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Single Cell Sequencing: Genomic and Transcriptomics Approaches in Cancer Cell Biology

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Abstract: This article reviews the transformative impact of single-cell whole genome sequencing (scWGS) on cancer biology research. ScWGS has revolutionized our understanding of tumor heterogeneity, clonal evolution, and the tumor microenvironment by enabling high-resolution analysis of individual cells' genomic, transcriptomic, and epigenomic profiles. The technology's unique capabilities, including detection of rare genomic events, simultaneous capture of multiple genomic features, and association with phenotypic data, have opened new avenues for cancer research and precision medicine. Integration of scWGS with other single-cell omics technologies has provided a multidimensional view of cellular states and regulatory mechanisms in cancer. Advanced data analysis tools, including machine learning and AI algorithms, have been crucial in interpreting the vast amounts of data generated, leading to the identification of new biomarkers and development of predictive models for patient stratification. The article also discusses emerging technologies like spatial transcriptomics and in situ sequencing, which promise to further enhance our understanding of tumor spatial organization and cellular interactions. As scWGS and related technologies continue to evolve, they are expected to drive significant advances in personalized cancer diagnostics, prognosis, and therapy, ultimately improving patient outcomes in the era of precision oncology.

Keywords: single-cell whole genome sequencing; tumor microenvironment; tumor heterogeneity; cancer research; precision medicine; artificial intelligence

1. Introduction

Human cancers are complex ecosystems composed of cells with distinct phenotypes, genotypes, and epigenetic states. Current cancer study models do not adequately reflect tumor composition in patients (Navin et al. 2011). Single-cell sequencing has revolutionized our understanding of cellular heterogeneity within cancer tissues, unlike traditional bulk RNA-seq analysis that provides an average transcriptional profile of a tissue sample, masking cellular diversity (Suvà and Tirosh 2019). Single cell RNA sequencing (scRNA-seq) enables the dissection of complex tumor ecosystems at single-cell resolution, revealing rare cell types, transition states, and intercellular interactions vital for cancer progression and therapeutic response (Patel et al. 2014; Tirosh et al. 2016; Puram et al. 2017).

Single-cell whole genome sequencing (scWGS) has revolutionized cancer research by providing a powerful platform to investigate clonal evolution, intratumoral heterogeneity, and treatment resistance (Navin et al. 2011). This technology enables the analysis of genomic and transcriptomic profiles at the individual cell level for the identification of specific cellular subpopulations within tumors associated with disease progression and treatment response (Han et al. 2022).

The advent of scWGS has transformed our understanding of cancer biology by providing an unprecedented view of the complex genetic landscapes of tumors at a single-cell level (Fu et al. 2020). This technique involves isolating individual cells, amplifying their DNA, and then sequencing the genomes to understand genetic variations at a single-cell level (Han et al. 2022). It has also revealed

the remarkable heterogeneity present both within and between cancer cells (Loo et al. 2019). Meticulously analyzed genomic profiles of single cells, uncovered the trajectories driving tumor progression, the mechanisms underlying cancer cell metastasis, and the development of resistance to medical treatments (Tritschler et al. 2017; Rognoni and Watt 2018; Loo et al. 2019).

This review examines how single cell technologies have changed cancer biology research. It explores the technology's key contributions to understanding critical aspects of cancer, including tumor heterogeneity, evolution, and the tumor microenvironment through its ability to analyze genomic, transcriptomic, and epigenomic profiles at the individual cell level. The review covers the essential capabilities of single cell technology, its integration with other single-cell omics approaches, and the crucial role of advanced computational methods in data analysis. Additionally, it discusses the emergence of complementary technologies like spatial transcriptomics and in situ sequencing, while also addressing the potential future impact of these advancements on personalized cancer medicine. Throughout, this review emphasizes how these technological developments are driving progress in cancer diagnostics, prognostics, and therapeutic strategies, ultimately aiming to improve outcomes for cancer patients through more precise and personalized approaches.

2. Cancer Research

Within the conceptual framework of scWGS, three central capabilities stand out: fidelity, co-presence, and phenotypic association. These capabilities are essential for understanding the unique advantages of scWGS. The fidelity capability refers to its ability to detect DNA features such as mutations or modifications existing at low levels of mosaicism within a sample. Unlike bulk DNA sequencing (Evrony, Hinch, and Luo 2021)., the co-presence capability in single-cell DNA sequencing refers to its ability to capture multiple genomic features within a single cell (Evrony, Hinch, and Luo 2021). This capability allows the analysis of different aspects of the genome simultaneously, including copy number variations (CNVs), single nucleotide variants (SNVs), structural variations such as insertions and deletions, chromosomal rearrangements, and DNA methylation patterns.

Providing a more comprehensive understanding of the genetic landscape of individual cells. Lastly, the phenotypic association capability allows linking genomic information with cellular phenotypes (Macaulay et al. 2015; Evrony, Hinch, and Luo 2021). Connecting genetic data with specific cellular characteristics, researchers can gain insights into the functional implications of genomic variations at a single-cell level (Macaulay et al. 2015; Demaree et al. 2020; Evrony, Hinch, and Luo 2021). The co-presence and phenotypic association capabilities are crucial in heterogeneous samples, spanning from profiling cells within an organism to distinguishing between tumor and normal cells or analyzing cellular mixtures in complex environments like soil samples or the gut microbiome (Evrony, Hinch, and Luo 2021).

One of the fundamental applications of scWGS in cancer research is the characterization of circulating tumor cells (CTCs). These are defined as tumor cells that have been sloughed from the primary tumor into the circulatory or lymphatic systems (Ni et al. 2013; Dago et al. 2014; Lohr et al. 2014; D. Lin et al. 2021). Understanding their part in metastasis may contribute to better therapeutic management (Ju et al. 2022). In a study conducted by Polzer et al., scWGS technique was used to analyze the genomic profiles of CTCs in breast cancer patients, allowing the identification of fundamental principles guiding the evolution of individual tumors. These principles include the ability of tumor cells to generate genetic diversity through mutations and chromosomal rearrangements, as well as clonal selection of subpopulations with evolutionary advantages, such as resistance to therapy or increased metastatic capacity. These findings offer a new perspective on CTC dynamics, highlighting the coexistence of genetically distinct subpopulations, each with different metastatic potentials and therapeutic vulnerabilities (Polzer et al. 2014).In addition to its application in studying CTCs, scWGS has enabled researchers to unravel the complex clonal architecture of solid tumors. This has revealed the hierarchical organization of genetically distinct subclones within a single tumor mass (Saadatpour et al. 2015). This approach has unveiled rare subpopulations of cells such as cancer stem cells or therapy-resistant clones, which can drive disease progression and treatment failure (Kharchenko, Silberstein, and Scadden 2014; Shekhar et al. 2014; Saadatpour et al.

2015). By reconstructing the evolutionary trajectories of these subclones, scWGS has provided valuable insights into the mechanisms underlying tumor heterogeneity and the emergence of resistant phenotypes (Y. Wang et al. 2014).

By comparing single cell data across multiple patients, researchers can identify common mutational patterns and chromosomal mutations that may contribute to the development and progression of specific cancer types (Wills and Mead 2015; S. Chen et al. 2023). This knowledge can inform the development of targeted therapies and guide personalized treatment strategies tailored to each patient's unique genetic profiles (Thiele et al. 2019; Reza et al. 2021; S. Chen et al. 2023).

