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Innovative Advancements in Single-Cell Sequencing Technologies for Comprehensive Analysis of Cellular Heterogeneity and Its Role in Cancer Progression and Therapeutics

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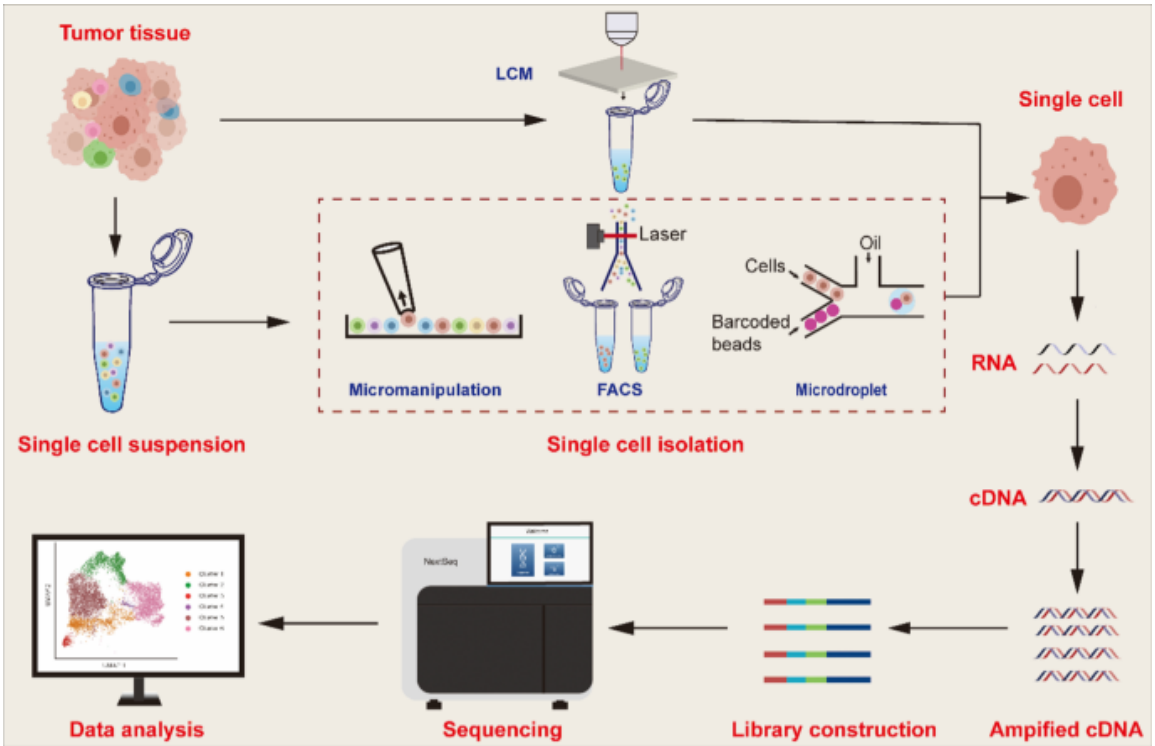
Abstract

Cancer is a group of rather diverse diseases that develop from normal to malignant state by means of functional capacity generation. Understanding the mechanisms of emergence, progression, metastases, and treatment resistance remains challenging despite such robust experimental and computational approaches. But since big scale RNA sequencing technology only shows an average gene expression of the sample, they cannot grasp the heterogeneity of the tumor microenvironment. The chance to grasp the minor tumor biology differences has come from the recent arrival and development of single cell RNA sequencing (scRNA-seq) technology that can identify different cell subpopulations, evaluate the tumor microenvironment, and characterize cellular genetic changes. The cancer research progressively started using more and more scRNA-seq technology to investigate the tumor microenvironment and heterogeneity in recent years in order to improved knowledge of carcinogenesis and evolution of cancer. The basics, development and increasing use of scRNA-seq technology in clinical practice and cancer research are described in this work. Because of high resolution transcript sequencing, ScRNA-seq has been shown to be a useful technique as it offers heretofore unheard mapping data for cancer study. New cell subpopulations and markers, gap intra, and inter-tumor heterogeneity, tumor microenvironment, intercellular communication, and lineage trajectories may all be defined using it. Our knowledge of cancer development, diagnosis, prognosis, therapy and medication resistance has been much improved by such investigations. Still, there are several restrictions that prevent the scRNA-seq from finding general use. First, while it does not data cancer biology at the most understandable level (in the level of proteomics, epigenomics, genomics, etc.), scRNA-seq is the most developed and extensively utilized single cell omics in cancer research. Second, scRNAseq only uses living cells as detection object and has great sample demand, hence there will be significant batch effects on samples examined at various times. Thirdly, RNAs could be broken down and single cell suspension produced from enzymatic digestion of cells could be killed. Furthermore, producing this effect is the loss of morphological and spatial information.

