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Review Article

Innovative aspects and applications of single cell technology for different diseases

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Abstract: Recent developments in single-cell technologies have provided valuable insights from cancer genomics to complex microbial communities. Single-cell technologies including the RNA-seq, next-generation sequencing (NGS), epigenomics, genomics, and transcriptomics can be used to uncover the single cell nature and molecular characterization of individual cells. These technologies also reveal the cellular transition states, evolutionary relationships between genes, the complex structure of single-cell populations, cell-to-cell interaction leading to biological discoveries and more reliable than traditional bulk technologies. These technologies are becoming the first choice for the early detection of inflammatory biomarkers affecting the proliferation and progression of tumor cells in the tumor microenvironment and improving the clinical efficacy of patients undergoing immunotherapy. These technologies also hold a central position in the detection of checkpoint inhibitors and thus determining the signaling pathways evoked by tumor invasion. This review addressed the emerging approaches of single cell-based technologies in cancer immunotherapies and different human diseases at cellular and molecular levels and the emerging role of sequencing technologies leading to drug discovery. Advancements in these technologies paved for discovering novel diagnostic markers for better understanding the pathological and biochemical mechanisms also for controlling the rate of different diseases.

Keywords: NGS, RNA-seq, tumor heterogeneity, cancer immunotherapy, drug discovery

Introduction

Bulk technologies are not reliable due to low expression of unique cells, tumor heterogeneity, and poor accessibility in presenting the molecular nature of single-cell populations [1]. Single-cell analysis has opened an opportunity in the development of cancer research and infectious diseases by detection of single-cell composition and characterization of different tumors [2]. Recent advances in the fields single-cell technologies including the RNA-seq, next-generation sequencing (NGS), genomics, and transcriptomics have resolved the issues of single cell based detection of the nature of different tissues in the tumor microenvironment and hold a central position in the detection of checkpoint inhibitors and thus determining the signaling pathways evoked by tumor invasion. These single cell approaches are helpful in measuring the nature of clinical samples for cell transition states and enhance the therapeutic efficacy of drugs against treating of blood and solid malignancies [3].

Single-cell technologies are helpful in revealing the three-dimensional molecular profile of individual cells, gene expression, cellular state

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transitions, distinct cell populations and associated molecular pathways [4]. Emerging single-cell technologies are cytometry by time-of-flight (CyTOF), split-pool ligation-based transcriptome sequencing (SPLiT-seq), RNA sequencing (RNA-seq), and topographic single-cell sequencing (TSCS). RNA-seq is used for exploring the transcriptional profiling of the single-cell populations and cancerous cell-type markers; CyTOF is applied for the investigation of proteomics and spatial cell profiling; SPLiT-seq is used for gene expression and transcriptome sequencing; TSCS is applied for revealing the spatial information about single cells and SCI-seq is applied for accurate construction of thousands of single-cell libraries. The single cell-based detection through these technologies also requires computational techniques for determining the checkpoint inhibitors acting in different cellular and molecular pathways [5].

Single-cell technologies are utilized for the treatment of different diseases [6]. RNA-seq is applied in understanding the maturity and functioning of human gut epithelial cells during fetal development [7]. SmFISH is helpful for early detection of Myh7 in the cardiac tissue as it detects the mRNA expression. This approach leads to understanding the mechanism of cardiac dysfunction in oxidative stress conditions [8]. Single-cell RNA-Seq database (ScREAD) is the first novel database and helpful in understating the pathogenesis of Alzheimer's disease by collecting the existing genes information from data sets of RNA-Seq of the neuroganglion tissues of human and mouse models [2]. Next-generation technologies are becoming the first choice for early detection and treatment of tuberculosis. Next generation sequencing techniques are used to identify the mutated genes in pancreatic cancer such as SF3B1, MLL3, TP53, KRAS, SMAD4 and SLIT2, ROBO2. These genes are highly associated with the pathogenesis of pancreatic cancer [9]. Different strategies need to be developed to enable cell-to-cell interaction and provide therapeutic targets for cancer immunotherapy.

RNA-sequencing technologies are widely applied for the detection of drug-induced gene expression and are thus valuable in drug discovery [10]. Nicotinamide (NAM) is a potential biomarker in drug development through RNA-seq technology and involved in age macular degeneration (AMD) through inhibition of the chromatin-modifying genes and complement factors [8]. Celastrol is a biomarker in cholestatic liver injury and involved in the activation of cellular pathways including Sirtuin 1 (SIRT1) & Farnesoid X receptor (FXR) and inactivation of nuclear factor-kappa B (NF-κB) and P53 [11]. The gene expression of polyphenon E in the human lung cancer cell line, H1299 cells through RNA-seq technology. Sequencing analysis revealed that polyphenon E inhibited the expression of activator protein 1 (AP-1) and potential biomarker in lung cancer [12]. Therefore, single-cell technologies can be manipulated for the treatment of different diseases.

This review addressed the emerging approaches of single cell-based analysis in cancer immunotherapies and different human diseases. We explored the recent developments of the role of sequencing technologies in molecular mechanisms leading to drug discovery. Single-cell sequencing technologies are helpful for exploring the nature of single cell types and relationships between cell-to-cell communications among individual cells. These technologies paved for discovering the novel diagnostic markers for better understanding the pathological and biochemical mechanisms for controlling the rate of different human diseases.

Emerging high-throughput single-cell sequencing approaches

RNA sequencing technologies are used for gene expression of individual cells in the tumor microenvironment and reveal the transcriptional profile of tumor heterogeneity [13]. RNA sequencing has several advantages over traditional bulk sequencing such as enabling the early detection of novel inflammatory biomarkers affecting the proliferation and progression of tumor cells and improving the clinical efficacy of patients undergoing cancer immunotherapy [14]. In RNA-seq platform, specific tissues are isolated from tumor biopsy and identified as single-cells. Among single cell technologies, 10× Genomics chromium single cell sequencing allows the transcriptome measurement in individual cells while sequencing large numbers of single cells can recapitulate bulk transcriptome complexity. **Figure 1** shows the principle of the 10× Genomics single cell sequencing. The process begins with tissue digestion and generation of single-cell suspensions, followed by the creation of GEMs (gel bead-in-