Furthermore, single cell sequencing has allowed exploration of the intricate interaction between cancer cells and their microenvironment. By analyzing the genomic profiles of individual cells within the tumor environment, researchers can elucidate the complex dynamics between cancer cells, immune cells, and stromal components components (Figure 1) (Saadatpour et al. 2015). These recent developments have improved the understanding of complex interactions between tumors and their immune microenvironment in various human cancers, which would allow the development of effective immune therapies (Chew, Toh, and Abastado 2012; Bai and Cui 2022).
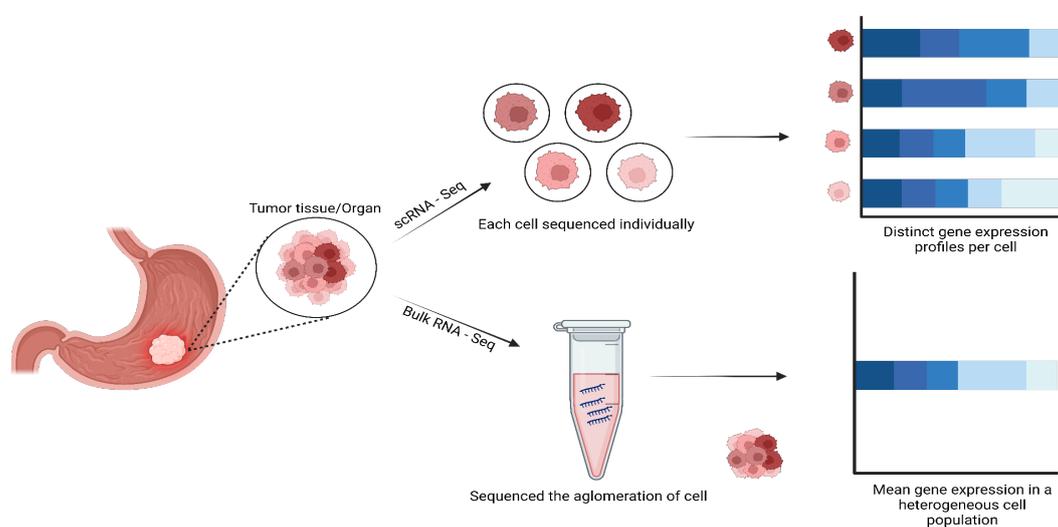

Figure 1. Workflow for single-cell RNA sequencing (scRNA-seq) and bulk RNA sequencing. scRNA-seq reveals the distinct transcriptome of individual cells (top), while bulk RNA sequencing captures the average gene expression profile across all cells combined (bottom). Created in BioRender. Lab, G. (2024) https://BioRender.com/u93e754.

As the field of single cell technologies advances, the integration of multi omic data at a single-cell level, including transcriptomics, epigenomics, and proteomics, holds great promise for unraveling the complex regulatory networks driving cancer cell behavior (Evrony, Hinch, and Luo 2021). By combining these complementary datasets, researchers can gain a comprehensive understanding of the molecular mechanisms underlying tumor heterogeneity, paving the way for more effective and personalized cancer therapies.

3. Applications of Single-Cell Sequencing for Genomic Profiling in Human Cancer Cells

Single cell sequencing (SCS), especially single Cell DNA sequencing (scDNA-seq) technologies have impacted cancer analysis through the genomic profiling of single cells in the tumor and cancer. However, unlike the traditional genomics, single-cell genomics targets intratumoral heterogeneity and microenvironmental elements involved in response and resistance to treatment, providing a broader picture of cancer-related processes (Kim, Eum, and Lee 2021). Here we describe in detail some of the applications of scDNA-seq related to cancer:

(a) Tumor heterogeneity and clonal evolution

Single-cell DNA sequencing has proven to be useful for interrogation of intratumoral heterogeneity and clonal evolution in a plethora of malignancies. Studies pointed out that tumor heterogeneity is due to clonal evolution, creating a fractal pattern of the primary and secondary main clones, sub-clones and single cells, raising issues on the best sampling strategy for tumor sequencing for clinical use (Munkácsy et al. , 2020). A great example is the use of single-cell DNA amplicon sequencing to track clonal heterogeneities of B-Cell Acute Lymphoblastic Leukemia (B-ALL) at diagnosis and during the course of chemotherapy treatment to investigate clonal evolution profile in response to therapy (S. Meyers et al. 2022).

The ability of scDNA-seq method has recently been demonstrated to track clonal evolution and identify dynamics of oncogenic cells in cancer progression and treatment   (L. Xu et al. 2019). It is important to emphasize the applicability of single-cell DNA sequencing, where it is possible to directly obtain clonal genotypes at the cellular level and detect branches in clonal evolution, demonstrating that this approach is superior to other massive sequencing methods, such as bulk sequencing, for study clonal evolution (Borgsmüller et al. 2020). Rajan et al. (2023) analyzed, on 10 tumor samples and 12 019 tumor cells, intratumoral genomic heterogeneity and the evolution of somatic copy number alteration (SCNA) in structurally complex osteosarcoma genomes. In this study they describe genomic homogeneity on these, with a surprising remarkably conserved profiles of SCNA with a limited variability among the subclones; also they find a whole genome duplication (WGD) in many of tumors, these appear to be a mechanism to mitigate the effects of genetic deletions. The most genomic alterations in tumors were acquired early in the oncogenic process and remained stable over time, both in tumor progression and in response to therapy; this suggests an early catastrophic event, rather than sustained genomic instability as the primary cause of the tumor structural complexity. And these tumors exhibited a remarkable stability in their SCNA profiles from diagnosis to relapse, indicating that there is no significant evolution in response to treatments (Rajan et al. 2023).

(b) Identification of Rare Mutations

ScDNA-seq has become a powerful tool in the identification of rare mutations in cancer, offering a high-resolution approach to uncover genomic complexity and compound mutations within individual cells (A. Y. Huang and Lee 2021). Examining cells individually provides the ability to reveal diverse evolutionary trajectories between primary and metastatic tumor cells, yielding insights into genetic heterogeneity within cancer populations (J. Tang et al. 2021). scDNA-seq has been instrumental in revealing the depth of genomic complexity and compound mutations within tumors, providing insights into the unique mutational profiles of individual cells (A. Y. Huang and Lee 2021). This technique has proven to be valuable in the study of somatic mutations allowing the detection of rare mutations that may go undetected with traditional massive sequencing methods (Jaberi et al. 2020). Gráf et al. (2021) illustrates how single-cell analysis can be used in conjunction with next-generation sequencing to identify mosaicism of BRCA2 mutations and decode the cellular processes underlying murine tumorigenesis (Gráf et al. 2021). Furthermore, this strategy improves the sensitivity of rare mutation detection of limited tumor cells by integrating laser capture microdissection (LCM) and NGS technologies, which shed light on BRCA1/2 mutations and the process of tumorigenesis.

