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AELIAN BIOTECHNOLOGY

Functional genomics at single-cell resolution

Using CRISPR screening and single-cell RNA sequencing Aelian have created a high-throughput engine for target discovery in immuno-oncology.

Combining CRISPR screening with single-cell RNA sequencing, Vienna-based Aelian Biotechnology has developed a new discovery tool for functional genomics. It enables biotech and pharma companies to identify drug targets or reveal the mechanism of action of existing drugs, particularly in the immuno-oncology space where there is a need for screens in primary human cellular material.

The company's technology platform, known as CRISPR droplet sequencing (CROP-seq) was developed in the laboratory of co-founder Christoph Bock, at the Research Center for Molecular Medicine of the Austrian Academy of Sciences (CeMM), to assist in mapping the impact of thousands of CRISPR perturbations on the transcriptome of a single cell. "We can now provide this powerful tool to other biotech and pharma companies," said co-founder and CEO, Thomas Moser, a veteran of several genomics-based start-ups. "We can find novel targets and create hypotheses that connect a gene or target to a given disease of interest."

The CROP-seq workflow represents a step forward from first generation CRISPR screening approaches, which are limited to the assessment of cellular fitness phenotypes. By combining CRISPR with single-cell RNA sequencing, Aelian's high-throughput platform can profile the transcriptional outcomes in a pool of cells, after an individual gene has been silenced in each one. This is achieved by infecting a pool of Cas9- expressing cells with a lentiviral guide RNA library. Each cell is then encapsulated in a lipid droplet, together with a barcoded bead and subsequently sequenced in isolation.

"We're able to look at the functional consequences of each gene knockout by single cell RNA sequencing. In the end, we get a transcriptomic profile from each single knock-out and that allows us to map phenotypes we observe in single cells back to the guide RNA. It really creates causal links between genes and phenotypes," explained Tilmann Buerckstuemmer, Aelian's Chief Scientific Officer.

What CROP-seq can do for you

The method has the advantage that it can look at the whole transcriptome in one experiment, unlike existing CRISPR screens which score for the presence or absence of a single phenotype. 'This means once we've done a CROP-seq screen, we don't need to redo it," said Bürckstümmer. "All the information is right there in front of us and alternative phenotypes can be queried in hindsight."

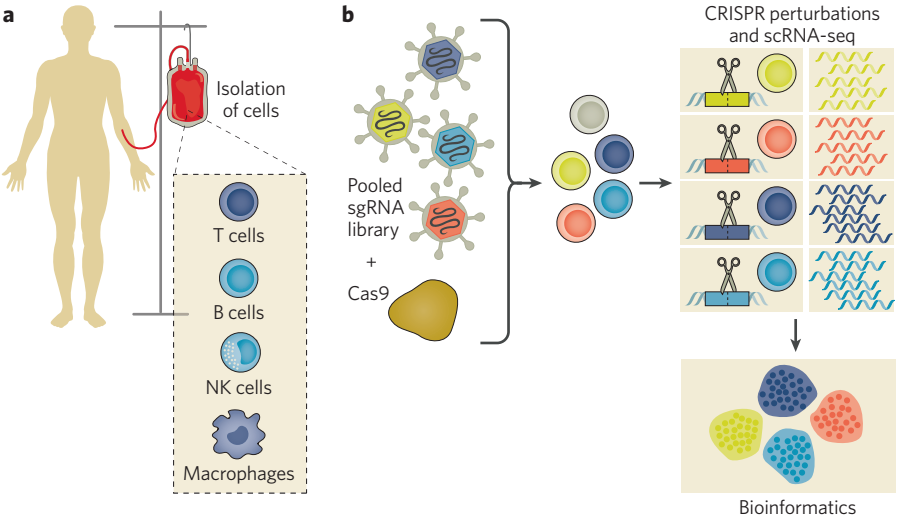

Fig. 1 | Single-cell CRISPR screens in primary human blood cells. (a) Primary T cells, B cells, NK cells or macrophages are isolated from human blood. (b) Following isolation, cells are transduced with Cas9 and a suitable guide RNA (sgRNA) library in a pooled fashion. Transcriptomic phenotypes are then assessed by single-cell RNA sequencing (scRNA-seq) and bioinformatics analysis. NK, natural killer cells.

CROP-seq is particularly attractive for the discovery of novel targets for immunotherapy, because of the relative ease with which immune cells can be recovered from blood. "Historically such experiments have been conducted using cancer cell lines, but we need to move to primary human blood cells to capture relevant biology. At Aelian, human T cells are up and running, NK cells and macrophages will be next," said Bürckstümmer.

The workflow starts with blood cells obtained from healthy donors, or even patients, and allows for individual immune cell types to be profiled (Fig. 1). "Single cell RNA sequencing captures a lot of the heterogeneity that is built into cells. For example, T cells come in several flavors, they can be cytotoxic cells or helper cells or even subtypes thereof. Capturing this heterogeneity with single-cell RNA sequencing, rather than brushing over it, is really the only way to provide meaningful CRISPR screening data," added Bürckstümmer.

The company has been working with a large pharmaceutical company to produce proof-of-concept data in primary human T cells, from blood obtained from healthy donors. Importantly, Aelian does not only cover the wet-lab work, but has assembled a dedicated bioinformatics team. This is necessary to deal with the enormous next generation sequencing datasets that emerge from each single-cell CRISPR screen. "These days, we routinely create close to 10 billion sequencing reads in one experiment," said Moser.

Aelian's partnering criteria

Aelian is looking to work with biotechnology and pharma companies seeking to identify or validate novel drug targets in immuno-oncology. "We can use our CROP-seq workflow to decipher the critical genes and pathways driving disease phenotypes and understanding better how a drug acts in complex cell environments," said Moser.

Moving forward, the company is developing further screens for other immune cell types, including natural killer cells, B cell and macrophages, so they can offer partnering companies an even wider range of cell types for functional genomic screens. "Now our ambition is to become the foremost technology company for functional genomics, with a significant emphasis on immunology and immuno-oncology," said Moser.

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