LAB-ON-A-CHIP DEVICES

Immunomagnetic cell sorting

For genome-wide screens and other applications that require the processing of a large number of cells, the immunomagnetic sorting of cells on a microfluidic chip is a scalable, rapid and cost-efficient alternative to fluorescence-activated cell sorting.

Mateusz Legut and Neville E. Sanjana

Since the completion of the Human Genome Project, one challenge in modern genetics is to link individual genes to the specific biological processes that they control. This can be done at high throughput via phenotype-to-genotype approaches. For example, forward genetic screens using pooled libraries perturb multiple genes in parallel, followed by the selection of the phenotype of choice, and then by the identification of genetic perturbations linked with the selected phenotype. Initially, genome-scale pooled screens in human cells used RNA interference (RNAi), which allows for the identification of genes contributing to a phenotype in a genome-wide manner and in virtually any cell type[1]. However, RNAi methods suffer from the incomplete knockdown of target genes and from widespread off-target effects, which confound the interpretation of the results of the screen[2].

DNA-specific programmable nucleases, in particular the CRISPR (clustered regularly interspaced short palindromic repeats) nuclease Cas9, make the detection of phenotypic changes more straightforward, owing to permanent gene knockout (rather than transient gene knockdown), less off-target activity and high consistency between different individual perturbations targeting the same gene[3]. Since Cas9 is targeted to specific regions of the genome, via short guide sequences that are easily produced through oligonucleotide library synthesis, the CRISPR–Cas9 system has been repurposed to conduct genome-wide loss-of-function screens in mammalian cells[4]. However, the number of cells per screen can become prohibitive. The human and mouse genomes contain approximately 20,000 protein-coding genes, and because each gene is typically targeted with several independent perturbations to ensure statistical power, hundreds of millions of cells are typically used in a single experiment. Consequently, genome-wide CRISPR screens have been predominantly restricted to phenotypes related to cell growth or cell survival[4–6].

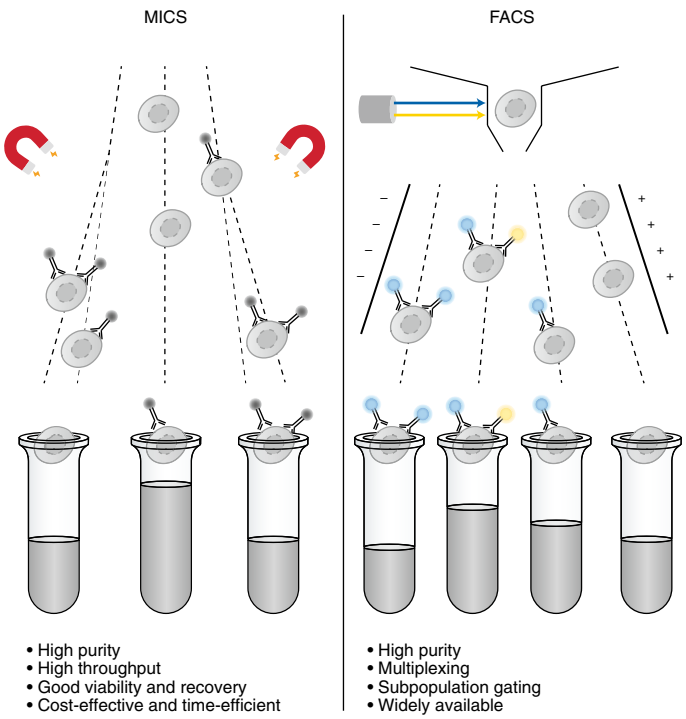

Fig. 1 | MICS versus FACS. In FACS, cells with fluorophore-bound antibodies targeting protein markers expressed on their surface are sorted according to fluorescence via a charge-based deflection system (a cell is given an electric charge that depends on the emitted fluorescence when the cell is excited by laser beams of different wavelengths). In MICS, cell sorting is based on the strength of the magnetic field, which depends on the number of magnetic nanoparticles attached to each cell via antibodies targeting a specific cellular protein.