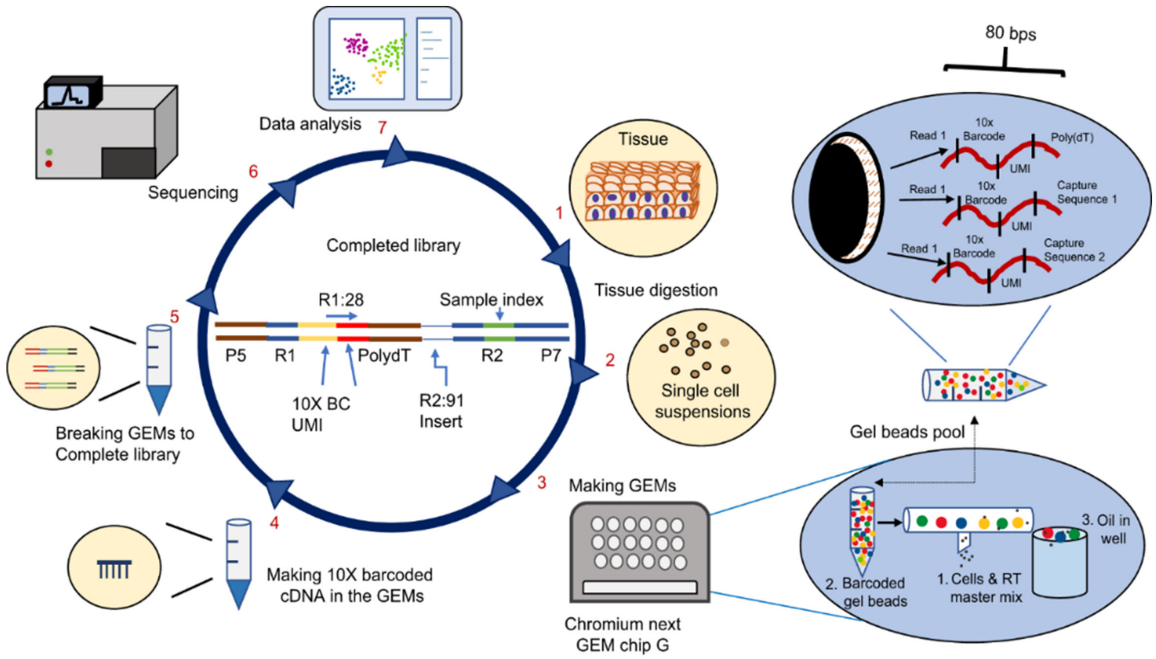
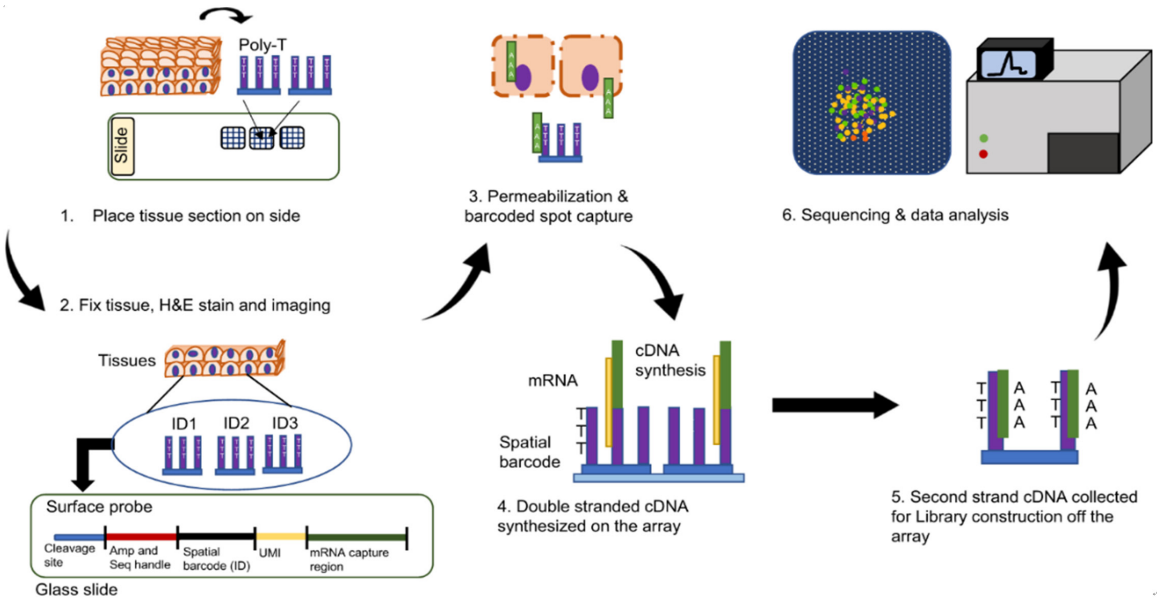


Figure 1. The image shows the principle of the 10× Genomics single-cell sequencing. It is followed by tissue digestion and generation of single-cell suspensions by the creation of GEMs (gel bead-in-emulsions) using the chromium Next GEM chip G. 10× barcoded cDNA is generated within the GEMs, which is then used to complete the library. Sequencing is carried out using Novaseq 100 cycle kits, with specific cycles allocated for cell barcode, sample index, and insert. The final step involves data analysis, which is conducted employing Cell Ranger, Loupe Browser, and other relevant software tools.

emulsions) using the chromium Next GEM chip G. Then, 10× barcoded cDNA is generated within the GEMs, which is then used for library construction. Sequencing is carried out using Novaseq 100 cycle kits with specific cycles allocated for cell barcode, sample index, and insert. The data analysis is conducted by employing the Cell Ranger, Loupe Browser, and other relevant software tools [15]. These advances in single-cell technologies offer an invaluable insight into the sequential procedures integral to single-cell sequencing, facilitating a better understanding of its methodology and workflow [6].

Another evolutionary advancement in single-cell technologies is the development of 10× spatial transcriptomics which enables the interrogation of gene expression within the context of tissue architecture, tumor microenvironment and cell groups especially when coupled with single-cell sequencing [16]. **Figure 2** shows the principle of the 10× spatial transcriptomics workflow, delineated into two distinct stages: on the array and off the array. Initial steps involve sample preparation, staining & imaging, permeabilization, and cDNA synthesis directly on the array. Subsequent procedures of array encompass library construction, sequencing, and meticulous data analysis. Utilizing Novaseq V1.5 100 cycle kits, whole transcriptome libraries undergo sequencing with specified parameters (read 1: 28 cycles, i7 index: 10 cycles, i5 index: 10, read 2: 90 cycles) [17]. The generated data undergoes comprehensive analysis and visualization using Space Ranger and Loupe Browser, enabling direct correlation of gene expression patterns with histological features. This structured representation elucidates the intricacies of spatial transcriptomics methodology, facilitating its effective application in clinical research settings [6].

SCI-seq technology is used for the construction of single-cell libraries and the detection of variations in somatic cells. This technique has several advantages over traditional sequencing techniques such as low cost for the construction of multiple libraries and reliability for the detection of somatic cell biology. This technology is also used for targeting tumors at early stages of invasive tumors and cancers [18]. Single cell WGA technology is widely used for the detection of mutations in a variety of can-



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cers including breast and colorectal cancers [19]. Single-cell multi-omics sequencing technology (Sc-COOL-seq) is widely applied for efficient detection of different patterns of chromatin localization and DNA methylation in a cost-effective manner [20]. TSCS based sequencing technology is widely used for the detection of variations occurring during metastasis and invasion in tumor cells. This technology is used for the identification of invasive tumor tissue and differentiates them from normal cells and thus targeting the proliferating tumor tissues [21]. SiC sequencing technology is used for the development of cellular lineage to differentiate the normal and disease cell, and is thus effective for genomic studies and the establishment of genotypic and phenotypic relationships [22]. The micro-well sequencing technology is applied for detection of clonal evolution and tumor heterogeneity. This high-throughput technology uses the agarose microplate for capturing the transcriptional profile of single cell [23].