INTRODUCTION

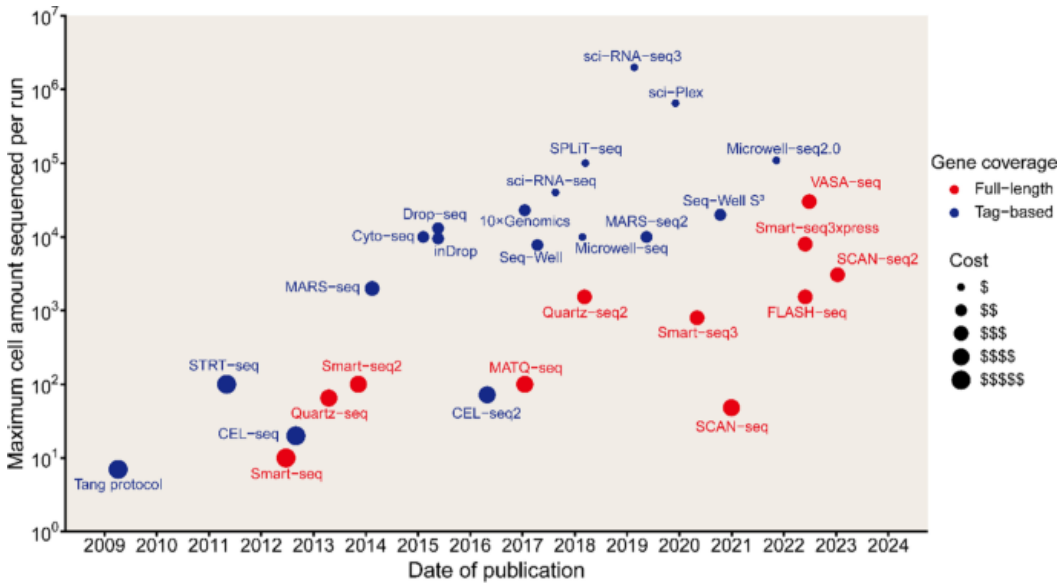
One of the main global problems of the kind affecting the whole body is cancer. There were a few quite significant modifications throughout its development and progression (that happened at precancer to cancer, local to metastases, and treatment responsive to therapy resistant tumors) (Rozenblatt-Rosen O, Regev A,2020). While cancer is notoriously difficult to cure, the tumor is clonally diverse, and the tumor microenvironment (TME) is complicated (Liu J, Xu T, Jin Y, Huang B,2020). Part of the explanation for drug resistance, invasion, metastases, carcinogenesis and progression is EMT and tumor heterogeneity (Erfanian N, Derakhshani A, Nasseri S, Fereidouni M, Baradaran B, Jalili Tabrizi N, et al, 2022). The era of precision medicine is finally brought up by the advent of vast amounts of molecular data from one single cancer sample resulting from the rise of sequencing technologies in clinical oncology (Amelio I, Bertolo R, Bove P, Buonomo OC, Candi E, Chiocchi M, et al,2020). For what is known as "precision medicine" (Cirillo M, Craig AFM, Borchmann S, 2020), it will be required to handle with a very full knowledge of patient's biological features. Large-scale RNA sequencing (RNA-seq) may provide little information on tumor clonal makeup and stages of EMT (Bühler MM, Martin-Suero JI, 20211). Due of reasons to switch from scRNA to snRNA the advantages of scRNA are obvious- to identify genes and networks activated in each cell or cell type to characterize cell subtypes and the fraction of one cell type in a patient (or the fraction of cell types per patient) and explore correlations between cell types (Ding J, Sharon N, Bar-Joseph Z,2022). Traditional scRNA-seq analysis was aimed at identifying if there are features that a cell lacks in the average of cells, generally with features that the average cell has. Since its first paper emerged in 2009 in this regard on solve the disease heterogeneity in human research using the cancer transcriptomes at the single cell resolutions (Tang F, Barbacioru C, Wang Y, Nordman E, Lee C, Xu N, et al2009). Sc RNA sequencing reveals biological information at the resolution of single tumor cells themselves as well as the molecular foundation of various neoplastic diseases and variables of intratumor gene