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Current research development of single cell genome in urological tumor

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ABSTRACT

The technique of whole genome amplification is advancing rapidly and generating attention on detecting genomic lesions in individual cancer cells. Also, single-cell genome could label the uniqueness of each cell, its individual mutations and structural variations especially in cancer studies. In this Review, we provide the insight into the current state of single-cell genome in urological tumor mainly including kidney cancer, bladder cancer and prostate cancer. We put more forward on the new progress of the technique used by single-cell genomes and different results of the genes transform on random tumor tissue from single cell isolated on account of tumor heterogeneity. With the advent of more complete and accurate genome information, single-cell sequencing will become a standard tool in early diagnosis and targeted therapy and prognosis judgement.

1. Introduction

Heterogeneity between cells has been a primary factor in the phenotypic profiling used for discerning different tissues. Single-cell genome is an advancing powerful technique which can give a better analysis of complex genetic variability by individual cells (Yilmaz and Singh, 2012; Bhattacharya et al., 2012). There are four pivotal technical challenges for single-cell sequencing such as cell isolation, whole-genome amplification, single-cell variant calling and interpreting the data (Gawad et al., 2016).The developing method aims to identify the modern genetics and genomics, and assess the contributions between the genetic heterogeneity and tumorigenesis (Gawad et al., 2016; Lawson et al., 2015). The genomes of individual cells show stochasticity, so single cell should be focused rather than the average of a large ensemble of cells (Huang et al., 2015). Additionally, the method of single- cell sequencing usually led to some unanticipated discoveries in terms of early diagnosis (Zhang et al., 2016a). In this review, we describe the technical development of single-cell sequencing and breakthrough in urological tumors.

2. Technical development of single- cell sequencing

2.1. Single cell isolation

The first step in single cell genomics requires the isolation of single cells, and the methods of single cell isolation have been developed a lot such as serial dilution, micromanipulation, microfluidics, and so on (Table 1)(Wang and Song, 2017). Among the methods above, serial dilution is an ordinary and cheap technique used widely in several studies. Micromanipulation is mainly applied to isolate individual cell of a number of organisms from surrounding samples (Woyke et al., 2010). Microfluidic, widely used for analysis of individual cell, has a sealed environment for separation and amplification, which benefits to the environmental protection (Lan et al., 2017). Fluorescence Activated Cell Sorting (FACS) and Laser Capture Microdissection (LCM) are new methods with most efficient (Zhu et al., 2017).

2.2. Whole genome amplification

In the aspect of whole genome amplification, there are three major chemistries including the degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR), multiple displacement amplification (MDA) and multiple annealing and looping-based amplification cycles (MALBAC). DOP-PCR has been used to amplify picogram quantities of human genomic DNA for genotypic analyses, which can be suited for measuring CNVs on a large genomic scale. MDA was firstly used for analyzing the cancer genome at a single-cell nucleotide level in 2012 (Hou et al., 2012). Now MDA was applied in the research of colon cancer, clear cell carcinoma of kidney, and myelodysplasia syndrome (Yu et al., 2014; Xu et al., 2012). However, MDA has the shortage of lacking completely biased and precise (Yilmaz and Singh, 2012). In the same year, a new technique called Multiple Annealing and Looping Based Amplification Cycles (MALBAC) was published in *Science*, which tells quasilinear pre-amplification to reduce the partial associated with

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Table 1
Comparison among different methods in cell isolation.

Method	Characteristic
Serial dilution	Acquiring a single cell in a single well via serial double dilution; It was hard to control the quality and takes too much time.
Micromanipulation	Sorting single cell by capillary pipettes under a high-powered microscope; It could easily cause the target cell injured.
Microfluidic	Isolating single cell by ultrasonic with high separation effect and fast separation speed.
FACS	Isolating special single cell from heterogeneous cell samples with specific fluorescent antibodies.
LCM	Staining the targeted single cell with fluorescent and isolate individual cell directly from tissues by external magnetic field.

Table 2
Comparison among DOP-PCR, MDA and MALBAC.

	DOP-PCR	MDA	MALBAC
Amplification	Exponential amplification	Exponential amplification	Linear Amplification
Primer	A random six-base sequence at the 3′ end and a fixed sequence at the 5′ end	A random hexamer and φ29 DNA polymerase	A common 27-nucleotide sequence at the 5′ end and 8fancom nucleotides at the 3′ end
Template	Copy genomic DNA	Copy genomic DNA	Original genomic DNA
Template Amount	pg	pg	μg
Characteristic	Classical method; Low genome coverage	Higher genome coverage than DOP-PCR; Amplification bias	Less sequence-dependent bias

nonlinear amplification (Zong et al., 2012; Lu et al., 2012). Compared to MDA, MALBAC has a sharp lower probability of Allele Dropout and a clear bigger range covering more genes, which yielded 93% genome coverage at an average 25X sequencing depth (Leung et al., 2017). MDA could not apply in CNV analysis. Nevertheless, MALBAC did not have any limits and may be used for next generation sequencing, chip inspection, qPCR, CNV analysis, SNP analysis, and common PCR (Grun and van Oudenaarden, 2015) (Table 2).