Approaches of single-cell technologies for different diseases

Single-cell technologies are used for the treatment of different diseases. Recent advances in single cell technologies including transcriptomics and proteomics approaches have resolved the issues of single cell based detection of the nature of different tissues in the tumor microenvironment and hold a central position in the detection of checkpoint inhibitors and thus determining the signaling pathways evoked by tumor invasion [21]. **Table 1** shows the various applications of single cell technologies and is illustrated in the following sections.

Approaches for inflammatory bowel diseases (IBD)

IBD is a group of intestinal disorders that are characterized by chronic inflammation in the digestive tract [40]. The risk of IBD increases day by day all around the world and remains challenged due to high-risk factors, and poor understanding of molecular pathogenesis. These factors are driven by the environmental and genetic factors that cause mutational changes in individuals carrying the genes responsible for IBD. The incidence of IBD in children against intestinal microbes was high due to low immunity [41].

Single-cell technologies are used for early diagnosis and different biomarkers associated with inflammatory bowel diseases. **Figure 3** illus-

Table 1. Shows the recent studies of Ss-RNA sequencing for treating different diseases and clinical aspects

Biological systems	Study Design	Samples	Target Cells	Gene Markers	Principle Technique	Findings	Reference
Digestive system	GWAS	Intestinal biopsies from UC as well as healthy persons	Epithelial cells	BEST4	Single RNA-sequencing for investigation the expression of BEST4 and JAK2 genes and showed over expression in the epithelial cells causing ulcerative colitis.	Clinical findings included the $CD8^+$, TNF were increased, CRP, B cells increased in IBD patients.	[24]
	Clinical, Pathological	Healthy donor & inflamed cells of ileum	Stromal cells	MERTK and FLT3	Single cell technique enables the MERTK and FLT3-genes expresses in tissues of inflamed ileum in Crohn's disease.	Activated DCs lead to aggregation of B and T cells, reduction in monocytes, increased the expression of TNF in Crohn's disease patients.	[25]
	GWAS, Clinical	IBD biopsies of healthy samples	Epithelial	LGR5 and EPHB2	Single cell technique revealed the high expression LGR5 and EPHB2 genes in human embryonic tissues leads to impaired devolvement in Crohn's disease.	CD patients showed increased fibroblasts, reduction in enterocytes, FOXM1 showed expression in embryonic development in IBD patients.	[2]
Cardiovascular Diseases	Molecular, Pathological	Cardiomyocytes	Embryonic tissues	HAND1	Single cell sequencing helpful to find the expression of different genes involved in human cardiac development.	Expression of HAND1 genes providing evidence that fibroblasts and cardiomyocytes are contributed to cardiac tissues formation in atherosclerosis.	[26]
	Clinical	Carotid artery tissues	Carotid artery cells	TREM2	SsRNA seq for identification the expression of TREM2 macrophages.	TREM2 macrophages that occurred during different events of atherosclerosis in carotid artery tissues of human.	[27]
	Clinical	Myocardial tissues	Cardiomyocytes	Myh7	SsRNA seq with smFISH for expression of Myh7 in the cardiac tissue.	Conjugation of ssRNA with smFISH is helpful to increase the expression of mRNA for better understanding the mechanism of cardiac dysfunction in oxidative stress conditions.	[28]
Neurodegenerative Disorders	Clinical	Brain tissues from the cortices	Microganglia	NFKB, IRF	Ss-RNA seq based platform, SCENIC successfully identified the microglial regulators such as NFKB, IRF of the TFs.	SCENIC data base identified 3005 different types of brain cells from the cortices of adult mice. This approach helps for understanding pathogenesis of Alzheimer disease.	[29]
	Clinical	Mouse single cell atlas	Mouse single cell atlas	TREM2, MAFB and Nfkb1	Ss-RNA seq based platform SCENIC identified the TREM2, MAFB.	SCENIC identified from the information available about different genes from mouse single cell atlas and identified the genes networks of TREM2, MAFB and NFKB1 involved in pathogenesis of Alzheimer disease.	[30]
	Clinical	Human Brain Tissues	Human embryonic stem cells	hESCs	ScRNA-seq for diversity and characterization of intracerebral grafts from human embryonic stem cells.	Clinical trial stages in rat model on human brain cells and characterized the intracerebral grafts of human embryonic stem cells (hESCs) also identified the perivascular cells that resembled to the hESC-derived grafts to Parkinson disease.	[31]

Tuberculosis & Pulmonary Infections	Retrospective study	TB Samples	Sputum	Mtb	mNGS allows the early detection of confection of microbial pathogen (Mtb) from other ones such as aspergillus through mutational analysis.	Retrospective on TB patients for detection of Mtb through mNGSs technology found that sensitivity & specificities by mNGS, and smear was 59.9%, 24.6%, and 100%, 93%.	[32]
	Clinical	TB Samples	Sputum	Mtb	NGS is helpful for sequencing of each part of genome through detection of mutational changes in pathogenesis of TB.	The sample analysis through NGS follows the extraction of DNA from sputum, target enrichment and assisted in diagnosis of Tuberculosis.	[33]
Cancers & Tumors	Clinical	Breast Tissues	CTCs cluster was analyzed from breast cancer patient blood	Estrogen receptor	Ss-RNA seq identified the CTCs expressing metastasis estrogen receptor (ER) from BC patients.	The Androgen receptor pathway was activated by CTCs clusters as biomarkers in breast cancers.	[34]
	Clinical	Breast Tissues	CTCs clusters	BRAC1 and BRAC2 genes	scRNA-seq method and extracted and transcriptomic analysis of CTCs.	CTCs revealed increased proliferation was associated with estrogen reactivity and decrease proliferation with epithelial mesenchymal transition.	[35]
	Clinical	Tissues from colon	Epithelial cells, Fibroblasts, CRC cells from patients	RET gene	Majority of the genes expressed by CRC cells was detected by scRNA-seq.	It was found that RET gene rearranged during transfection related to epithelial mesenchymal transition were overexpressed in colorectal cancer.	[36]
		Samples from liver metastasis tissue	Samples from liver cells	Metastatic CRC	ScRNA and ScDNA sequencing to find out the heterogenicity of colorectal cancer cells through.	This study revealed the mutational changes in DNA and RNA changes are involved in colorectal cancer progression.	[37]
	Clinical	Pancreatic ductal tissues	Pancreatic ductal adenocarcinoma compartment	SMAD4, CDKN2A	Single cell transcriptome analysis of SMAD4, and CDKN2A genes to determine the intratumor heterogenicity for pancreatic ductal adenocarcinoma compartment.	It was found that stroma of tumor cells contains variety of cells with increase heterogenicity was associated with pancreatic cancers.	[38]
	Clinical	Pancreas tissues	Pancreatic islets	SF3B1, MLL3, TP53	Next generation sequencing techniques identified the mutated genes in pancreatic cancer such as SF3B1, MLL3, TP53, KRAS.	This study identified the mutated genes in pancreatic cancer highly associated with the pathogenesis of pancreatic cancer.	[39]