expression (Hong M, Tao S, Zhang L, Diao L-T, Huang X, Huang S, et al, 2020). We examine the present situation and evolution of scRNA-seq, its use in clinical practice and cancer biology research, and upcoming studies on how this method could support the precision medicine strategy in the clinic. Here, artificial intelligence and machine learning find a pancreatic cancer biomarker using this fresh study data of 591 samples drawn from a Kaggle Pancreatic Cancer Urine Biomarkers dataset. Using those important biomarkers—CA19-9, LYVE1, REG1B, REG1A, TFF1—predictive model was created to distinguish pancreatic ductal adenocarcinoma from positive, negative, and control cases. With 89% accuracy and 91% sensitivity, the Boost classifier proved to be outperformed by conventional statistical approaches. This work emphasized one of the main conclusions: a new predictive marker was REG1B/REG1A ratio. Sensitivity studies and cross-valuation shown that model is strong over patient populations. For the diagnosis and tailored therapy in pancreatic cancer, the metabolome may be more precisely studied using artificial intelligence. Commonly, scRNA-seq comprises sample collecting, single-cell isolation, lysis, reverse transcription (RT), cDNA amplification, building a library, sequencing, and data analysis (Fig. 1) (Yeo AT, Rawal S, Delcuze B, Christofides A, Atayde A, Strauss L, et al, 2022). The fast, exact and efficient capture of single cells is clearly the main obstacle to single cell sequencing (Kolodziejczyk AA, Kim JK, Svensson V, Marioni JC, 2015). Today, there are several methods available to individually separate cells: limiting dilution, laser capture microdissection (LCM), magnetic activated cell sorting (MACS), fluorescence activated cell sorting (FACS), manual cell sorting (Tang F, Barbacioru C, Wang Y, Nordman E, Lee C, Xu N, et al, 2009) and microfluidic. Among others they are popular because they provide inexpensive running cost, fine fluid control, and low sample consumption (Qi Z, Barrett T, Parikh AS, 2019). Microdroplets (droplet-based microfluidics) are the most popular among all the current high-throughput technologies as single cells are contained in nanoliter droplets with lysis buffer and barcoded beads in a reverse emulsion in microfluidics (Choe K, Pak U, Pang Y, Hao W, Yang X,2023).