(c) Drugs Resistance and Mechanisms

ScDNA-seq is a powerful tool that helps understand the resistance that occurs against cancer drugs. The analysis of individual cells offers information about the heterogeneity within tumors and reveals which subpopulations can generate resistance to treatment (L. Liu et al. 2023). It has been shown that cancer stem cells, a small fraction of the tumor cell population, can cause progression, metastasis, and drug resistance (Pang et al. 2019). Therefore, it is extremely important to identify and target these specific cell populations to improve treatment results.

Another exemplary case is the study by Lee et al. (2022). In this study they employed a scDNA-seq approach to study clonal heterogeneity and clonal evolution in two patients with Myelodysplastic syndrome (MDS) refractory to hypomethylating agents (HMAs). Importantly, HMAs are the mainstay of treatment for MDS, however, in most patients, resistance to treatment and transformation

of the disease into acute myeloid leukemia (AML) was observed. Two patients with HMA resistance and progression to AML were studied. By bulk sequencing, different single nucleotide variations (SNV) or insertions and deletions (INDEL) are detected in these patients, but using the scDNA-seq approach, rare cell clones with mutations that cannot be detected by bulk sequencing was detected, identifying pathogenic copy number variation (CNV) of GATA2, DNMT3A and TET2 which are associated with resistance against HMA but these CNVs could be coupled with small SNVs or INDELs of FLT3 and IDH2, all of this mutations works together, having an impact on disease progression and drug resistance (P. Lee et al. 2022).

Likewise, scDNA-seq also helps us discover and elucidate the mechanisms involved in these resistances, as in the case of quizartinib where on- and off-target mechanisms of resistance which can preexist therapy were identified. In this study they analyzed over 103 000 cells from 16 timepoints across 8 patients, identifying pathogens variants undetected by bulks. In this work they identify FLT3 tyrosine kinase domain (TKD) as a primary mechanism of resistance to quizartinib. The on-target resistance mechanism are: Kinase Domain (KD) Mutation, seven of eight patients developed at least one additional mutation in the FLT3 Kinase domain, most commonly at the D3895 locus, these KD mutations can occur on the native a (FLT3-ITD (internal tandem duplication) negative), in cis with the FLT3-ITD (Peretz et al. 2021).

(d) Detection and Diagnosis the presence of Cancer

scDNA-seq has become a powerful tool to aid and complement cancer diagnosis by providing information on the genetic heterogeneity and molecular characteristics of tumors at the single cell level. Circulating tumor DNA (ctDNA) has been recognized as a valuable biomarker for molecular diagnosis and monitoring of cancer progression through blood samples (Grant et al. 2024). Single-cell sequencing techniques have enabled the detection of circulating tumor cells (CTCs) for liquid biopsy-based diagnosis of multiple cancers, allowing identification of genuine CTCs through concordant copy number alteration profiles (Shen et al. 2024).

Wang et al., (2022) presented a molecular algorithm based on single-cell genomics for early cancer detection (Z. Wang et al. 2022). The study uses single-cell sequencing to examine somatic copy number alterations (CNA) or oncogenic driver mutation profiles at the single-cell level to confirm cellular malignancy. The application of these technologies has also been seen in pancreatic cancer, significantly improving the sensitivity and specificity of cancer cell detection, allowing the analysis of rare cancer cells, circulating tumor cells and metastatic cells (Y. Liu et al. 2022; K. Zhang et al. 2023).

4. Applications of Single-Cell Sequencing for Transcriptomic Profiling in Human Cancer Cells

SCS has emerged as a pivotal method for exploring gene regulatory networks (GRN) and cellular dynamics (S. Chen et al. 2023). Among these technologies, single-cell RNA sequencing (scRNA-seq) provides a detailed view of transcript expression levels and patterns within individual cells across different subpopulations (Kuksin et al. 2021; Lähnemann et al. 2020; F. Tang et al. 2009). Here, we describe applications to sc-RNA related to cancer.

(a) Identifying cancer stem cells and cells rare populations

CSCs (Cancer Stem Cells) are primitive, undifferentiated cells with characteristics like normal stem cells (Ren et al. 2021; Boesch et al. 2016). However, CSCs are biologically characterized by self-renewal, multi-directional differentiation and infinite proliferation, inducing anti-tumor drug resistance and metastasis (Pan et al. 2020). Pan et al., characterized and identified primary and metastatic duct   renal cell carcinoma (CDRCC) and observed CSC specific markers to be correlated with poor prognosis of CDRCC and pinpointed inhibitors for effectively targeting CSCs as   potential therapeutic strategies for CDRCC (Pan et al. 2020).

Other CSC malignancies such as pancreatic cancer have been studied by Ren et al., (2021), specifically pancreatic duct adenocarcinoma (PDAC) which is an aggressive and lethal malignancy (Ren et al. 2021). This study revealed the heterogeneity of ductal cells and identified nine main clusters: endocrine, acinar, endothelial, ductal, myeloid, fibro- blast, pericyte, T, and B cells and then were reclustered identifying 6 different clusters. Most enriched clusters in tumor tissue exhibited

remarkably high copy number variations (CNV) levels. The expression of specific marker genes found to be particular from each cluster type for example on cluster 1 acinar epithelial related genes expressed PRSS1, CLPS (Segerstolpe et al. 2016); heat shock protein-related genes were expressed in cluster 6 are involved in protein transport and folding of ductal cells (Rosas et al. 2016). Clusters 3, 4, and 5 primarily expressed genes related to the proliferation and invasion potential of cells. single-cell trajectory analysis showed pancreatic duct cells originated from clusters 1 and 6, transitioned to cluster 2, and finally evolved into clusters 3, 4, and 5. In this study there could also be identified 202 cancer related genes (CRGs), including 140 upregulated CRGs and 62 downregulated CRGs and establish a relationship between key genes and patient survival. Frequency of mutation events in the high-risk cohort was significantly higher. Kras mutation indicated poor survival in patients. LY6D and MET were significantly more highly expressed in tumor tissues than in normal pancreatic tissues.

(b) Determining heterogeneity within a cell population

Heterogeneity between different malignant cells is one of the fundamental characteristics of almost all human cancers (El-Sayes, Vito, and Mossman 2021; Lenz et al. 2022; Wen et al. 2022). By measuring transcriptomes at the single-cell level, scRNA-seq enables identification of cellular heterogeneity in far greater detail than conventional methods (Haide Chen, Ye, and Guo 2019). Furthermore, scRNA-seq can be used to identify specific modes of gene expression authorized for the elucidation of molecular mechanisms underlying tumor migration and invasion (Y. Zhang et al. 2021; Wen et al. 2022). Also, constructing gene regulatory networks (GRNs) from scRNA-seq data is used to explore intratumoral heterogeneity and can elucidate the critical genes involved in cancer development (Wen et al. 2022). For instance, Wouters et al. (2020) used single-cell transcriptomics in combination with GRNs and trajectory inference to study 10 melanoma cultures that have mapped the gene regulatory landscape of relapsed melanoma (Wouters et al. 2020; Wen et al. 2022).

(c) Tumor immunology

The immune system is composed of a complex hierarchy of cell types that protect the organism against disease and maintain homeostasis (Haide Chen, Ye, and Guo 2019). Understanding how the immune system affects cancer development and progression has been one of the most challenging questions in immunology (Schreiber, Old, and Smyth 2011). Identifying heterogeneity of immune cells is the key to understanding the immune system. Advanced scRNA-seq technologies are revolutionizing our ability to study immunology. Recent studies from Hui et al. used scRNA-seq to reveal how neoadjuvant PD-1 blockade combined with chemotherapy remodels the tumor microenvironment in non-small cell lung cancer. They identified significant remodeling of immune cells within the tumor, including the expansion, activation, and phenotypic alterations of cytotoxic T cells, CD16+ natural killer (NK) cells, and regulatory T cells (Tregs) following therapy (Hui et al. 2023).