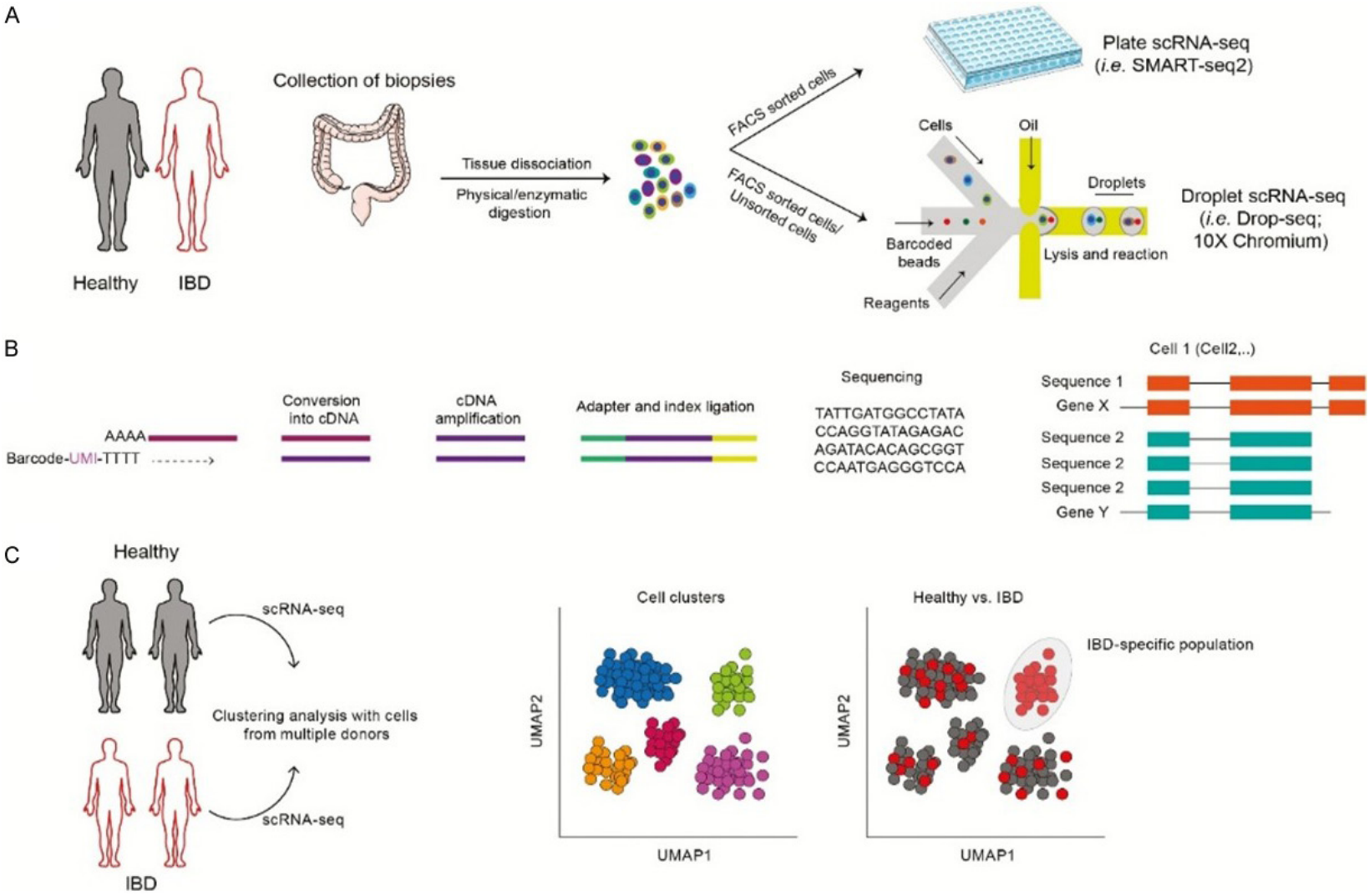

Figure 3. The single-cell RNA sequencing role for inflammatory bowel disease (IBD). A: Illustrates the initial steps, wherein intestinal biopsies from healthy donors and patients with inflammatory bowel disease (IBD) are dissociated to yield cell suspensions. B: Elucidates the subsequent stages, involving the conversion of RNA from single cells to cDNA, amplification, addition of adapters, and pooling of samples for sequencing on high-throughput platform. C: Highlights the computational analysis of scRNA-seq data, facilitating the unbiased mapping of novel cell subsets, detection of rare cell populations, and identification of disease-specific cell subpopulations.

trates the principal role of single cell technologies for inflammatory bowel diseases. Panel A illustrates the initial steps, wherein intestinal biopsies from healthy donors and patients with inflammatory bowel disease are dissociated to yield cell suspensions [42]. These suspensions can be further sorted into specific subsets such as by fluorescence-activated cell sorting (FACS), undergoing RNA sequencing via either plate-based or droplet-based methods. Panel B elucidates the subsequent stages, involving the conversion of RNA from single cells to cDNA, amplification, addition of adapters, and pooling of samples for sequencing on high-throughput platforms [43]. The panel C highlights the computational analysis of the RNA-seq data, facilitating the unbiased mapping of novel cell subsets, and identification of disease-specific cell subpopulations. This systematic approach provides insights into the cellular landscape of the intestinal mucosa, crucial for understanding health and disease states [44].

A GWAS-based cohort study in patients suffering from bowel inflammatory disease with single cell clustering analysis and found that PSMA6 gene expressed in stem and epithelial cells is associated with Crohn's Disease (CD). Other clinical findings included increased levels of Hb, Alb, CRP, and inflammatory responses such as TNF, $CD41^+$ and B cells as well as increased inflammation in stem cells [45]. RNA-seq was applied to inflamed and non-inflamed ileum for investigating the cellular and molecular events of Crohn's disease. Lamina propria cells and stromal cells were isolated from inflamed and non-inflamed Ileum tissue. This cohort-based study showed that MERTK and FLT3 genes expressed in inflamed ileum tissues in Crohn's disease. Activated DCs lead to aggregation of B and T cells, reduction in monocytes, and increased the expression of TNF [25]. RNA-seq is helpful to understand the maturity and functioning of human gut epithelial cells during fetal development. It was found that patients showed increased fibroblasts, reduction in enterocytes, and high expression of LGR5 and EPHB2 genes in human embryonic tissues leading to impaired fetal devolvement thus increasing the incidence of Crohn's disease. FOXM1 as a main transcription factor that showed high expression in embryonic development due to oxidative stress [2]. BEST4 and JAK2 genes showed their expression in the epithelial cells in ulcerative colitis. They found that inflammatory markers such as $CD8^+$, TNF increased, CRP, and B cells also increased during the proliferating of ulcerative colitis [24].

GIMATS is a novel single cell-based module used for the identification of single cell populations in IBD [45]. This module comprises cellular populations activated DCs, activated T cells, IgG PCs, and endothelial cells. Activated DCs within the GIMATS module stimulates the aggregation of B and T cells, reduction in monocytes, expressed high levels of the T cell ligands CCL19, CCL17, and CCL22 and resultantly increased the expression of TNF. The inflammatory macs within the lymphocyte containing activated DCs, aggregates and receptors within the T cells control the shaping of the T cell polarization in crown disease patients with high GIMATS module scores. Therefore, this module identifies the lesions and subpopulations in crown disease [2].