Technology	Year	Single-cell Isolation	Gene Coverage	Library Amplification	Throughput	Advantages	Disadvantages
10x-Genomics	2017	Droplet	3' or 5'	PCR	Very high (> 10,000)	High throughput; identifies cells well; ease of use; high cell flux; short library construction cycle; ultra-high capture efficiency	Many steps for DNA library construction; high sample requirements; specialized experimental equipment; non-full-length information
CEL-seq	2012	Micromanipulation	3'	In vitro transcription	Low (1-200)	High specificity and accuracy; first method to use IVT for expression transcripts	Low efficiency, reduced sensitivity for low expression transcripts
CEL-seq2	2016	FACS	3'	In vitro transcription	Low (1-200)	High sensitivity; low hands-on input	Strong 3' preference; high-abundance transcripts are preferentially amplified
Cyto-seq	2015	Microwell platform	3'	PCR	High (1000-10000)	Direct analysis of complex samples	Expensive and time-consuming
Drop-seq	2015	Droplet	3'	PCR	High (1000-10000)	High throughput; low cost; fast amplification; equipment is easily obtained	Low mRNA capture efficiency and low sensitivity

FLASH-seq	2022	FACS	Full length	PCR	High (1000-10000)	Increased sensitivity and reduced hands-on time compared to Smart-seq3	High manual technical requirements
inDrop	2015	Droplet	3'	In vitro transcription	High (1000-10000)	High throughput; strong cell capture capabilities; simplified process	Extremely low cell capture efficiency
MARS-seq	2014	FACS	3'	In vitro transcription	Median	Reduced amplification bias and labeling errors; high reproducibility	High manual technical requirements
MARS-seq2	2019	FACS	3'	In vitro transcription	High (1000-10000)	Greatly reduced background noise compared with MARS-seq; simplifies steps	High manual technical requirement
MATQ-seq	2017	Micromanipulation	3'	PCR	Low (100-200)	High sensitivity and accuracy; high transcript capture rate	Inefficient cell lysis
Microwell-seq	2018	FACS	3'	PCR	High (1000-10000)	High throughput; low cost; high sequencing quality	Presence of 3' bias; FACS requires skilled operators
Microwell-seq2	2020	FACS	3'	PCR	High (1000-10000)	Higher utilization of microprobes and higher throughput than Microwell-seq; high sensitivity and stability	Presence of 3' bias; FACS requires skilled operators

In general, there are two categories of the procedures, which are (i) 3′/5' end transcript sequencing techniques (i.e., tag-based techniques) and (ii) full length transcript sequencing techniques (Jia Q, Chu H, Jin Z, Long H,2022). Besides this, the scRNA-seq technologies that use tag-based approaches are slightly cheaper than full length scRNA-seq methods. Each of these approaches has its own drawback as well as features and benefits (Table 1; Fig. 2). Hereafter below, we will explain a few traditional or promising scRNA-seq techniques based on the current advances in those scRNA-seq technology.



Timeline and throughput of various scRNA-seq methods. Scatterplot depicts the published date and throughput of sequencing for each technology. The color indicates the different gene coverage. Size indicates the cost per sequenced cell of scRNA-seq methods.

METHODOLOGY

Tag-based methods

The primary benefit of tag-based methods is their ability to be coupled with unique molecular identifiers (UMIs), which can lower labor and overhead expenses, enable multiplexing of additional samples, and boost throughput and gene-level quantification. However, because mappable reads are restricted to one end of the transcript, tag-based methods have a comparatively low sensitivity. As a result, tag-based methods are primarily employed for measuring gene expression and are not suitable for splicing or isoform identification.

CEL-seq and CEL-seq2

Linear amplification from in vitro transcription (IVT) is preferable to PCR exponential amplification. CEL-seq (cellular expression by linear amplification and sequencing) was the first scRNA-seq procedure to use IVT for single cell RNA linear amplification, CEL-seq is a sensitive, precise and reproducible method for measuring single cell transcriptomes. Particularly, the process that we brought to bear was laborious and intricate. It was poor performance and had a 3' bias. Another single cell on-chip barcoding technique CEL-seq2, which improved the time and cost of CEL-seq, was developed by the same team four years later by combining CEL-seq2 with the C1 system of the US company Fluidigm. In addition, CEL-seq2 increased the accuracy and markedly raised RT efficiency, thus increasing detection sensitivity, by including 5-base UMI tags.

Full-length methods

Full-length transcription protocols are more suitable for detecting allelic expression, examining alternative splicing patterns, and discovering RNA editing because they have higher transcription coverage than methods that solely capture and sequence the 3' or 5' ends of cDNA (Sasagawa Y, Nikaido I, Hayashi T,2013). Full-length scRNA-seq methods like Smart-seq2 are also widely used in tumor research.

Applications of scRNA-seq in human cancer biology

In human cancer research, scRNA-seq has been widely used to study heterogeneity, the TME, gene expression profiles, transcriptome profiles, and cell–cell interactions, and other biology related to cancer research (Fig. 3). The applications of scRNA-seq in cancer research are explored and discussed in this section.