(d) Cancer progression, drug development and cancer treatment

There is an increased interest in potential clinical application of single-cell techniques reflected in collaborative initiatives like LifeTime. LifeTime aims to understand the complex behavior of human cells during disease progression and analyze their response to the therapy, all at single-cell resolution (Rajewsky et al. 2020; Stein et al. 2021). To achieve that, further development and integration of multiomics methods is urgently needed (Stein et al. 2021).

ScRNA-seq has enabled identification of molecular pathways that allow prediction of survival (Sade-Feldman et al. 2018; Van de Sande et al. 2023), response to therapy (Jang et al. 2019; Van de Sande et al. 2023), likelihood of resistance (Tanaka et al. 2018; Jerby-Arnon et al. 2018; Van de Sande et al. 2023) and candidacy for alternative intervention (Cohen et al. 2021; Van de Sande et al. 2023).

Recently, an increasing number of clinical trials have been integrating RNA-seq in their design with various objectives; either biological description of the effect of treatments or with the intent to treat patients (Kuksin et al. 2021). Single-cell genome-plus-transcriptome sequencing is also a valuable tool to study the efficacy and safety of genome editing in germline therapy. Genome editing of human embryos or germ cells provides the means for introducing heritable genetic alterations, which may reduce the burden of genetic disease in specific familial situations (Vandereyken et al.

2023; Bekaert et al. 2022). For instance, Vishnubalaji and Alajez (2023) used computational algorithms to decipher the cellular composition of various Breast Cancer (BC) subtypes, including estrogen receptor-positive (ER+), HER2+, ER+HER2+, and triple-negative (TNBC) (Vishnubalaji and Alajez 2023). They analyzed transcriptomic data from 49,899 single-cells, derived from 26 BC patients, and integrated differentially expressed genes with CRISPR-Cas9 perturbational gene effects data from the Achilles project (R. M. Meyers et al. 2017). The key discoveries include ER+ BC: 13 targets, with RPS4X, RPL34, and VMP1 being more effective than ESR1. HER2+ BC: 44 targets, some stronger than ERBB2, with enriched processes in mRNA decay and protein targeting. TNBC: 29 targets, enriched in processes like protein targeting and mRNA processing. Overall, these findings point to new potential targets for treating each subtype.

Other studies are using tumor model systems exposed to treatment, or direct longitudinal sampling of patient tumor specimens before and during treatment to analyze single-cell genome-plus-transcriptome sequencing to understand the genetic subclones resistant to drug selection. Additionally, it will allow the study of how cells within these   genetic subclones putatively apply cell plasticity to change their gene   expression repertoire and accommodate different phenotypic cancer   cell states able to withstand drug treatment and, eventually, acquire   resistance (Rambow et al. 2018; Vandereyken et al. 2023). In turn, these approaches might enable the identification of potential cancer cell vulnerabilities, such as druggable molecular players involved in the acquisition of drug tolerance (Vandereyken et al. 2023).

Single-cell DNA sequencing enhanced our understanding of cancer in human cells, this technology allows tracking clonal evolution, detecting rare mutations, understanding drug resistances, improving cancer diagnosis with the implementation of single-cell RNA sequencing, to get an integral understanding of treatment in cancer (figure 2).
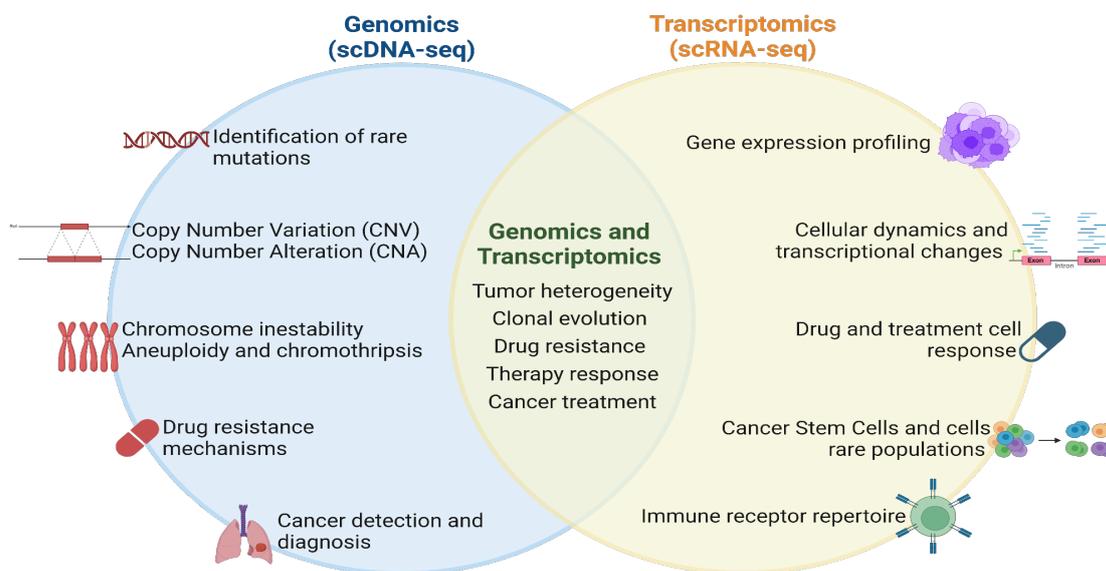

Figure 2. Applications of single-cell sequencing for genomic and transcriptomic profiling in human cancer cells. Applications that are carried out by both technologies, independently or integrated, are found in the middle. Created in BioRender. Lab, G. (2024) https://BioRender.com/y81r919.

5.. 'Co-Presence' and 'Phenotypic Association' Capability of scSeq Technology

The 'c'o-presence' functionality of SCS technology refers to its ability to identify the various genomic features or variations concurrently present within each cell analyzed(Evrony, Hinch, and Luo 2021). For example, it can detect the simultaneous presence of multiple or single mutations(Petti et al. 2019; X. Xu et al. 2012), copy number alterations(Yan Gao et al. 2017; Wu et al. 2021), and structural variants(Aganezov et al. 2020) within the same single-cell. This capability provides insights into the interplay between different genomic aberrations and their potential combined effects on cellular processes or disease development(Funnell et al. 2022). By capturing the co-occurring genomic

variations within individual cells, researchers can better understand the heterogeneity and complexity of genomic landscapes, particularly in diseases like cancer, where cells can harbor diverse combinations of mutations and other alterations. Furthermore, the ability to detect co-presence in SCS enables scientists to concurrently identify the manifestation of both genetic alterations and epigenetic changes within each cell analyzed(Shema, Bernstein, and Buenrostro 2019). As well as uncovered widespread variation among malignant cells concerning their cellular identity and developmental staging(Schuster 2022). Therefore, co-presence is a crucial capability of SCS that enables many of its unique applications, revolutionizing our understanding of genomics and disease development.