2.3. Data analysis

The data analysis is associated with scRNA-seq typically (Ofengeim et al., 2017; Ellsworth et al., 2017). A table with relative expression of each gene per cell is necessary. The next step in single-cell genomic analyses is variant calling and interpreting the data, which are important to produce the results and determine genetic relationships between single cells. SNV calling and CNV detection both appeared the genetic differences (Cai et al., 2014; Kolinko et al., 2013). Nowadays, single-cell sequencing also meets the challenges which need to be overcome mainly in resolving mutations at base-pair resolution and making a missing values including false positive and false negative mutations (Navin, 2015a; Navin, 2015b). Sometimes false positives even can outnumber true somatic variants (Hou et al., 2012; Li et al., 2012; Lohr et al., 2014).

3. Single-cell genome in cancer research

Cancer is a genetically related disease initiating from a single cell and accumulating sufficient genetic damage (Lozano and Behringer, 2007). Cellular heterogeneity is developing appreciated as a factor of tumor diagnosis and tumor therapeutic schedule which was affected by different target spots (Bedard et al., 2013; Kim et al., 2011). The cellular heterogeneity will bring fundamental perception for therapy because drug resistance is often suffered by heterogeneous responding at the cellular level (Navin et al., 2010; Zheng et al., 2010).

Recently, single-cell genomes in cancer have made great progress and acquire the certain capacity to resolve complex mixtures of cells in tumors, and many studies have showed that single-cell analysis might be applied to characterize the cellular heterogeneity in different cancers (Van Loo and Voet, 2014; Patel et al., 2014). The mutations could be used as biomarkers for diagnosing the types of tumors and providing novel insights into dynamics of mutation in many kinds of cancers, such as breast cancer (Eirew et al., 2015; Voet et al., 2013; Navin and Hicks, 2011), colon cancer (Yu et al., 2014) and renal cell carcinoma (Xu et al., 2012). Another function of single-cell genome is detecting new targets, including circulating tumor cells, cancer stem cells (Qian et al., 2017). Circulating tumor cells (CTC) derived from primary and metastatic tumor cells which are disseminated into the peripheral blood. We can detect tumor progression by peripheral blood at an early stage (Kidess and Jeffrey, 2013). Recently, more and more researchers put their attention towards CTC to understand the metastatic process and detect early tumor. For example, initial studies on CTCs as a biomarker in pancreatic cancer are available (Court et al., 2015), and conventional therapies targeted at proliferating cells may be inadequate for eliminating metastatic seeding by CTCs (Powell et al., 2012).

Lately, a new synthetic system, Engineered Mutagenesis with Optical in situ Readout (MEMOIR), has been reported in Nature, which can enable cells into record lineage information and event histories in the genome in a format that can be subsequently read out in single cells in situ. The system could alter Cas9-based targeted mutagenesis, and read out in single cells through multiplexed single-molecule RNA fluorescence hybridization (smFISH) (Frieda et al., 2016). In the process of tumor progressing, cells could receive special molecule signals, but not all cells could acquire mobility and metastasis. The new technique which catch the signals of each affected cell will give a new concept on space-time of tumorigenesis.

Urological cancers are mainly comprised of kidney, bladder, prostate, which has made great progress in molecule level of diagnosis and therapy. Single-cell genome as a new technique has been used for urological cancers for some time. In this review, we detailed the current research development in urological cancer.

4. Single-cell genome developed in kidney cancer

Renal cell carcinoma (RCC) is a heterogeneous disease including clear cell, papillary, chromophobe, translocation and collecting duct tumors (Hancock and Georgiades, 2016). The clinic-pathological appearance of RCC is unpredictable, generating up to one-third of patients present with metastatic disease, especially part of the patients have followed curative surgery (Siegel et al., 2016). The disease remains hard to detect and difficult to treat, and researchers still struggle to find new ways to predict early kidney tumor and reduce the endanger factors (Owens, 2016).

A single cell RNA-sequencing strategy can be used to create a series of gene expression patterns in the kidney cancer. The research about the single cell RNA-sequencing analysis of total mouse kidneys has been reported, which performed at E11.5 and E12.5, and the renal vesicles at P4 (Brunskill et al., 2014). There are many interesting heterogeneities