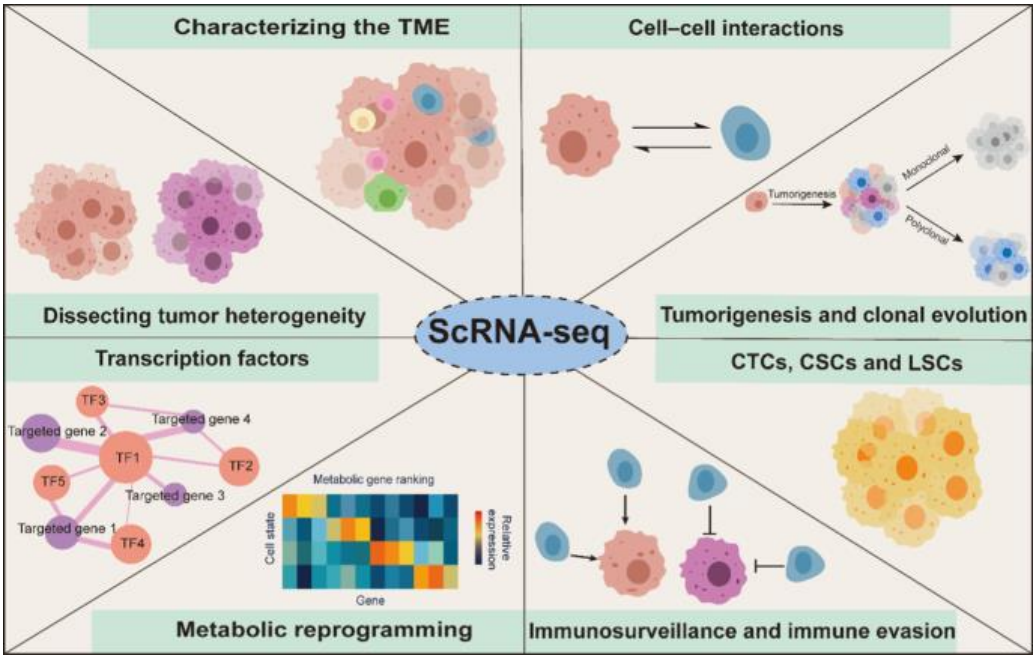


Fig 3. Applications of scRNA-seq in human cancer biology

Table 2: Key findings related to tumor heterogeneity among various tumors using scRNA-seq

Tumor	Year	Species	Protocol	Accession Number (custom database if available)	Key Findings
Lung cancer	2020	Human	10x Genomics	EGAD00001000554	Identified a cancer cell subtype deviating from the normal differentiation trajectory and dominating the metastatic stage, and revealed potential diagnostic and therapeutic targets in cancer-microenvironment interactions
	2020	Human	Smart-seq2	NCBI BioProject #PRJNA591860	Identified that individual tumors and cancer cells exhibit substantial molecular diversity and that tumor microenvironment cells exhibit marked therapy-induced plasticity
	2022	Human	STRT-seq	HRA000270	Provided novel insights into the tumor heterogeneity of NSCLC in terms of the identification of prevalent mixed-lineage subpopulations of cancer cells with combined SCC, ADC, and NET signatures, and offered clues for potential treatment strategies in these patients
Gastric cancer	2020	Human	10x Genomics	PRJEB04146	Highlighted response heterogeneity within MSI-H gastric cancer treated with pembrolizumab monotherapy; supported the potential of estimated baseline and early on-treatment biomarker analyses to identify responders
	2021	Human	10x Genomics	EGAS00001004443	The links between tumor cell lineage/state and ITH were illustrated at the transcriptome, genotype, molecular, and phenotype levels
	2021	Human	10x Genomics	HRA000051	A panel of differentiation-related genes revealed large differences in differentiation degree within and between tumors
Liver cancer	2022	Human	Seq-Well S^3	GSE186975	Identified five hepatoblastoma tumor signatures that may account for the tumor heterogeneity observed in this disease, and used patient-derived hepatoblastoma spheroid cultures to predict differential responses to treatment based on the transcriptomic signature of each tumor
Esophageal cancer	2022	Human	10x Genomics	GSE196756	Revealed intratumoral and intermural epithelium heterogeneity and tremendous differences between

					the tumor and normal epithelium. Epithelium cells and myeloid cells had more frequent cell-cell communication than epithelium cells and T cells
Melanoma	2016	Human	Smart-seq2	DUOS-000002; GSE720565	Malignant cells within the same tumor displayed heterogeneity in the transcription of proteins related to the cell cycle, context, and drug resistance program
	2020	Human	10x Genomics	GSE139829	Analysis of tumor cells revealed previously unappreciated subclonal genomic complexity and transcriptional states

Samples from seven patients with Sézary syndrome (SS) were analyzed in scRNA-seq and multicolor flow cytometry by Booth et al,2018. The malignancies of these individuals had been demonstrated to possess highly heterogeneous malignant T cell populations at the level of individual cells. However, some subsets of malignant T cells expressing HDACi were greatly decreased, while others were little affected. Among these subsets, there was heterogeneous expression of surface markers and mRNA. The heterogeneity of intertumoral T cells in cutaneous T-cell lymphoma (CTCL) skin tumors and the heterogeneity of tumor infiltrating CD8+ T cells in effector and exhaustion programs across all CTCL patients was provided by Gaydosik et al,2011, affording totally new insights into lymphocyte heterogeneity of individual CTCL patients. scRNA-seq analysis and TCR profiling defined the profile of malignant T cell activation/proliferation programs in each patient and associated with intrinsic and extrinsic heterogeneity of CTCL.