Conventional sequencing methods, which analyze entire cell populations, often face significant limitations. One such limitation is their inability to detect the simultaneous presence of multiple mutations within individual cells.   This drawback results in a loss of information about the cellular diversity within the population(Misra, Jadhav, and Bapat 2022). In contrast, single-cell omics offer a superior approach to unraveling cellular diversity. SCS, in particular, preserves this information, allowing for a more holistic study of cells. Interestingly, studies have begun to use both, single-cell sequencing and multi-omics (bulked sequencing) datasets to analyze the molecular diversity of hepatocellular carcinoma (HCC) across inter- and intra-tumor levels(T. Wang et al. 2022). This approach exemplifies how we can enhance the understanding of co-presence in cell studies by integrating cutting-edge and traditional technologies.

The capability of SCS to associate phenotypes is a significant advancement in genomics. It allows for integrating genomic data from individual cells with simultaneous phenotypic profiling as a crucial link between a cell's genotype and phenotype(Evrony, Hinch, and Luo 2021). This feature aids in identifying cellular identities or states characterized by specific genomic features. However, it's important to note that existing single-cell data cannot directly associate cell clusters with phenotypic characteristics. Unfortunately, due to feasibility and sample size limitations, single-cell technology is not yet suitable for extensive cohort studies, with most experiments involving fewer than twenty patient samples, thereby lacking the statistical power needed to pinpoint the cell subpopulations driving the phenotype of interest(Redavid et al. 2022).

The complexities surrounding co-presence and phenotypic association analysis encompass the widely recognized technical noise, intricacies of data analysis, and the necessity for integrative approaches. Regardless of these challenges, SCS persists as a potent methodology for comprehensively investigating co-presence and phenotypic association in cellular entities.

6. Immune Cells Response in Tumor Microenvironment Using scWGS

Beyond the important roles immune cells play in determining the immune responsiveness of tumors, they are also important direct targets of immunotherapeutic strategies. It is thus pivotal to identify molecular mechanisms that modulate immune responsiveness and therapeutic performance of the tumor microenvironment (TME) immune cells. These mechanisms involve non-genetic events that pose significant regulatory elements to be characterized. For instance, Huang et al., highlighted the role of microvascular invasion-related malignant cells, which are driven by MYC pathway activation and MIF signaling within the TME of hepatocellular carcinoma (HCC). Their findings underscore how specific malignant subpopulations interact with immune cells, shaping prognosis and immune responsiveness(H. Huang et al. 2024). At the same time, cellular transition trajectory analysis in single-cell epigenomes further demonstrates that epigenetic features do not always directly predict future cell-fates or gene activities but are reciprocal to transcriptional dynamics. Identification and decoding of the epigenetic features associated with unique TME states and functions in vivo would thus require extensive studies of the single-cell TME epigenomes and their interplay with the transcriptional landscape(M. Kumar 2023). Because of these important roles, TME immune cells play in predicting the prognosis and therapeutic performance of distinct human cancer types, their molecular properties are urgently needed to be characterized with a personalized precision medicine, so that therapeutic strategies to modulate, within TME immune cells efficiently, can be designed(Sierant and Choi 2018).

It has been documented that various immune cells that are part of the TME can play important roles in promoting or inhibiting tumor development and metastasis. These cells, collectively called tumor-infiltrating immune cells 1 (TIICs), are also significantly associated with clinically relevant features, such as patient survival and response to immunotherapy. Efforts aiming to understand the molecular characteristics of immune cells, especially those within the TME have thus gained significant traction. To date, single-cell genomics has emerged as a widely used tool to study TIICs, and has identified various subpopulations of T cells and myeloid cells and shown spatial, differentiation, and functional heterogeneity within the TME(A. W. Zhang and Campbell 2020). For instance, Zhang et al. (2021) demonstrated age-related differences in the immune landscape of melanoma-bearing mice, where older mice exhibited a higher proportion of cytotoxic CD8+ T cells and fewer exhausted cells, contributing to enhanced antitumor immunity. This highlights the importance of dissecting immune cell subpopulations at the single-cell level to better understand their role in tumor progression and response to immunotherapies(C. Zhang et al. 2021).

Similarly, Chen et al. (2024) demonstrated that the upregulation of HMGB2 shapes the immunosuppressive microenvironment in HCC, correlating with exhausted T cells and poorer clinical outcomes. This underscores how epigenetic and transcriptional regulation of factors like HMGB2 can hinder immune cell functionality and drive resistance to immunotherapy, making it a key target for therapeutic intervention (Y.-Z. Chen, Meng, and Xiang 2024). Multi-omics technologies that provide single-cell data at the transcriptional/genomic, epigenetic, proteomic, and other levels have been pivotal in exploring the states and functions of TME immune cells. These have shown that epigenetic dynamics have a prominent influence on the cellular status of TME immune cells. Furthermore, parallel profiling of chromatin state and gene expression in single-cells revealed that chromatin modifications could be predictive of cell state dynamics and that cellular transcriptomes might not always reflect their epigenetic status. Myeloid-lineage immune cells are also influenced by epigenetic chromatin modifications, which can directly control their TME functions.

(a) Tumor microenvironment

Tumors are highly complex entities composed of heterogeneous tumor cells, stroma cells and immune cells(Wolfe et al. 2022). Intertwined biological processes between different types of cells facilitate tumorigenesis, tumor progression and resistance to therapy, as well as, contributing to tissue homeostasis maintenance of normal physiological processes. Consequently, for effective therapeutic intervention, it is crucial to understand the underlying cellular and molecular knowledge and their roles in orchestrating the tumor microenvironment(S. Wang et al. 2023). The constituents of the TME and their role in tumorigenesis and in response to therapy are a rapidly evolving field, thanks to cutting-edge methodological advances. single-cell analysis has facilitated the understanding of immune cell responses in the TME and has allowed researchers to study the functional and molecular aspects of cancer immunologists in greater detail. For instance, Pires et al. (2020) demonstrated how T cells drive ECM remodeling and CSC reduction in fibrosarcomas, revealing how immune activity in the TME directly impacts tumor rejection and progression(Pires et al. 2020). A thorough understanding of immune cell activity in the TME is essential in developing newer drugs and treatment options in cancers.

(b) Cellular Components of Tumor Microenvironment

These immune cells interact with each other and with cancer cells, secreting a variety of soluble factors, contributing to tumor initiation, progression, and invasion. The function of these cellular components and the tissue-specific microenvironment are deregulated in TME, with pro-tumorigenic and anti-tumorigenic activities being intertwined.