Approaches for cardiovascular diseases

Single-cell techniques emerged for the treatment of cardiovascular diseases through analysis of gene expression of each cell type. These techniques are also helpful for early detection of atherosclerosis by targeting the heavy plaques in arteries thus reducing the risks of heart failure and following up the different stages of heart development [46].

Approaches in cardiac dysfunction and atherosclerosis

Single-cell technologies are helpful to find the expression of different genes involved in human heart development. Expression of HAND1 gene provides evidence that fibroblasts and cardiomyocytes contribute to the extracellular matrix of cardiac tissue formation [26]. Genes involved in the formation of extracellular matrix in human cardiac development showed high expression. This difference is helpful in finding out the gene markers in murine models for the investigation of diseases associated with the cardiovascular system [47]. SmFISH is helpful for early detection of Myh7 in the cardiac tissue as it increases the expression of mRNA after loading the pressure. This approach leads to understand the mechanism of cardiac dysfunction in oxidative stress conditions. It was found that after applying oxidative stress or pressure

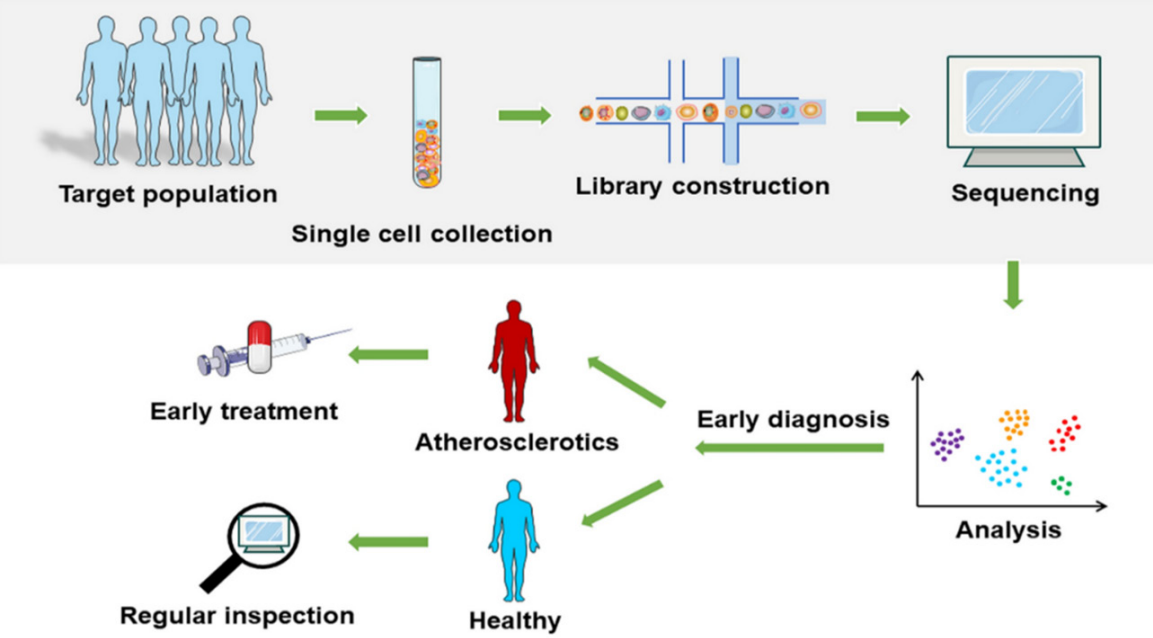

Figure 4. Depicts the future decision-making process for atherosclerosis detection, emphasizing advancements in single-cell sequencing technology. Initially, individual cells are collected from the targeted population, and libraries are constructed from the single cells collection. Subsequently, sequencing and data analysis are conducted to identify molecular signatures associated with atherosclerosis.

to myocardial tissues, expression of Myh7 gene potentially increased in the middle layer of the myocardium [28].

Atherosclerosis is caused by the deposition of fats, cholesterol, and other substances in artery walls in the form of plaque. It is a serious medical condition and can affect arteries that become blocked and lead to clot formation that increases the risk of heart attack, and peripheral artery disease. Single-cell techniques are used to discover the inflammatory mediators evoked in atherosclerosis [48]. **Figure 4** illustrates the fundamental role of single-cell sequencing technology in atherosclerosis detection, and emphasizing advancements [49]. Initially, individual cells are collected from the targeted population, and libraries are constructed from the single cells collection. Moreover, precise sequencing and data analysis are conducted to identify molecular signatures associated with atherosclerosis. This approach is helpful for early diagnosis and intervention by healthcare professionals, facilitating timely treatment. Additionally, it offers an opportunity for proactive health monitoring among asymptomatic individuals, allowing for early detection and intervention in at-risk populations [48].

Literature depicted the role of single cell sequencing technology for atherosclerosis detection, risk of plaque ruptures during atherosclerosis leads to severe inflammation and remains challenged for clearing the dead cells [50]. RNA sequencing in conjugation with nanoparticles for clearing the dead cells by restoring the phagocytic activity of the apoptotic cells during atherosclerosis. The aortic cells of mice were detected through single analysis of RNA-seq with nanoparticles. This strategy leads to a decrease the inflammation in aortic cell by reducing the cytokine that induces severe inflammation [51]. Nanoparticles binding to the inflamed cells are helpful for treating atherosclerosis as well as clinical markers for early diagnosis of inflammatory diseases.

RNA seq is helpful for identification of TREM2 macrophages occurred during different events of atherosclerosis in carotid artery tissues of human. TREM2 macrophages showed a foamy appearance like those plaques found during atherosclerosis in carotid tissues of the human. This approach mimicked the pathophysiological process of mice to atherosclerosis in humans. TREM2 macrophages caused calcifications during the plaque formation. TREM2