Clinical applications of scRNA-seq in solid tumors

Solid tumors account for the vast majority of cancer incidence and death. ScRNA-seq has been used in the study and application of various solid tumors (Table 3).

Table 3 Clinical significance of scRNA-seq in various solid tumors

Tumor	Year	Species	Protocol	Accession Number (custom database if available)	Clinical Significance
Lung cancer	2021	Human	10x Genomics	Correspondence with authors	Provided single-cell transcriptomic profiles of SSNs and their TME that helped advance lung cancer immunotherapy
	2022	Human	10x Genomics	GSE180963; GSE182228	ICAM1 on tumor cells activates immune response, especially in adaptive immunity
	2022	Human	BD Rhapsody Single-Cell Analysis	HRA000103	Neoadjuvant PD-1 blockade combined with chemotherapy was associated with the emergence of distinct NSCLC tumor microenvironment transcriptional states that correlated with therapy response
Colorectal Cancer	2020	Human	Smart-seq2	PRJEB34105; GSE146771	Two distinct MSI tumors showed inflammatory and angiogenic signatures, and showed differential sensitivity to CSFR1 blockade. Anti-CD40 therapy activated specific CD4+ Th1-like and CD8+ memory T cells
	2020	Human	STRT-seq	HRA000201	SCNAs were prevalent in immune cells, fibroblasts, and endothelial cells in the TME of CRC, and the proportion of SCNA fibroblasts in tumors was much higher than in normal tissue. There was also clonal expansion of fibroblasts with SCNAs in the tumor, especially in those tumors with chr7 gain
	2021	Mouse	10x Genomics	SUB8898393	The intratumoral immunomodulation induced by CD73 inhibition is distinct from that induced by PD-1 inhibition, and agents inhibiting CD73 have potential as novel anticancer immunotherapies for CRC that may have synergistic effects when combined with PD-1 blockade treatments
	2021	Human	SMART-seq v4	PRJN475964	Identified 59 single CTCs which were classified into four groups based on EMT and stem cell related gene expression. Patients receiving second

					or later-line treatment who had CTCs expressing EMT genes had significantly shorter PFS and OS
Gastric cancer	2021	Human	Correspondence with authors	Correspondence with authors	Tumor mutational burden was associated with distinct immune profile patterns in human CRC, and identified phenotypic and functional diversity of tumor-associated macrophages and T cells
	2021	Human	Modified STRT-seq and SMART-seq2	HRA000183	Provided insights into how driver mutations interfere with the transcriptomic state of cancer cells in vivo at a single-cell resolution knowledge on metastatic mechanisms as well as potential markers and therapeutic targets for CRC diagnostics and therapy
	2022	Human	10x Genomics	GSE156831	Discovered gastric cancer lymph node metastasis marker genes (ERBB2, CLDN11, and CDK12), as well as potential gastric cancer evolution-driving genes (FOS and JUN)
	2022	Human	10x Genomics	Correspondence with authors	PD-1 expression in CD8+ T cells might predict clinical responses to PD-1 blockade therapy in gastric cancer

FINDINGS

Drug resistance

The majority of patients either acquire or already have immunotherapy resistance. In three pre-treatment and twelve post-treatment samples from NSCLC patients who underwent neoadjuvant PD-1 inhibition in addition to chemotherapy, Hu et al. described the transcriptomes of almost 92,000 single cells. Two cell types (FCRL4+FCRL5+ memory B cells and CD16+CX3CR1+ monocytes) may be indicators for "positive feedback" and "negative feedback" immunological responses, respectively, they discovered, while serum estradiol levels were elevated in the TME.

