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Recent progress in single-cell cancer genomics

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The advent of single-cell sequencing has been revolutionary to the field of cancer genomics. Perfectly suited to capture cancer's heterogeneous nature, single-cell analyses provide information bulk sequencing could never hope to uncover. Many mechanisms of cancer have yet to be fully understood, and single-cell approaches are showing promise in their abilities to uncover these mysteries. Here we focus on the most recent single-cell methods for cancer genomics, and how they are not only providing insights into the inner workings of cancer, but are also transforming individualized therapy and non-invasive monitoring and diagnosis.

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Introduction

Genomic analysis has been widely applied in cancer studies. The identification of genomic, epigenomic, and transcriptomic changes in cancer has led to precise classification, biomarker discovery, and mechanical understanding of cancer, and has played an essential part in cancer diagnosis, monitoring, and treatment [1]. However, until recently, bulk sequencing has been the only viable option for cancer genomic analysis. One major limitation is that bulk sequencing cannot detect the heterogeneity within a tumor. This limitation has important clinical consequences. For example, cancer is often composed of multiple clones, and the most aggressive clone is difficult to identify and target since it may not be the one that metastasizes.

Throughout every stage of cancer, cells accumulate distinct mutations, which define the further evolution and progression of the disease. It is commonly viewed that cancer originates from an accumulation of mutations in oncogenes and tumor suppressors such that cell growth becomes unregulated and invasive [2]. The progeny of these cells in turn accumulate further mutations and selective pressures drive clonal evolution. The cancer will eventually metastasize, spreading to other parts of the body through the circulatory or lymphatic systems to form further distinct subpopulations. In addition, targeted cancer therapy may drive further evolution and eventually lead to drug resistance.

The recent advent of single-cell sequencing has revolutionized the field of cancer genomics, opening the door to a vast number of possibilities (Table 1). From the ability to resolve intra-tumoral heterogeneity [10•,17••,27•,34••], map clonal evolution [50,51], and track the development of therapy resistance [10•,58•], to the capacity to analyze rare tumor cell populations such as tumor stem cells and circulating tumor cells [47,48], single-cell techniques have opened new avenues for cancer research. A better understanding of the mechanisms of cancer can in turn inform more effective and personalized treatments.

In this paper, we review recent progress in single-cell analysis techniques and their applications in cancer genomics (Figure 1), focusing on topics that have not been covered by previous reviews [3–6].

Intra-tumor genome sequence heterogeneity

Understanding the genomic heterogeneity of cancer cells first and foremost necessitates methods for single-cell DNA sequencing. The earliest developments for single-cell genomics involve whole genome amplification, providing ample amounts of DNA for subsequent sequencing. Degenerate oligonucleotide primed PCR (DOP-PCR) is appropriate for CNV detection, with low coverage but uniform amplification [7]. Multiple displacement amplification (MDA) is a linear amplification method capable of higher coverage through the use of Phi-29 polymerase, making it suitable for SNP detection [8]. MALBAC (multiple annealing and looping-based amplification cycles) combines MDA and PCR for a high coverage, uniform amplification method suitable for either CNV or SNP detection [9]. These methods have been extensively applied to the characterization of intra-tumor CNVs and SNPs in various cancer types.

However, one major limitation of the aforementioned methods is that spatial information is lost as soon as single cells are isolated. Such information is integral to understanding the interaction of the cell with its microenvironment and may prove valuable for evaluating drug responsiveness. Recently, a new technology,

Table 1

A summary of relevant single-cell methods and their applications to cancer

Method type	Specific methods	Application to cancer genomics	Refs.
Experimental methods			
Single-cell whole genome amplification	DOP-PCR, MDA, MALBAC	Used in conjunction with next-generation sequencing to detect intra-tumor CNVs and SNPs.	[7–9]
Single-cell spatial genomics	STAR-FISH	Detects the spatial distribution of intra-tumor CNVs and SNPs. Can be combined with longitudinal analysis to reveal migratory cells.	[10•]
Single-cell transcriptome amplification	Smart-seq, Tang *et al*. method, single-cell qPCR	Identifies cancer-specific gene expression signatures, cancer cell types, alternative-splicing events.	[11–13]
Single-cell spatial transcriptomics	smFISH, SeqFISH, MERFISH, FISSEQ, TIVA	Can provide spatially-resolved gene expression signatures in tumors. Has potential applications in tracing cell migratory paths and locating tumor-like stem cells.	[16,17••,18•,19,20•,21,22]
Single-cell DNA methylomics	scRRBS, PBAT	Enables the discovery of differential methylation in cancer cells. Potential for broadening understanding of phenotypic plasticity of cancer cells.	[25,26,27•]
Single-cell chromatin accessibility	ATAC-seq, Pico-Seq	Can give insight into the differential binding of transcription factors in cancer cells.	[29•,30•,31]
Chromosome conformation capture	Hi-C, ChIP-seq	Potential for understanding the mechanisms of cancer heterogeneity through mapping transcription factor-regulatory element interactions.	[32,33]
Simultaneous multiple single-cell omics	G&T-seq, scTrio-seq, Darmanis *et al*. method	Provides an integrated view of intra-tumoral heterogeneity through measuring direct interactions between genomic, transcriptomic, epigenetic, and proteomic variation.	[34••,27•,36•]
Computational methods			
Single-cell spatial transcriptomic inference	Seurat, Achim *et al*. method	Infers cell location through scRNA-seq data and an *in situ* RNA reference map of several landmark genes, enabling mapping of intra-tumor spatial heterogeneity.	[37•,38]
Pseudo-time ordering	Monocle, TSCAN, Waterfall, SCUBA, Wanderlust, Wishbone	Projects gene expression values from a single time-point to a continuous trajectory over cell differentation. Potential use in understanding differentiation from stem-like cancer cell to matured cancer cell.	[39,40–43,44•,45]
Rare cell-type detection	RaceID, StemID, GiniClust	Potential use in the detection of circulating tumors cells and stem-like cancer cells.	[46–48]
Clonal evolution inference	SCITE, OncoNEM	Builds lineage trees for understanding evolutionary events such as the development of therapy resistance.	[50,51]

STAR-FISH (specific-to-allele PCR-FISH) [10•], has been developed which can detect the spatial distribution of both SNVs and CNVs using a combination of *in situ* PCR and FISH. PCR primers are built to target mutant and wild type mRNAs, one gene at a time. Amplification is followed by hybridization of fluorophores to a 5′ overhang built into each probe. Janiszewska *et al*. use their method to study the commonly reported His1047Arg mutation in *PIK3CA* and *ERBB2* (commonly known as *HER2*) amplification in *HER2*+ breast cancer, before and after chemotherapy. They were able to identify changes in mutational frequency of mutated cells, which help gain an understanding of the development of drug resistance in *HER2*+ breast cancer [10•]. When combined with longitudinal analysis, this method was used to pinpoint migratory cells [10•]. Currently, the technology can only be used to detect the location of known mutations.

The introduction of spatial methods to single-cell cancer genomics allows genomic heterogeneity to be mapped in space. This presents new opportunities in studying cell-to-cell interactions, and in identifying migratory cancer cells and their roles in metastasis.

Intra-tumor transcriptomic heterogeneity

Like single-cell genome analysis, the first efforts in single-cell transcriptomics were in the amplification of the transcriptome to allow for quantification and sequencing of the transcriptome. Whole transcriptome amplification methods include poly-A tailing methods