Yet CRISPR screens can also be powerful when used to discover genome-wide regulators of other phenotypes of interest, in particular genes that govern the expression of therapeutically relevant proteins[7]. In contrast to growth-related phenotypes, CRISPR screens probing the expression levels of a target protein typically require antibody labelling and the fluorescence-activated cell sorting (FACS) of the desired cell population. However, it is challenging to process the hundreds of millions of cells required for genome-wide CRISPR screens via FACS, and prolonged cell sorting can reduce cell viability and cause undesired metabolic perturbations in the cells[8]. Reporting in *Nature Biomedical Engineering*, Shana O. Kelley, Jason Moffat and colleagues now show that, for loss-of-function CRISPR–Cas9-mediated phenotypic screening, cell sorting via the use of antibodies coupled to magnetic particles in a microfluidic chip is a high-throughput and cost-and-time-efficient alternative to FACS[9].

Sorting via FACS involves processing the fluorescent signal from each cell, one cell at a time, which limits the technique's throughput. In Kelley and co-authors' approach, which they termed 'microfluidic

immunomagnetic cell sorting' (MICS), a set of magnetic guides in the microfluidic chip deflect millions of cells simultaneously on the basis of the number of antibodies, labelled with magnetic nanoparticles, bound to each cell (Fig. 1). The authors show that MICS can separate the magnetically labelled cell population into three categories, on the basis of the expression level of the target protein: a population representing the baseline level of the target marker, and subpopulations expressing either a lower or a higher level of the target. They also show that MICS performs well for a range of cell sizes and expression levels of target markers, and that 30 chips in parallel can sort approximately one billion cells per hour, a level of throughput more than sufficient for genome-wide CRISPR screens that would otherwise take several days with standard FACS. Compared with FACS, MICS also offers substantial improvements in cell recovery and cell viability while maintaining similar purity and efficiency of cell sorting.

Kelley and co-authors used MICS to perform a genome-wide loss-of-function CRISPR screen for the identification of modulators of the expression of CD47 — a surface marker ubiquitously expressed across different tissues that signals to macrophages and other cells of myeloid lineage via SIRPα to inhibit phagocytosis[10]. Antibody blocking of CD47 on cancer cells promotes phagocytosis by macrophages, which in turn prime $CD8^+$ T-cell responses to cancer antigens, initiating a potent immune response[11]. By performing a MICS-assisted genome-wide screen of CD47 modulators, the authors identified QPCTL as a crucial enzyme in the post-translational modification of CD47 (which has been hypothesised to be required for CD47 interaction with SIRPα), thus making QPCTL a potential therapeutic target[12]. Since the high expression of the *QPCTL* gene is a poor prognostic factor in some cancers[13], and pharmacological modulators of *QPCTL* are readily available, small molecules targeting the protein could amplify the therapeutic effect of anti-CD47 antibodies.

MICS lacks some of the control, customizability and flexibility that are inherent to FACS. For instance, operators of FACS can adjust the distributions of cell populations sorted by FACS to desired percentages, and further fine-tuned adjustments can be made in real time if required by the experiment. In contrast, the fixed geometry of the MICS chip limits how the sorted cell population can be fractionated. Also, FACS enables easily adjustable sequential gating strategies, including (but not limited to) the selection of cells based on their granularity and size, the exclusion of doublets and dead cells, and the selection of specific populations on the basis of the expression patterns of several different markers. The routine multiplexing of different cellular markers is crucial for the analysis of heterogeneous cell populations, and such multiplexing cannot be achieved with MICS alone. A possible solution would involve the use of MICS as an initial enrichment strategy for processing a large number of cells on the basis of a single marker in order to isolate a rare cell population, followed by further deconvolution with FACS or single-cell-sequencing technologies. Such a combined strategy could be used to pull out and analyse neoantigen-reactive or autoimmune T-cells, the frequency of which can be as low as one in tens of thousands. In this case, a bulk T-cell population would be stained with DNA-barcoded, magnetically labelled antigen multimers[14], followed by the enrichment via MICS of the T cells labelled with any multimer. This pre-enrichment would reduce the number of cells to a quantity amenable for single-cell sequencing, which in turn would determine antigen identities (via DNA barcodes) and the corresponding T-cell-receptor sequences.

FACS and experienced operators of the technique are readily available in any major research institution. At present, MICS chips would need to be manufactured in-house, and specific cell types or marker expression patterns may require a bespoke design. Yet, as with single-cell sequencing, which used to require a highly specialized setup, often involving microfluidic devices, should MICS prove widely useful it would not be surprising if fully automated MICS setups eventually become commercially available. If so, MICS would allow the mapping of the genetic regulators of every intracellular or surface-expressed marker that is targetable with an antibody. This would be conceptually similar to how growth-based genome-wide screens have been applied to map genetic dependencies in hundreds of cancer cell lines[15]. ❐

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Competing interests

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