Single-cell RNA Sequencing to Investigate Human Disease

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The last two decades has seen the continual improvement of profiling gene expression at a genome-scale using hybridization-based microarrays (Schena M et al, 1995) and, more recently, RNA sequencing (RNA-Seq) (Wang Z et al, 2009), a technique for unbiased sequencing of expressed genomic loci at high coverage. Regarded as the industry standard for gene expression profiling via measurement of messenger RNA (mRNA), RNA-Seq also allows for analysis of non-coding RNA classes (Ozsolak F and Milos PM, 2011; Wang Z et al, 2009). However, this technique conventionally requires millions of cells (∼1 μg of total mRNA) and therefore the output for each gene is an average expression level across the population of input cells (Wilhelm BT and Landry JR, 2009). Now often referred to as ‘bulk’ RNA-Seq, it does not account for the stochastic nature of gene expression, cellular diversity (i.e. differences between cells of the same ‘type’), or cellular heterogeneity (i.e. different cell types within the same tissue/cell population).

In recent years, technological advances in next generation sequencing have allowed for unbiased profiling of single cells at multiple layers (i.e. the genome, epigenome and transcriptome) (Linnarsson S and Teichmann, 2016). Although single-cell RNA-Seq (scRNA-Seq) was first published by Tang et al. in 2009, it only started to gain widespread popularity several years later following low sequencing costs and refinement of protocols (Tang F et al, 2009). Earlier scRNA-Seq approaches such as Smart-Seq (Ramskold D et al, 2012), MARS-Seq (Jaitin DA et al, 2014) and Fluidigm C1 (Xin Y et al, 2016), were well-based, but recent droplet-based approaches such as Drop-Seq (Macosko EZ et, 2015.), inDrop (Klein AM et al, 2015) and Chromium (Zheng GX et al, 2017) have significantly increased the number of cells that can be profiled in parallel for a single experiment. So far, scRNA-Seq has already yielded insight into a number of different areas that could not be achieved using bulk transcriptome profiling including, for example, the stochastic nature of gene expression (Shalek et al, 2017; Kar et al, 2017). To reveal complexity in the brain, studies in the central nervous system have successfully mapped cellular diversity and have even identified novel cellular subtypes (Zeisel A et al, 2015; Lake BB et, 2016). Similarly, studies in embryonic and immune cells have also revealed new levels of heterogeneity (Jaitin DA et al, 2014; Deng Q et al, 2014; Yan L et al, 2013). In a scRNA-Seq analysis of ~2400 immune cells, a subpopulation of dendritic cells were identified that could potentially stimulate T-cell activity (Villani AC et al, 2017), which has therapeutic implications against cancer. In several different contexts, scRNA-Seq has been used to infer cellular lineages and developmental relationships (Treutlein B et al, 2017; Venteicher AS et al, 2017). This approach has also been used in cancer to investigate the cellular heterogeneity in the tumour microenvironment (Patel AP et al, 2017; Puram SV et al, 2017) and for profiling individual circulating tumour cells (Miymoto DT et al, 2015). These are a just few examples of how single cell analysis, in particular scRNA-Seq, is transforming how we perform genomic profiling. The future looks bright for this emerging technology in investigating human disease, alone or in combination with other ‘omics analysis. For example, as scRNA-Seq can resolve each clone within a tumour, it could potentially be used for longitudinal monitoring of tumour relapse, reveal subsets refractory to therapy, and be used in a clinical setting for detection of rare disease-associated cells.

References