COLORECTAL CANCER

Tumorigenesis and metastasis

The main reason for death from colorectal cancer is still metastasis, even after curative treatments. In 12 mCRC patients, Wang et al. use matched primary, metastatic and surrounding normal tissues for whole genome sequencing (WGS), multi region whole exome sequencing (WES), simultaneous scRNA-seq sequencing, and single cell targeted cDNA Sanger sequencing. It was found that the abnormal activation of the PPAR signaling system plays a major part in the carcinogenesis of colorectal cancer. Analysis of matched samples from the same patient argued against the classic theory that metastasis to lymph nodes seeds the distant organ by revealing that cancers metastasizing to the lymph nodes were not from the same source as cancers that metastasized to the liver. These results provide new insights into the mechanics of metastasis, and potential therapeutic targets and markers for the diagnosis and therapy for colorectal cancer.

Treatment

ScarNA-seq has not, however, hitherto been employed to identify immunomodulating therapy strategies. SC Zhang et al. investigated stromal and immunological populations in CRC patients using scRNA-seq; reduction of SPP1+ TAMs may finally enhance outcome either in conjunction with ICBs or myeloid focused immunotherapy. Anti CSFR1R monotherapy selectively depletes C1QC+ TAMs with inflammatory characteristics while suppressing expression of proangiogenic/tumorigenic genes produced by SPP1 + TAMs, in both mice and humans. Furthermore selectively stimulating the Ccl22+ cDC population, the CD40 agonist antibody raised Bhlhe40+ T helper 1 (Th1) like cells and CD8+ memory T cells. In the framework of a greater microsatellite instability (MSI), the study of BHLHE40+ Th1 like cells in tumor samples of CRC patients considerably addresses these issues by course of a response to ICBs. In a distinct scRNA-seq research of mice, PD-1 blocking reduced Malat1high Treg cell amount and M2 macrophage quantity; AB680, a selective CD73 exoenzyme inhibitor, increased anti-cancer efficacy of immunosuppressive cells like exhausted T cells, and Treg cells. Because of their natural intratumorally immunomodulating effects, AB680 might be a

possible therapy for individuals with resistant colorectal cancer (CRC) who cannot react to the present cancer chemotherapeutic drugs and PD-1 antagonists. Wu et al. investigated 97 matched samples using scRNAseq and spatial transcriptome to find that MRC1+CCL18+ suppressive macrophages had the greatest metabolic activity and were the cells most subjected to geographic reprogramming. While the responding patients, who would have had their anti-tumor immunological balance restored by neoadjuvant chemotherapy (NAC) to stop this activity, the nonresponsive patients stayed quiet.

GASTRIC CANCER

Cancer Metastasis

However, since data from metastatic studies were collected by mass method, which may obscure the contribution of the subpopulation, it is still unclear how lymph node metastasis is initiated in gastric cancer. Wang et al. subjected the samples of the main tumor and metastatic lymph node (MLN) from three patients with gastric cancer to scRNA-seq. Scientists found a cell subpopulation between the main and metastatic groups and potential driver genes for advancement of gastric cancer (FOS and JUN) or for lymph node metastasis in gastric cancer (ERBB2, CLDN11 and CDK12). Finally, we further investigated one organ by one organ metastases of gastric cancer in liver, peritoneum, ovary and lymph nodes, and found that stromal cells and immune cells were molecularly heterogeneous and created an immunosuppressive and pro-tumorigenic milieu. Additionally, a 20 gene profile of CD8+T cells originating from tired lymph nodes may predict for lymph node metastasis. To investigate the variation of tumor cells and TME in primary tumor tissue and malignant lymph node from main gastric cancer tumors and malignant lymph node from patients with gastric cancer. The scientists found a malignant subgroup that had high translation start and high protein activity, and could invade to lymph nodes. Besides gastric cancer cells, abnormal neutrophil polarization and differentiation as well as the immunological checkpoint activation SPP1 may also contribute to the disseminated gastric cancer to lymph nodes.

Disease Monitoring

In a phase II study of pembrolizumab, patients with MSI-H advanced gastric cancer underwent serial peripheral blood analysis by Kwon and colleagues; serial and multi-regional tissue analysis were performed with WES and scRNA-seq. It was found that responders and non-responders differed significantly in the composition of baseline and adaptive TME. CAF abundance was higher in non-responders and the Wnt/β-catenin pathway was frequently mutated and active. Interestingly, non-responders had lower NK cell count and T cell infiltration.