TME consists of non-cancerous cells, their associated cell products, and extracellular matrix within and around the tumor mass. The cellular components of TME are macrophages, granulocytes, mast cells, myeloid-derived suppressor cells, dendritic cells, T cells, B cells, natural killer cells, cancer-associated fibroblasts, mesenchymal stromal cells, endothelial cells, pericytes, and nerve cells. These cells perform various functions such as immune response, cell signaling, and extracellular matrix remodeling, crucial for tumor growth and progression. For instance, Anderson and highlighted that macrophage, especially M2 macrophages, along with myeloid-derived suppressor cells (MDSCs),

contribute to immunosuppression by secreting cytokines such as IL-10 and TGF-β. These cytokines activate key signaling pathways like NF-κB and STAT3, facilitating tumor growth and immune evasion. Additionally, cancer-associated fibroblasts play a significant role in ECM remodeling, supporting metastasis and tumor invasion(Anderson and Simon 2020). Alcantara et al. (2023) further demonstrated that targeting STAT3 activation in MDSCs through CpG-STAT3 antisense oligonucleotide (ASO) therapy significantly reduces immunosuppression and enhances T-cell activity in renal and bladder cancer. This combination therapy, when used with anti-PD-1, improved tumor control by reducing MDSC-driven immune resistance and promoting tumor regression(Alcantara et al. 2023). Li et al. (2020), emphasize that tumor-associated macrophages (TAMs) and MDSCs, along with cytokines like IL-6 and TGF-β, contribute to chronic inflammation and immunosuppression within the TME. Targeting these cells and pathways could improve cancer therapies by disrupting the immunosuppressive TME(L. Li et al. 2020).

(c) Overview of tumor microenvironment at single-cell resolution

The accessibility of single-cell sequencing to mixed cell populations in a non-perturbing manner has made it an attractive technique to rationalize the dynamics of cell heterogeneity in the TME. Due to their relatively low assay cost and high-throughput performance compared with imaging technologies, single-cell sequencing has been widely applied to study various types of tumors and mouse models, demonstrating that single-cell sequencing enables research of tumor cells andTME's cellularity and spatial distribution. Moreover, SCS reveals signaling interactions among different cell types, potentially uncovering novel factors driving the initiation and progression of cancers besides uncovering novel cell subsets with unknown functions.(V. Kumar et al. 2022; Nofech-Mozes et al. 2023)

In a translational perspective, single-cell sequencing provides vast advantages over the traditional bulk RNA-seq assay since the former could identify the complex heterogeneity in not only the cancer cells themselves but also historically stroma cells, opportunities, as well as challenges for developing novel cancer treatments. Single-cell sequencing provides comprehensive views on statics and dynamics of the cell composition in the TME along with their spatial distributions, which not only facilitate the discovery of potential biomarkers in a clinical routine but also advances researchers from bench to bedside, which would subsequently benefit cancer patients in the future.(J. J. Lee et al. 2021; Qian et al. 2020; Jianhong Zhang et al. 2021).

A central question addressed by single-cell sequencing of the tumor microenvironment is the cellular heterogeneity among stromal and immune cell types. Importantly, single-cell sequencing allows defining cell populations at higher resolution than possible by bulk sequencing, offering potential identification of biomarkers that would be otherwise diluted when bulk material is used. To this end, single-cell sequencing of dendritic cell subsets is a perfect test case. Dendritic cells are generally a rare cell type in the whole blood (0.36%) and single-cell technology could offer a better representation of the subsets such as plasmacytoid dendritic cells (pDC). The ability to define these dendritic cell subsets would have great implications in allowing personalized treatment of cancer through the application of active immunotherapies. Recently, single-cell RNA sequencing has been used to define dendritic cells, monocyte, and other diverse cell populations in triple-negative breast cancer (TNBC) and murine melanoma tumors. For instance, Gao et al. (2021) utilized single-cell RNA sequencing (scRNA-seq) to reveal the heterogeneity of monocyte-derived dendritic cells (moDCs) and type-2 conventional dendritic cells (cDC2s). Their study identified seven distinct DC subtypes, with functional differences in antigen processing and immune regulation, especially during maturation. This demonstrates the utility of scRNA-seq in uncovering novel immune cell subsets and their potential roles in shaping tumor immunity, offering insights into how these cell populations could be targeted for immunotherapy(Yuehan Gao et al. 2021). scRNA-seq allows to gain insight into spatial localization of tumor and stromal cells in the tumor microenvironment over bulk RNA sequencing technology. Distinct cellular localization within different parts of the tumor microenvironment, for example, within the tumor, invasive margin, and the center of the tumor, or between epithelial and stromal compartments, may allow a dissection of the distinct functions and mechanisms of tumor-associated cells.(Sun et al. 2022; Jian Liu et al. 2020; Ke et al. 2022).

7. Role of Single-Cell Data Analysis Technologies in Cancer Therapy

Cancer is a complex and heterogeneous disease, characterized by diverse cellular populations within a tumor and dynamic interactions between cancer cells and their microenvironment. This heterogeneity poses significant challenges in understanding disease mechanisms and developing effective treatments. The advent of single-cell data analysis technologies has revolutionized our ability to dissect the complexities of cancer at an unprecedented resolution, unveiling new insights into tumor biology and paving the way for personalized cancer therapy. The increase in SCS data and methodologies for wet-lab applications has been paralleled by advancements in data analysis tools to interpret this data(Svensson, Vento-Tormo, and Teichmann 2018; Zappia and Theis 2021).

The data analysis options available for a particular biological sample are largely dictated by the sequencing technique used to generate the raw data and depend on the research question and study objectives. The type of cell isolation employed in a study is closely aligned with its performance regarding capture efficiency and purity of target cells, which directly affects the output data and consequently the data analysis approach(G. Chen, Ning, and Shi 2019; Gross et al. 2015). For example, a droplet-based method is particularly effective for characterizing tissue composition, as it enables the capture of a substantial number of cells(Wolfien, David, and Galow 2021).

Single-cell data analysis generally involves distinct preprocessing and integration stages. Preprocessing focuses on preparing individual datasets through demultiplexing, quality control, filtering data and normalization. Integration then combines these datasets, using methods like data alignment and correction to account for technical variations (e.g., batch effects) and/or integrate data from different omics (Figure 3). Feature selection and dimensionality reduction are often performed after integration to facilitate downstream analyzes like clustering and trajectory inference. The pipeline choice can have a comparable or even greater impact on the ability to detect biological signals in scRNA-seq data than increasing the cell count from 96 to 384 cells analyzed(Vieth et al. 2019).
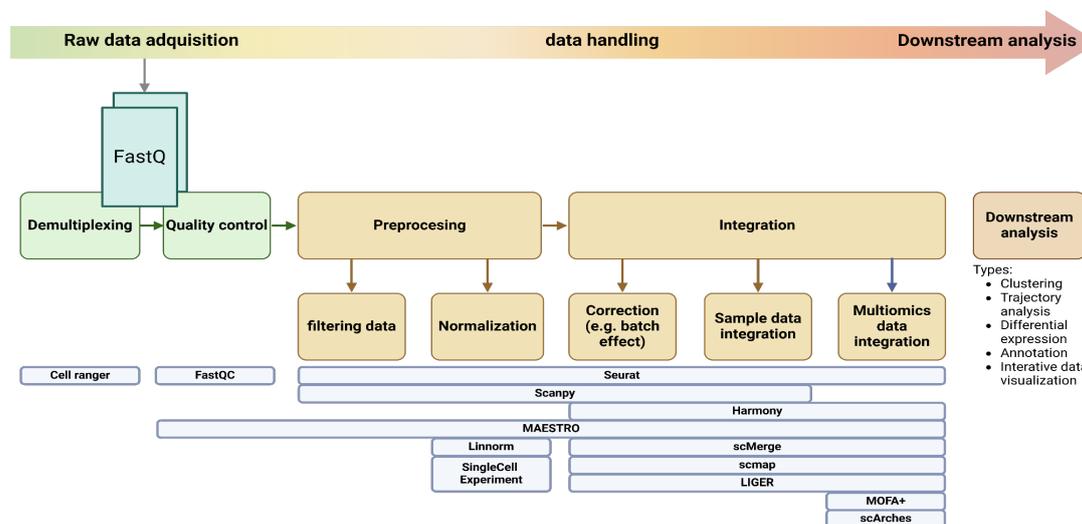

