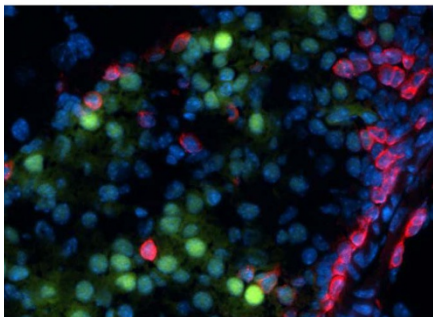
IMMUNOLOGY

Jedi cells patrol the mouse

Engineered Jedi T cells scour the body to eliminate GFP-expressing cells.

Nearly ten years ago in Milan, Brian Brown was working on better gene therapy vectors when he noticed that transgenes, when delivered to different cell types, triggered very different immune responses. Targeting transgenes to hepatocytes could even give security clearance; the immune cells believed that the genes were the body's own. His work touched on a long-standing question: how do immune cells interact with various cells of the body?



J. Agudo, Icahn School of Medicine at Mount Sinai

Jedi T cells (red) target GFP-expressing beta cells (green) in the mouse pancreas.

So-called naive T cells prowl the tissues of the body in search of infected or malignant cells that express foreign antigens. Binding of an antigen with its specific T cell receptor activates the T cell and usually turns it into a killer that targets the antigen-producing cell type. To study these immune interactions, researchers have generated mice with T cells specific for chicken ovalbumin. The T cells can be injected into host mice, where they attack tissues engineered to express the egg protein—but the target cells cannot be easily imaged. Brown came up with the idea of combining cell labeling with targeting by simply asking, "Can we turn GFP into a model antigen?"

Now at the Icahn School of Medicine at Mount Sinai, New York, he and his colleagues have produced 'just EGFP death–inducing' (Jedi) T cells. They generated the cells by immunizing mice with enhanced GFP (EGFP) and then using major histocompatibility complex (MHC) protein tetramers that recognize the immunodominant epitope of

GENOMICS

A SINGLE CELL'S OPEN CHROMATIN

Increasing the sensitivity of DNase-seq allows chromatin accessibility to be profiled from very low numbers of cells.

Although cells derived from the same clone, tissue or organism have identical genomes, their gene expression varies, as single-cell RNA-sequencing data have shown. "We need to understand the determinants of that heterogeneity in expression," says Keji Zhao at the US National Institutes of Health, "and we need to understand single-cell chromatin states, chromatin accessibility."

Comparing cellular expression profiles yields information on the genes actively expressed in each cell, but identifying regions of open chromatin provides additional information about where regulatory regions such as enhancers and promoters lie. It also reveals a gene's potential. "At the moment of the analysis, a gene may not be expressed, but it may be expressed under the right conditions," says Zhao.

The traditional method for probing open chromatin (termed DNase-seq) has been to digest the DNA with DNase I nuclease and then isolate and sequence short DNA fragments (fewer than 200 base pairs (bp)) that comprise regions not protected by histones and thus are open to cleavage. Large consortia such as ENCODE have profiled such DNase-hypersensitive sites (DHSs) in populations of cells and drawn what Zhao considers a "pretty comprehensive" map of cells' DHS landscapes. ENCODE profiled around 90,000 DHSs in one cell type, but a critical limitation is that they needed 10 million cells to do so. "You want a more sensitive method," explains Zhao, "because practically for cells from primary tissue the numbers are very limited."

To increase the sensitivity of DNase-seq, Zhao's team added large excesses of circular carrier DNA to minimize loss of the tiny amounts of nuclease-digested fragments and to specifically amplify and enrich only those short DHS fragments. PCR adaptors do

EGFP to isolate antigen-specific T cells. Selected cells were used to generate transgenic mice by somatic cell nuclear transfer.

The chosen Jedi strain produces gifted killers. Transplanted Jedi cells destroyed cells in the pancreas that express EGFP from the insulin promoter, causing a drop in insulin and modeling type I diabetes. Impressively, they were also able to target a very rare EGFP-expressing cell type in the heart and clarify its role in cardiac conduction and pacemaking.

A great strength of the Jedi cells is their generality—researchers can now subject the large catalog of tissue-specific EGFP mouse models to T cells, and the system is designed to work in a number of genetic backgrounds. "A lot of people are excited about targeting rare cell types," says Brown. "One thing we'll be able to see is … cells that are resistant to T cell killing," he adds, which is critical for understanding immune evasion and residual cells that enable tumor survival.

Specific cells can be ablated in other ways, such as by inducing expression of the diphtheria toxin receptor and adding its cognate toxin, but repeated application can lead to general toxicity. Jedi cells seem to behave in a physiological way; "once most of the [target] cells are gone, we see the T cells contract very quickly," notes Brown.

The Jedi system also makes it possible to directly confirm and quantify cell death. "We immunologists love flow cytometry," says Brown. "It's the first time that a model antigen has been visualizable, and that allows us to do flow analysis."

Although a healthy brain is generally considered to be immune privileged ("If you open up a brain, you will not see T cells there," says Brown), the researchers showed that EGFP-expressing microglia can recruit naive Jedi T cells. This important finding suggests an immunosurveillance role for naive T cells that extends beyond the blood-brain barrier.

Jedi T cells will enable new studies of T cell interactions as well as of tissue heterogeneity and function at a granular level across a wide range of cell types.

Tal Nawy

RESEARCH PAPERS

Agudo, J. *et al.* GFP-specific CD8 T cells enable targeted cell depletion and visualization of T-cell interactions. *Nat. Biotechnol.* **33**, 1287–1292 (2015).

not ligate to circular DNA, so it cannot be amplified, and nonspecific DNase fragments amplify inefficiently because they tend to be much larger than 200 bp. With this single-cell (sc) DNase-seq strategy, the researchers were able to profile DHSs using very low input—from 10,000 cells down to a single cell—and their results agree with those from ENCODE data. In a single cell they identified more than 30,000 DHSs, from 100 cells they detected 50,000 DHSs, and with 1,000 cells they detected 60,000 DHSs, which comes close to ENCODE's 90,000.

Of course, it is difficult to validate a DHS unique to a single cell. Unlike transcriptional profiling, which can be validated with imaging techniques such as fluorescence *in situ* hybridization, there is no orthogonal technique for DNase-seq. Zhao's team used DHSs derived from bulk genomic DNA or ENCODE data to confirm their results and saw that DHS patterns even from single cells were highly reproducible and correlated with modifications on histones typical for active chromatin.

Changes in gene expression and chromatin accessibility are also a hallmark of cancer cells, and the researchers tried scDNase-seq on formalin-fixed paraffin-embedded (FFPE) tumor samples. Such samples present a particular challenge to molecular analysis, because the DNA is damaged during the harsh fixation procedure. "We did not expect that it would work," recalls Zhao, but they were pleasantly surprised to see that chromatin features such as DHSs were retained in the samples.

They found a mutation in a patient with thyroid cancer that affected the binding of a tumor suppressor. Opening the huge repertoire of FFPE samples to DHS analysis is likely to yield a wealth of important information on how the regulation of gene expression goes awry in disease.

Nicole Rusk

RESEARCH PAPERS

Jin, W. *et al.* Genome-wide detection of DNase I hypersensitive sites in single cells and FFPE tissue samples. *Nature* **528**, 142–146 (2015).