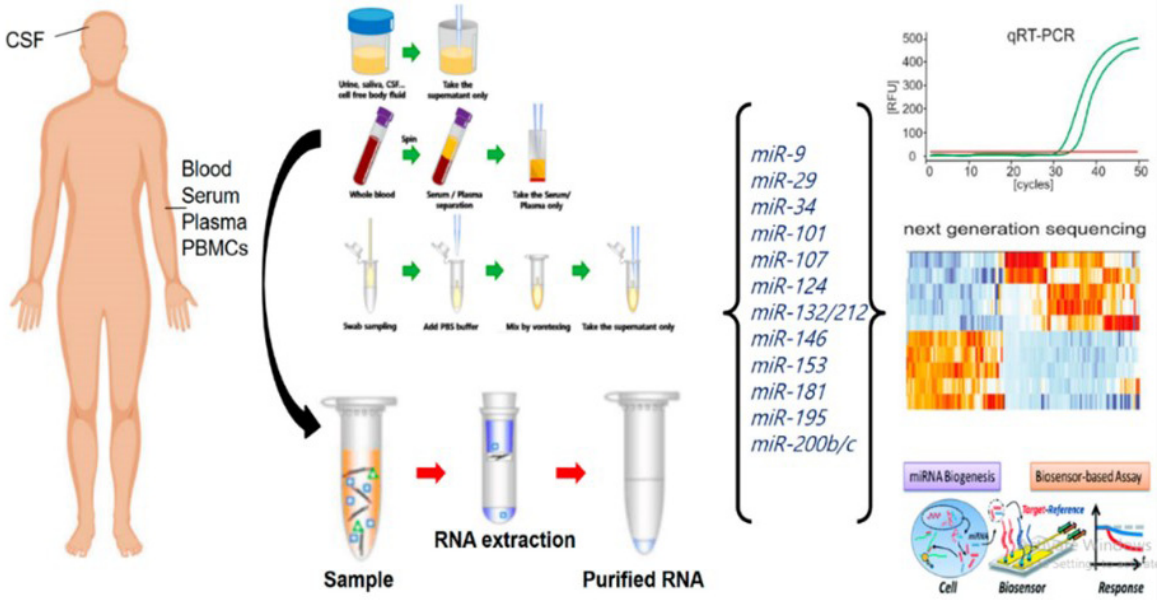

Figure 5. The image shows the principle for miRNA expression analysis in Alzheimer's Disease (AD). Initial steps involve sourcing fluid samples like CSF, blood, or plasma, followed by RNA isolation and purification. Subsequent analysis employs various techniques, including qRT-PCR, next-generation sequencing, miRNA biogenesis studies, and biosensor-based assays. These approaches enable the identification of miRNAs associated with AD pathogenesis, shedding light on their roles in aging, neuroprotection, inflammation, and protein misfolding.

macrophages gained special interest as biomarkers for predicting the different levels of atherosclerosis [52].

Approaches for neurodegenerative disorders

Neurodegenerative disorders are the leading cause of the death among aged individuals and becoming an alarming threat to mental health [53]. These are characterized by the deposition of abnormal proteins such as beta-amyloid and hyperphosphorylated tau in brain tissues and lead to the deaths of neurons. The most common forms of neurodegenerative disorders are Alzheimer's and Parkinson's disorders. Single-cell techniques are helpful for early diagnosis of neurodegenerative disorders through the gene expression analysis of various cell types involved in the progression of the disease [54].

Approaches for Alzheimer's disease (AD)

Alzheimer's disease is a cognitive neurodegenerative disease that is characterized by the deposition of beta-amyloid proteins in the form of plaques and hyperphosphorylated tau protein in the form of insoluble filaments. RNA-sequencing technology is the most emerging technique for treating the Alzheimer's disease by transcriptomic analysis of neuronal cell populations which would not be possible by microarray and bulk RNA-sequencing technology [54]. Single-cell technology is used for miRNA expression analysis in Alzheimer's Disease and is thus helpful for the disease progression of different stages [55]. **Figure 5** depicts the workflow for miRNA expression analysis in Alzheimer's Disease (AD), delineating key methodologies and pathways. Initial steps involve sourcing fluid samples like CSF, blood, or plasma, followed by RNA isolation and purification [56]. The subsequent analysis employs various techniques, including qRT-PCR, next-generation sequencing, miRNA studies, and biosensor-based assays. These approaches enable the identification of miRNAs associated with AD pathogenesis, shedding light on their roles in aging, neuroprotection, inflammation, and protein misfolding. Notably, miRNAs impact AD through amyloid formation, and Tau pathology [57]. The figure underscores the significance of exosome miRNAs as potential AD markers and highlights specific miRNAs implicated in disease mechanisms. Ultimately, this comprehensive analysis aids in early AD detection and diagnosis, facilitating targeted therapeutic interventions [58].

Different novel databases are helpful in investigating the pathogenesis of AD by collecting the existing genes. Single cell RNA-Seq database (SCREAD) as the first novel database helpful in the diagnosis of AD by collecting the existing gene information from data sets of RNA-Seq of the neuroganglion tissues of the human and experimental mouse models [53]. The principal network of the SCREAD can be designed by constructing the control atlas of different neuronal tissues of the human and mouse based on information available on 23 data sets as control, information of different types of neuronal cells involved in the pathogenesis of AD carefully operated to control atlas, identification of CTSRs and DEGs, functional enrichment of DEGs leads to store information in other databases such as GO & KEGGs [59]. SCENIC is another database network based on RNA-seq technologies and helpful for the identification of different genes involved in the pathogenesis of AD by establishing a network of gene modules. SCENIC data base analysis on 3005 different types of brain cells from the cortices of adult mice and successfully identified the microglial regulators such as NFKB, IRF of the TFs [29]. SCENIC network comprises the TREM2, MAFB and Nfkb1. Among these genes, TREM2 is used as a potential marker in the progression of AD [30].

Approaches for Parkinson disease (PD)

Parkinson's disease is a neurodegenerative disorder that affects 3% of the world population [60]. It is characterized by impaired functioning of dopaminergic neurons in the brain that leads to the occurrence of symptoms such as movements, anxiety, and tremors. The mechanism of action of dopaminergic drugs for the treatment of PD is poorly understood and fails to recover all patients with the progression of the disease. Different traditional methods such as microarrays and dopamine transporter scans are employed for diagnosis of Parkinson's disease. These methods are time-consuming and technical errors mostly errors that occurred during for analysis of neuronal tissues [61].

RNA-seq technologies are helpful in identing the complexity of neuronal cell types that assist in diagnosis and provide a better understanding of different stages of Parkinson's disease progression. These techniques enable the modifications in DNA as well as histone proteins to target the tumor cells [62]. The diversity and characterization of intracerebral grafts from human embryonic stem cells remains unclear at different clinical trial stages in rat models [31]. RNA-seq experimental-based study on human brain tissues to hiPSC-derived dopaminergic neurons identified the HDAC4 gene that protected the brain cells through the deactivation of abnormal proteins by removing their acetyl groups thus exerting the antitumor effects [63].

Approaches for tuberculosis and pulmonary infections

Tuberculosis is a bacterial infection known as TB and can be fatal if prolonged for an indefinite period. TB most often affects the lungs but can also affect other organs like the brain. Single-cell sequencing is used to reveal the pathogenesis and diagnostics of tuberculosis [64]. The resulting suspension is then subjected to barcoded library construction, which involves the addition of unique molecular identifiers (UMIs) to each RNA molecule. The cDNA libraries undergo next-generation sequencing, generating vast amounts of data that can be analyzed using various visualization tools. The data generated using RNA-seq techniques can be used to study cellular heterogeneity in granulomas, which are key structures in TB infection [65]. By analyzing the molecular signatures of individual cells within granulomas, researchers can identify biomarkers for vaccines and therapeutics in diagnostics and precision medicine. This detailed analysis not only enhances our understanding of TB pathogenesis but also paves the way for the development of targeted therapies and personalized treatment strategies based on individual patient profiles [66].