Figure 3. Overview of common tools used in single-cell RNA sequencing (scRNA-seq) data handling workflow. The workflow begins with raw data acquisition in FastQ format, followed by demultiplexing and quality control to ensure reliability. It then transitions to preprocessing and integration stages. In preprocessing, data undergo filtering to eliminate low-quality cells, normalization to address technical variations, and correction for batch effects. The integration phase combines data from different samples and/or incorporates multi omics data for a comprehensive analysis of cell populations, depending on the study. Finally, the workflow culminates in downstream analysis, encompassing clustering to identify distinct cell populations, trajectory analysis to infer developmental pathways, differential expression analysis for gene variation, annotation for cell type identification, and interactive data visualization to interpret complex datasets. This systematic approach provides a simplified overview of the steps used for processing and analyzing scRNA-seq data. Created in BioRender. Lab, G. (2024) https://BioRender.com/r42z271.

(a) Preprocessing and Integration

The analysis of single-cell data begins with robust and effective data handling and integration. CellRanger is a key pipeline that primarily does the initial step of preprocessing raw single-cell RNA-seq by performing demultiplexing, alignment, and quantifying gene expression(Zheng et al. 2017). It uses these barcodes and UMIs to find low-quality cells and identify reads originating from the same cell(Tjoonk 2023). It is also known that it can cluster similar or the same cells into non-overlapping groups. Some tools merge data handling and integration with the subsequent analysis for a multimodal approach(Wolf, Angerer, and Theis 2018). Seurat is a great example of a multimodal analysis tool that enables researchers to do further analysis from data handling and integration(Hao et al. 2024). However, there are tools dedicated just to data handling experiments, like SingleCellExperiment(Risso et al. 2018; Amezquita et al. 2020) that include specialized methods to store and retrieve spike-in information, dimensionality reduction coordinates, and size factors for each cell, along with the usual metadata for genes and libraries. This flexible data representation is compatible with the Bioconductor ecosystem, allowing seamless integration with other analysis tools and pipelines. Additionally, tidySingleCellExperiment bridges the gap between SingleCellExperiment objects and the Tidyverse ecosystem(Wickham et al. 2019), promoting reproducible and efficient data handling and integration workflows.

Due to the difference in experimental methods and computational analysis in SCS, comparing directly cell identities between different experiments can be challenging. For this issue, tools like scmap(Kiselev, Yiu, and Hemberg 2017) address this by allowing users to integrate cell-type or individual cells in different experiments. Similarly, LIGER (Welch et al. 2019) can be used to compare and contrast across experimental batches, individuals, sex, tissues, species, and is particularly useful for handling different modalities like RNA-seq and ATAC-seq.

Unlike the single-cell Experiment, scmap offers further data exploration (downstream analysis) to identify clusters, find shared gene markers, compare clusters, and visualize clusters and gene expression, foir. Similarly, scMerge(Y. Lin et al. 2019) offers a robust method for merging multiple batches of single-cell RNA-seq data, accounting for potential batch effects and enabling integrated analyzes across diverse datasets. MAESTRO(C. Wang et al. 2020) besides doing quality control, normalization, and filtering of single-cell data, specializes in the integration of single-cell transcriptome and regulome for evaluating scATAC-seq clustering, automatic cell-type annotation, integration between scRNA-seq and scATAC-seq.

Moreover, many specialized tools analyze specific modalities. DropletUtils(A. Lun, Griffiths, and McCarthy 2021) facilitates the analysis of droplet-based single-cell data, while zellkonverter(Zappia L 2024) enables interoperability by converting between Python and R environments, allowing seamless data transfer between these platforms. Furthermore, data normalization and transformation methods, such as Linnorm(Yip et al. 2017), play a crucial role in ensuring data quality and comparability across samples. Linnorm provides robust normalization and transformation of data based on linear models and normality assumptions, enabling accurate downstream analyses. It preserves biological variations in scRNA-seq data and removes technical noises simultaneously.

Many studies utilize a multi-omics approach for cancer research, highlighting the importance of accurately integrating diverse data types for effective analysis. By combining transcriptomics, epigenomics, and proteomics, researchers can gain a comprehensive understanding of cellular states and the regulatory mechanisms that drive them(Ruan et al. 2024; Xing et al. 2023; Warfvinge et al. 2023). Tools like MOFA+(Argelaguet et al. 2020) and scArches(Lotfollahi et al. 2022) enable the integration of diverse omics data, revealing shared and unique features across different molecular layers. Spatial analysis techniques, such as spatialHeatmap(Jianhai Zhang et al. 2024), spicyR(Canete et al. 2021), and SpatialExperiment(Righelli et al. 2022), facilitate the exploration of spatial patterns and heterogeneity within tumor samples, providing insights into the tumor microenvironment and its influence on cancer progression and treatment response.

(b) Clustering and downstream analysis

Identifying distinct cell populations and subgroups within heterogeneous tumor samples is a critical step in understanding cancer biology. Clustering algorithms like bluster (Aaron Lun 2024), celda (Campbell et al. 2021), and SC3 (Kiselev et al. 2017) group cells based on their transcriptome or genomic profiles, enabling the identification of distinct cell populations. Differential analysis methods, such as DEsingle (Z. Miao et al. 2018), and distinct (Tiberi et al. 2020) identify genes, pathways, or molecular features that are differentially expressed or regulated between cell populations or experimental conditions, providing insights into the molecular drivers of cellular heterogeneity.

In the other hand there a well-known multimodal tool like Seurat (Hao et al., 2024) and Scanpy (Wolf et al., 2018) that enable quality control, analysis, clustering and differential expression capabilities for scRNA-seq data, facilitating the identification and interpretation of cellular heterogeneity. Tools like SCENIC (Aibar et al., 2017) infer active transcription factors and gene regulatory networks, providing insights into gene regulation within tumor cells. CopyKAT (Gao et al., 2021) and InferCNV (inferCNV of the Trinity CTAT Project) specialize in inferring genome-wide copy number variation profiles from scRNA-seq data, a critical aspect of cancer genomics. Other recently used tools such as non-linear dimensional reduction (UMAP) cluster cells with a graph-based clustering approach (Khayatan, Hussain, and Tebyaniyan 2023; Y. Miao et al. 2024) and SingleR does cluster definition plus annotation (Aran et al. 2019).

(c) Single-cell DNA and Whole Genome Sequencing (scWGS)

Single-cell DNA-sequencing (scDNA-seq) and whole genome sequencing (scWGS) techniques offer unprecedented insights into genomic alterations, such as copy number alterations, copy number variations, and clonal heterogeneity within tumors. Here, we present some tools for the downstream data analysis of scDNA-seq. The tool Ginkgo (Garvin et al. 2015) identifies copy number variations, CHISEL (Zaccaria and Raphael 2021) infer copy number alterations including whole-genome duplications (WGDs), while PyClone (Roth et al. 2014) and SCCNV (Dong et al. 2020) elucidate clonal populations and their evolutionary trajectories. SCcaller (Dong et al. 2017) and SCCNV identify single-nucleotide variations and copy number variations, respectively, and SCITE (Jahn, Kuipers, and Beerenwinkel 2016) designed tool to infer the evolutionary history of tumors using noisy and incomplete mutation profiles of single-cells from scDNA-seq data.