Therapy

The mode of treatment for early-stage stomach cancer is surgery, whereas for advanced stage gastric cancer, there are no adequate treatments available at the present times. In a study by Li et al.2020, they showed that ACKR1 was particularly expressed in tumor endothelial cells of nine untreated non-metastatic gastric cancer patients that were sequenced through scRNA-seq. Since this gene was identified as a marker of a poor prognosis in the cohort data, it was proposed as a possible novel target of the therapy of stomach cancer. However, different research found that activating the SPP1–CD44 interaction in maxillary lymph nodes inhibited T cell activation in those nodes. Such a finding may serve as a therapeutic target for patients with gastric cancer lymph node metastasis (Datta S, Malhotra L, Dickerson R, Chaffee S, Sen CK,2015). Additionally, combination of immune checkpoint inhibitors and inhibition of Wnt/β-catenin pathway may be promising strategy for gastric cancer.

CONCLUSION

ScRNA-seq is a valuable tool because it provides previous unheard mapping data for cancer analysis, due to high resolution transcript sequencing. It can be used to define new cell subpopulations and markers, gap intra, and inter-tumor heterogeneity, measure tumor microenvironment, intercellular communication, and lineage trajectories. Such studies have significantly enhanced our understanding of cancer pathogenesis, diagnosis, prognosis, treatment and drug resistance. Nevertheless, there are some limitations that hinder the scRNA-seq from seeing widespread application. First, scRNA-seq is the most developed and widely used single cell omics in cancer research, but it does not data cancer biology in the most intuitive way (in the level of proteomics,

epigenomics, genomics, etc.). Second, there will be meaningful batch effects on samples tested at different time; scRNA seq has high sample demand and can only use live cells as detection object. Thirdly, the cells in single cell suspension created from enzymatic digestion of cells may be destroyed and RNAs degraded. The loss of morphological and spatial information also makes this result. Fourth, scRNA-seq data fail to provide a complete transcriptional profile and corresponding genetic information with coverage of the cancer samples, do not cover all nucleated cells and may be limited by sequencing depth. Homogeneity of data is also lacking because of the variety of patient malignancies as well as variation in detection platforms besides making it more difficult to understand results. In addition, scRNA seq is expensive, laborious, and it takes a lot of data processing work to learn from. But due to the fact that it with cancer, there is still ample area to advance the scRNA-seq technology. There are several directions for future research aimed at lowering costs, advancing single cell isolation technology, increasing sequencing depth and throughput, refining bioinformatics analysis procedures, and expanding the technology to apply to frozen and formalin fixed paraffin embedded (FFPE) tissues to increase the amount of accessibility and usability. In addition to the progress of scRNA-seq technology, scRNA-seq is advised to be integrated with other omics and artificial intelligence technologies. The dynamics between gene mutation and expression are documented by scRNA-seq-plus-genomics; the relationship between transcript abundance and protein content is revealed by scRNA-seq-plus-proteomics; the regulation of gene expression by chromatin structure or methylation is better understood by scRNA-seq-plus-epigenomics; and the mapping and quantification of cellular functions in highly heterogeneous cancers is sufficiently detailed by scRNA-seq-plus-metabolomics to yield valuable information [46, 47]. In order to better understand cell localization and many of the activities which occur within, we should also direct our efforts on spatial multiomics, alongside single-cell multiomics, and use it in conjunction with scRNA-seq or other single-cell multiomics; scRNA-seq has been used to integrate with other single cell labeling techniques such as nuclear hashing and CRISPR/Cas9. They can detect and describe the effect of hundreds of different genetic changes throughout the lives of thousands of individual cells with different cell functions in tissues or cells with different activities in vivo at single cell resolution. Certainly, the need to add additional samples to combine more omics makes it an ethical and financial barrier. The more technologies are used, the more the data that is produced. However, this also raises the demand of bioinformatics and intensifies the amount of data that researchers and practitioners have to study and use. scRNA seq will need to be more tightly linked to other omics and technologies in order to save expenses and sample sizes. Furthermore, machine learning and artificial intelligence-based data processing analysis needs to catch up with the rapid advancement of the technology of scRNA seq to give researchers more useful information to further our knowledge of cancer. Finally, scRNA-seq should be integrated to clinical needs for providing the more individualized and accurate therapy options to patients. Since this is relatively new technology, which is not extensively used in clinical practice it has to be developed and defined ethical guidelines for its clinical use.

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