Next-generation sequencing (NGS) technologies are becoming the first choice for early detection and treatment of tuberculosis [67]. Early detection of *Mycobacterium tuberculosis* through next-generation sequencing techniques is an emerging step in controlling the death rate among TB patients. Different traditional methods are employed for testing the Mtb such as Xpert, mycobacterial culture and smear microscopy. These methods have low sensitivity and time time-consuming as take about 3-7 weeks [68]. Thus, NGS is helpful for treating pulmonary TB through early detection of Mtb and patients as well as in those patients

who receive anti-TB therapy. NGS also helpful for sequencing each part of the genome through the detection of mutational changes in the pathogenesis of TB. The sample analysis through NGS followed the extraction of DNA from sputum, target enrichment and finally data analysis [33]. XT Target Enrichment System is the next generation-based approach that provides comprehensive information about the whole genome sequencing of *M. tuberculosis*. This method has an 83% success rate and high-quality genomic sequencing obtained in a short time. Another approach of NGS is the differential lysis of sputum samples that catalyzes the following extraction of DNA of the *M. Tuberculosis* and then lysis in the presence of DNase. These methods allow low-cost genomic analysis, and the success rate is about 87% [69].

Approaches of metagenomic next-generation sequencing (mNGS) for tuberculosis

Metagenomic next-generation sequencing is an emerging technology for the detection of TB samples in a short time with high septicity as it detects 10% additional cases rather than traditional methods [70]. It is helpful in mutational analysis of bacterial pathogens by generating millions of sequences of DNA/RNAs. It is also helpful detection of confections such as *Mtb*, and *Aspergillus fumigatus* [71]. The specificity of traditional methods such as smear and culture-based methods for the detection of Mtb is low as this method gives false positive results which can increase the chances of transmission of infections [32]. Thus, mNGS is helpful for treating the pulmonary TB through early detection of Mtb and patients as well as in those patients that receive Anti-TB therapy.

Approaches of single cell sequencing for cancer immunotherapy

Approaches for breast cancers

RNA-seq technology are used for the detection of mutational changes in the breast at early stages and are thus helpful for treating breast cancer [35]. RNA-seq technique and flow cytometry were used *in vivo* in mouse models revealing the circulating tumor cells (CTCs) in metastatic breast cancer. It was described that CTCs cluster originated from oligo clonal tumor cell groups. As compared to single CTSs, these clusters are rare in circulation and exhibit high metastatic tendency. CTCs cluster formation is mediated by Plakoglobin which facilitates metastasis [34]. CTCs from patients exhibit heterogenicity with the expression of androgen receptor mutation [72]. Hydro-seq technique is also used to analyze the CTCs with high throughput sequencing. This technique was used successfully to carry out RNA-seq analysis of 666 CTCs among 21 breast cancer patients. Transcriptomic analysis of these cells clarified the comprehensive tumor-targeted therapy and tumor metastasis process [72]. Transcriptome analysis of these CTCs revealed that increased proliferation is potentially associated with estrogen reactivity and decreased proliferation with epithelial-mesenchymal transition [35].

Approaches for colorectal cancer (CRC)

Colorectal cancer (CRC) is the second most common type of cancer and arises from mutations in the colonic and rectal epithelial tissues that target oncogenes, tumor suppressor genes and genes related to DNA repair mechanisms [73]. Single cell transcriptome analysis can analyze the circulating tumor cells and reveal the heterogeneity of tumor cells in CRC [74]. The second part focuses on the specific application of single-cell sequencing in CRC, highlighting its utility in investigating compositional evolutionary pathways, tumor heterogeneity, and the immune microenvironment comprising tumor-associated macrophages (TAMs), dendritic cells (DCs), cancer-associated fibroblasts (CAFs), T cells, and myeloid-derived suppressor cells (MDSCs) [75]. This integrated approach facilitates a deeper understanding of CRC pathogenesis and immune responses, providing crucial insights for the development of targeted therapies and personalized medicine strategies in combating this prevalent malignancy [76].

Literature depicted the role of single cell sequencing technology in the diagnosis of colorectal cancer. A comprehensive transcriptome-based study was conducted on single cell sequencing to determine the heterogeneity of tumor cells among patients diagnosed with colorectal cancer [36]. Through the reference component analysis method, seven cell clusters such as B cells, endothelial cells, fibroblasts, mast cells, T cells, myeloid cells and

epithelial cells were obtained. Most of the genes expressed by CRC cells were detected by RNA-seq and it was found that RET (gene rearranged during transfection) genes related to epithelial-mesenchymal transition were overexpressed in colorectal cancer samples [36]. In another experimental-based study, mouse models were used to find the heterogeneity of colorectal cancer cells through ScRNA and ScDNA sequencing. This study revealed the mutational changes in DNA and RNA lead to the progression of colorectal cancer [37].

Approaches for ovarian and testicular cancers

The complicated process of embryonic development is regulated by environmental factors, genes, and genetic predisposition. With the help of RNA-seq, key genes can be identified that are involved in the early stage of embryonic development. Different cell subtypes and molecular mechanisms of cell differentiation can be identified through RNA-seq analysis [77]. Single-cell tagged reverse-transcription sequencing (STRT-seq) is widely used for to find out the gene expression NOTCH1, SNAI2, TGFBR1, and WNT11 level of each cell. To understand the mechanism of ovarian aging, the gene expression of oocytes is deeply analyzed through advances in single cell technologies and new biomarkers can be used as a therapeutic target for ovarian disease related to aging [78].

Single-cell atlas for the development of testes as the common progenitor for spermatocytes (SYCP3+), Sertoli cells (C4; SOX9+), Leydig and myoid-like cells (C5; IGF1+ and/or ACTA2+) cells during puberty. After puberty, sex-related infections can be controlled through antimicrobial peptides and complex signaling pathways that are involved in spermatogenesis [79]. With the help of single cell sequencing technologies, infertility mechanisms can be efficiently developed for controlling the different diseases in germ-line cells [77].

Role of RNA-seq in drug screening and drug target genes

Drug screening and the therapeutic effects of different drugs can be accessed through RNA-seq technology [80]. Mubritinib inhibited the expression of $ERBB2^{+}$ in AML cancerous cells, OXPHOS and blocked the activity of complex I inhibitor during the formation of ETC. They conclude that mubritinib could be used as a therapeutic drug for the treatment of acute myeloid leukemia [81]. Sequencing analyses revealed that metformin-initiated interaction of cytokine-cytokine receptors and production of IgA antibodies with intestinal disorders [82]. Rifampicin possesses anti-virulence activity against hvKP activity and could be used as a potential drug candidate for the treatment of hvKP [83]. CEDR explored the 582 single cell data and 140 phenotypes in both humans and mice by searching the keywords of diseases, drug response, cells, tissues, and treatment [84]. SCREAD successfully optimized the concentrations of dose in an effective manner and thus valuable tool for drug screening. More research is needed for advancing the novel software's for drug delivery [85].

