and perhaps guide effective immunotherapy techniques [123].

Zhao et al. do scRNA-seq on tumor cells coupled with TCR scRNA-seq from immune cells, recognize the diverse T cell clonotypes and expansion distribution in individual cancers, and show different types of immune cells in the microenvironments of NPC. Their study of TCR variety revealed the asynchronous characteristics of polyclonal T cell activation or heterogeneity between different patients. They discovered that EBV-positive tumor samples had more T cell clonal expansion, suggesting that sequence analysis of these TCRs could be linked to EBV-specific antigens. Their research will aid in advancing immunotherapies for NPC, like adoptive T cell therapy [99].

ScRNA-seq is a helpful method for TCR sequencing in individual cells, which is essential for detecting viral antigens or cancer-specific neoantigens presented by the MHC. TCR-seq study has become an essential means for understanding T cell biology in healthy people and people with a variety of pathological conditions, and it is being used not just to investigate the biology of immunological-mediated diseases but also to track immune responses to treatments. Recently, there has been an increase in interest in T cells' function in TME and cancer immunotherapy, making it a hot issue for research (Fig. 3).

6. Challenges and future perspective

ScRNA-seq is a powerful new technique that allows for individual cell transcriptome research. It's a potent tool for dealing with the intrinsic complexity of malignancies and the tumor environment, paving the path for personalized treatment. However, as a newly advanced method, scRNA-seq still has limitations, such as preserving cell integrity and survival, critical for subsequent single-cell analyses. This means that single cells must be isolated from each other rapidly and correctly with minimal cell damage during the single-cell isolation procedure to

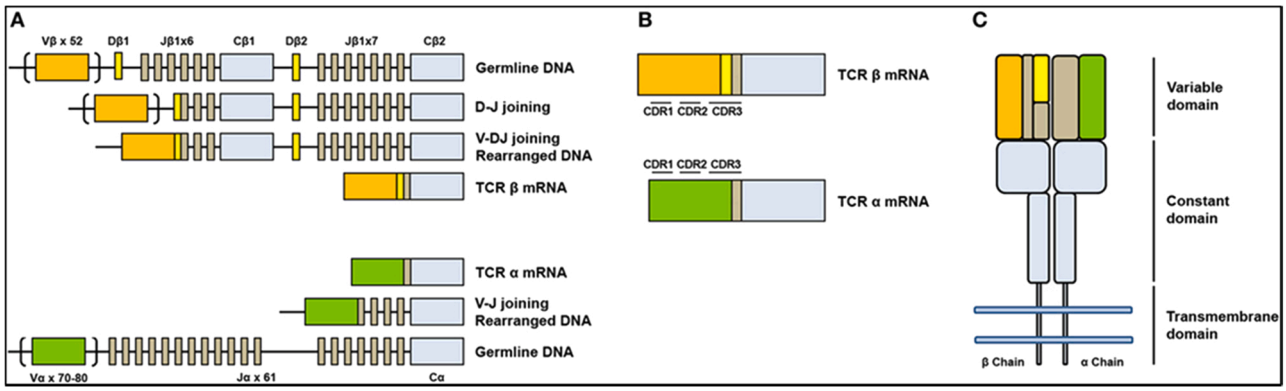

Fig. 3. Somatic V(D)J arrangement in the alpha and beta chains.

achieve the highest cell viability. Thus, there is a necessity to progress methods to empower the efficient "gentle" extraction and capture of living cells and avoid the possible damage to single cells. Another restriction is the considerably high cost of single-cell sequencing; nevertheless, modern technologies have reduced the cost of sequencing each cell to an acceptable level; yet, the total price is too high because tens of thousands of cells should be examined in some instances. Decreasing sequencing costs would make scRNA-seq more widely used in cancer research possible. ScRNA-seq also requires modern data processing techniques to exploit and quantify biological variation properly. Dropout difficulties in single-cell datasets, for example, provide serious challenges for downstream analysis such as data normalization, dimensional reduction, and clustering. It would be beneficial to develop techniques specifically for sparse single-cell data [124].

All of these restrictions, however, are expected to be overcome by fast scientific and technical progress. The use of scRNA-seq in cancer biology investigations might help us learn more about tumor heterogeneity, get insight into molecular processes of tumor development and metastasis, and identify new immunotherapy medicines for more successful personalized cancer care because tumor heterogeneity and the complexity of its ecosystem are significant biological challenges in the way of treatment. Considering tumor cell populations are very diverse both between tumors and within a tumor, identifying tumor subpopulations and uncommon cell types, such as cancer stem cells, can sometimes be challenging. Tumor plasticity and the continuous process of tumor development, in addition to heterogeneity, provide significant obstacles to establishing a tumor ecosystem. Other cells in the TME, such as T cells, are just as complex as tumor cells. While $CD4^+$ or $CD8^+$ T cell lineage commitment is permanent, some lineage commitments may be more adaptable than previously thought.

Nonetheless, instead of focusing on tumor heterogeneity and adaptability, the mystery of tumor biology may be addressed by looking for the key to creating such a complex environment. Recent single-cell research described here is beginning to show a network of cellular interactions in the cancer-immune landscape, which might help us better understand resistance mechanisms and create better combination treatments. In an ideal world, the single-cell analysis would be utilized to develop comprehensive cellular atlases for each patient, but this is still a concept for the future.

Despite the significant advancements in scRNA-seq fields, one of the most challenging aspects of this method is that it recognizes cell subpopulations within tissue but does not capture their spatial distribution or demonstrate local networks of intercellular communication acting in situ, so spatial information in the tissue context is lost. Multiplexed in situ hybridization, in situ sequencing, and spatial barcoding are just a few recently developed techniques for localizing RNA within the tissue that can aid with this problem [94]. The gene expression of individual cells at their original site can be distinguished using spatial transcriptome sequencing technologies. The technique is critical for determining tissue function, following developmental processes, and detecting clinical and molecular alterations. Because different approaches have different encoding efficiency and application contexts, the key to spatial transcriptomics is encoding the position information [125,126].

7. Conclusions

Up to date, immunotherapy therapies have been beneficial in treating a variety of malignancies, including melanoma, lung cancer, gastrointestinal tumors, non-nasopharyngeal squamous cell carcinoma of the head and neck, and urologic cancer. However, the underlying processes remain unknown due to tumor heterogeneity and TME, which limited the bulk investigations. Deep scRNA-seq is required to shed light on this intricacy and better comprehend the immune landscape in malignancies. ScRNA-seq provided enlightening insights into the analysis of tumor heterogeneity and enabled a more comprehensive understanding of tumor formation, progression, and resistance, all of which are critical for designing targeted therapy and achieving personalized management. The widespread adoption of scRNA-seq might result in a dramatic shift in our knowledge of cancer, paving the way for developing more effective treatment approaches to prevent cancer recurrence and increase survival rates.

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N.E and **A.D** provided the initial version of the paper. **S.N**, **M.F**, and **B.B** helped in data categorization and critically reviewed the manuscript. **N.J.T**, **O.B** and **R.B** revised the paper and left some comments. **H. S.** and **N.S.**, the corresponding authors of the manuscript, supervised the project and revised the manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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