The integration of single-cell data analysis technologies has revolutionized our understanding of cancer biology, enabling the exploration of tumor heterogeneity, clonal evolution, and cellular interactions within the tumor microenvironment. As single-cell technologies continue to advance, data analysis tools will play a crucial role in leveraging the wealth of information contained within these datasets, ultimately paving the way for personalized and targeted cancer therapies tailored to individual patients' tumor profiles.

8. Emerging Technologies and Future Directions in scWGS in Cancer Biology

The rise of single-cell whole-genome sequencing (scWGS) has transformed cancer biology, offering detailed insights into the molecular landscapes of cancer cells. Deep learning techniques have proven highly effective in analyzing the vast and complex data generated by scWGS (Erfanian et al. 2023; Molho et al. 2024). These methods have outperformed traditional computational approaches in various domains (Premkumar et al. 2024; Zhu et al. 2024). In cancer biology, deep learning has facilitated the study of tumor cell heterogeneity, uncovering gene expression patterns and regulatory networks governing cell behavior (Halawani, Buchert, and Chen 2023; Molho et al. 2024).

Artificial intelligence (AI) has emerged as a powerful tool in scWGS analysis, with machine learning (ML) algorithms excelling at extracting valuable insights from high-dimensional and complex data (Qi and Zou 2023). In cancer research, AI has contributed to the development of prognostic models, biomarker identification, and characterization of the tumor microenvironment (Jianlan Liu et al. 2023; Mou et al. 2023). For example, Chen et al. (2023) used AI to construct a communication signature derived from cancer-associated fibroblasts (CAFs), enabling the stratification of clear cell renal cell carcinoma patients based on their immune profiles and potential response to immunotherapy (Hualin Chen et al. 2023). Similarly, Kang et al. (2024) employed AI

techniques to identify tumor-associated macrophage subpopulations in prostate cancer, revealing subpopulations that may facilitate tumor progression by enhancing immune evasion and altering the tumor microenvironment, which can lead to therapeutic challenges in tumor progression and drug resistance (Kang et al. 2024).

Deep learning, a branch of AI, has emerged as a transformative force in scWGS, offering algorithms that can integrate multi-omics data, including genomics, transcriptomics, and proteomics, to unravel the complexities of tumor heterogeneity and identify cancer subtypes (He et al. 2023; J. Li et al. 2023). Danishuddin et al. (2024) explored the applications of deep learning in cancer genomic and proteomic studies, highlighting its potential to improve patient diagnosis, prognosis, and treatment strategies (Danishuddin, Khan, and Kim 2024). In glioma research, Luo et al. (2023) demonstrated the utility of deep learning in tumor segmentation, diagnosis, grading, and characterizing the tumor microenvironment, paving the way for personalized treatment approaches (Luo et al. 2023). As scWGS continues to evolve, deep learning will play a role in extracting meaningful insights from multi-omics data, driving the development of precision oncology.

In the future, developing technologies for single-cell analysis in cancer is crucial. Techniques in addition to DNA-based and RNA-based methods such as scEpigenetics, sc proteomics, sc metabolomics, sc CRISPR technologies and sc multi omics technologies, may be particularly helpful in advancing this field. Additionally, the advancements in machine learning, AI and Data Analysis will further enhance our understanding and capabilities in cancer biology and its implications. Consequently, more studies focusing on areas such as prostate cancer, breast cancer, cervical cancer, and solid tumors especially for complex matrices that are difficult to understand would be beneficial for future at the level of diagnosis, microenvironment, understanding of existing pre and post resistance, better targeted and efficient therapies, all supported by a robust bioinformatics analysis that will enhance these benefits through the incorporation of cutting-edge technologies such as artificial intelligence.

The integration of these tools into cancer profiling has greatly enhanced our ability to understand tumor complexity, genomic instability and clonal evolution. This comprehensive understanding of tumor complexity allows for more accurate predictions for treatment responses, identification of resistance mechanisms, and development of more personalized and effective therapeutic strategies, taking us to the way for precision oncology.

9. Conclusions

The introduction of single-cell whole genome sequencing (scWGS) has revolutionized our understanding of cancer biology, providing unprecedented insights into tumor heterogeneity, clonal evolution, and the complex interactions within the tumor microenvironment. This technological breakthrough has enabled researchers to analyze the genomic, transcriptomic, and epigenomic landscapes of single-cells and uncover the complex cellular dynamics that drive cancer progression, metastasis, and treatment resistance.

The unique capabilities of scWGS, particularly its accuracy in detecting rare genomic events, co-presence functionality in capturing multiple genomic features simultaneously, and phenotypic association potential, have opened new avenues for cancer research and precision medicine. These advances have facilitated the identification of rare cell populations such as cancer stem cells and elucidated the heterogeneous nature of tumors with unprecedented resolution.

Furthermore, the integration of scWGS with other single-cell omics technologies, including scRNA-seq, scATAC-seq and single-cell proteomics, has enabled a multidimensional view of cellular states and regulatory mechanisms in cancer. This integrative approach has enhanced our ability to decipher the complex interplay between genetic alterations, gene expression patterns and epigenetic modifications that contribute to tumor initiation, progression and therapeutic response.

The rapid development of data analysis tools and computational methods, including advanced machine learning and artificial intelligence algorithms, has been critical to harnessing the vast amounts of data generated by single-cell technologies. These sophisticated analytical approaches

have enabled the identification of new biomarkers, the creation of predictive models for patient stratification and the development of targeted therapeutic strategies.

As this field continues to evolve, new technologies such as spatial transcriptomics and in situ sequencing will further improve our understanding of the spatial organization and cellular interactions within tumors. In addition, the integration of single-cell multiomics data with clinical information and longitudinal studies will be critical to translate these insights into actionable clinical strategies.

In summary, scWGS and related single-cell technologies have ushered in a new era of cancer research, offering unprecedented opportunities to unravel the complexity of tumor biology. As these technologies continue to evolve and become more accessible, they promise tremendous advances in advancing personalized cancer diagnostics, prognosis and therapy, ultimately improving patient outcomes in the era of precision oncology.

The challenges regarding co-presence and phenotypic association analysis include the well-known technical noise, data analysis complexities, and the need for integrative approaches. Dropout events, amplification bias, and sequencing errors are common technical noises that compromise the data quality. Data analysis complexities stem from difficulties in accurate variant calling, interpreting cellular heterogeneity, and integrating genomic data with phenotypic traits, which will be discussed further in the subsequent section. Moreover, integrative approaches such as multi-omics integration, longitudinal studies, and functional validation add complexity but are essential to creating robust studies. Other issues like sample preparation, scalability, cost, statistical power in cohorts, and the need for standardized protocols also present significant hurdles. Despite these challenges, SCS remains a powerful methodology for delving deeper into the analysis of co-presence and phenotypic association in cells.

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