Single cell technology is helpful in revealing the development of different drugs for the treatment of genetic diseases. These drugs interact with specific cells, tissues, organs, and their effects can be exerted at the molecular level with the target [86]. Clinical and experimental investigation of genomic drugs leads to the discovery of novel drugs. **Table 2** shows the details of each drug, target gene and mechanism of action against disease. Bleomycin inhibits the growth of cancerous cells by binding to the ligase enzyme and is used for the treatment of Hodgkin's lymphoma and cervical cancer [87]. Olaparib is used for the treatment of breast cancer resulting in mutations in BRCA1 gene [88]. Methotrexate inhibited the growth of cancerous cells and reduced inflammation in joints. Cisplatin is used to inhibit the growth of cancerous cells by binding to DNA thus inhibiting DNA replication. Mianserin as an antidepressant is used to maintain mental balance and relieve pain. Kanamycin is used to control infections caused by *E. coli* and *S. marcescens* [89].

Limitations and future perspectives

Although single cell sequencing cannot be used on a large scale due to poor detection of cancer cells and loss of genetic material. For instance, single cell sequencing of the circulating tumor cells accounts for the mRNA of about 0.1 pique in the total sample of RNA about 8 piques. It becomes difficult to diagnosis the cancer stage

Table 2. The various types of single cell-based drugs with mechanism of action

Drug	Class	Target Gene	Mechanism of Action	Single Cell Technology	Clinical Applications	Reference
Rifamycin	Anaphylaxis	*rpoB*	Inhibit the bacterial RNA synthesis by binding with β subunit of RNA Polymerase	RNA-Sequencing	Tuberculosis	[90]
Methotrexate	Immunosuppressant/ antimetabolites	*HMGB1*	Inhibited the growth of cancerous cells	Next generation sequencing	Inflammatory bowel disease	[91]
Mianserin	Antidepressant	*5-HT*	It is used for the treatment of mental syndromes	Single cell RNA-Sequencing	Neurological disorders	[92]
Kanamycin	Aminoglycoside	*Eis*	Used to control bacterial infections	Single cell RNA-Sequencing	Tuberculosis	[93]
Haloperidol	Neuroleptics	*L-DOPA*	Used to relief mental pain by blocking dopamine receptors	Sc-RNA-Sequencing	Parkinson disease	[90]
Cisplatin	Antineoplastic	*NRF2*	Used to inhibit the growth of cancerous cells by blocking DNA synthesis	Single cell reverse transcription sequencing	Testicular, and ovarian cancers	[94]
Pyrazinamide	Antibacterial	*rpsA*	Tcontrol the growth of *M. tuberculosis*	Single cell RNA-Sequencing	Tuberculosis	[95]
Bleomycin	Cytotoxic	*LIG1*	It inhibits the growth of cancerous cells by binding to the ligase enzyme	Single cell RNA sequencing	Cancer therapeutics	[96]
Moxifloxacin	Fluoroquinolones	*Gyr*	It is used to block the bacterial replication by inhibit the DNA gyrase	Single cell RNA sequencing	MDR-TB	[97]
Diazepam	Anxiolytic	*CYP450*	Competitive inhibitor by blocking *CYP450* mechanism	Single cell RNA sequencing	Neurological disorders	[98]
Tranylcypromine	Immunosuppressant	*MAO*	Block the MAOs by improving the mental behavior	Single cell RNA sequencing	Alzheimer's disease	[99]
Delamanid	Nitroimidazole	*Ddn*	To block the cell wall synthesis of coughing causing bacteria	Single cell RNA sequencing	TB	[100]

due to the small amount of mRNA in the sample. Single cell sequencing also causes the loss of genetic material as mutations arise due to tumor cells that significantly increase the development of tumors [101, 102]. Metagenomic next-generation sequencing fails to identify the comparison between live *M. tubercuolsis* in respective to dead ones. Metagenomic next generation sequencing cannot correctly predict drug dosage in TB patients in the first, second and third stages due to which chances of drug resistance increase [103]. It is a great challenge to identify patients with increased drug-resistant TB as traditional molecular techniques are not reliable for detecting the mutations that arise due to *M. Tuberculosis*. It leads to an increased mortality rate among TB patients due to recurrent infection [104].

RNA-seq technology has certain limitations for gene expression such as signal dropouts occurring as a major issue due to low expression of gene in one cell while expression of gene fails to be observed in another [105]. DL models are designed to solve the problems of signal dropout issues. At single cells, DL models as an excellent way for extraction of dimensional features of genomic sequencing data and biological characteristics of other gene-drug relationships. DL model establishes a relationship at the bulk level by transferring the features of the drug to each cell type. It leads to improving the prediction of sensitivities for each cell type. Thus, DL models are helpful in the prognosis of heterogeneous diseases by providing bulk data information on the patients suffering from these diseases [106].

Single-cell biology is helpful in drug discovery for a better understanding of the mechanism behind drug resistance due to tumor heterogeneity. Advances in whole genomic sequencing have been accelerated to understand the molecular mechanism of drug resistance due to *M. Tuberculsis*. Some approaches to molecular techniques have been developed to express the functions of different proteins in cultured cells of humans. For instance, RNA interference (RNAi) and cDNA libraries are used for overexpression of the genes at the molecular level. Genomic library of yeast is helpful for the identification of genes that confer strong resistance

against specific drugs. This approach is helpful for the discovery of new drugs using the genomic information of yeast as in eukaryotes [107].

Conclusion

This review addressed the emerging applications of single cell-based technologies including cancer biology, nervous disorders, digestive tract abnormalities, cardiovascular dysfunction mechanisms, and TB drugs also highlighted of drug discovery platform. Single cell sequencing technologies are helpful for exploring the nature of single cell types and relationships between cell-to-cell communications in individual cells. These technologies paved as new platform for discovering novel diagnostic markers for better understanding the pathological and biochemical mechanisms for controlling the rate of different human diseases. In most cases, understanding the nature of heterogeneous tissues becomes crucial in TME. Furthermore, advancements in scRNA sequencing technologies should be made for easy operation, improving the multiplexing low detection cost; so that, these technologies could be applied for clinical diagnosis of infectious diseases and reduce the risk of spreading at the global level.

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Disclosure of conflict of interest

None.

Abbreviations

TREM2, Triggering Receptor Expressed on Myeloid cell; smFISH, Single molecule fluorescent in situ hybridization; LGR5, Leucine G-protein coupled receptor 5; EPHB2, Ephrin type-B receptor 2; PSMA6, Proteasome subunit alpha type 6; TNF, Tumor necrosis factor; MERTK, Mer proto-oncogene tyrosine kinase; FLT3, Fms Related Receptor Tyrosine Kinase 3; scRNA-seq, single-cell RNA-sequencing; LGR5, Leucine G-protein coupled receptor 5; EPHB2, Ephrin type-B receptor 2; FOXM1, Forkhead BoxM1; HAND1, Heart- and neural crest derivatives-expressed protein 1; smFISH, Single molecule fluorescent in situ hybridization; Mhy7, Myosin Heavy Chain 7; SCENIC, Single-cell regulatory network inference and clustering; hESCs, human embryonic stem cells; mNGS, Metagenomic Next-Generation Sequencing; SF3B1, Splicing factor 3b subunit 1; KRAS, KRAS proto-oncogene, GTPase.

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