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ATAC-seq, primary human T cells overexpressing BATF3

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Andrea R Daniel: This protocol was adapted form Sean McCutcheon's work in the Gersbach lab at Duke University.

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ABSTRACT

This protocol describes methods for performing ATACseq on human HER2 targeted CAR T cells overexpressing BATF3 or GFP. Chromatin remodeling was assessed in acutely and chronically stimulated cells.

Protocol status: Working
We use this protocol and it's working

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Transfections for high-titer lentiviral production

1 Plate 1.2 x 106 or 7 x 106 HEK293T cells in a 6 well plate or 10 cm dish in the afternoon with 2 mL or 12 mL of complete opti-MEM (Opti-MEM‰ I Reduced Serum Medium supplemented with 1x Glutamax, 5% FBS, 1 mM Sodium Pyruvate, and 1x MEM Non-Essential Amino Acids).

2 The next morning, transfect HEK293T cells with 0.5 µg pMD2.G, 1.5 µg psPAX2, and 0.5 µg transgene for 6 well plates or 3.25 µg pMD2.G, 9.75 µg psPAX2, and 4.3 µg transgene for 10 cm dishes using Lipofectamine 3000.

3 Exchanged media 6 hours after transfection and collect and pool lentiviral supernatant at 24 hours and 48 hours after transfection.

Primary human CD8+ T cell cultures

4 Isolated CD8+ T cells from individual donors were obtianed directly from vials purchased from StemCell Technologies.

5 Culture T cells in PRIME-XV T cell Expansion XSFM (FujiFilm) supplemented with 5% human platelet lysate (Compass Biomed), 100 U/ml penicillin and 100 µg/ml streptomycin. All media were supplemented with 100 U/ml human IL-2 (Peprotech).

Transduction of primary human CD8+ T cells

6 Centrifuged lentiviral supernatant at 600g for 10 min to remove cellular debris.

7 Concentrate lentivirus to 50–100× the initial concentration using Lenti-X Concentrator (Takara Bio).

8 Transduce T cells at 5–10% v/v of concentrated lentivirus at 24 h post-activation. For dual transduction experiments, T cells were serially transduced at 24 h and 48 h.

T cell stimulation with HER2+ tumor cells

9 HER2+ SKBR3 breast cancer cells were maintained in Dulbecco's modified Eagle medium (DMEM) GlutaMAX supplemented with 10% fetal bovine serum (FBS), 1mM sodium pyruvate, 1x MEM nonessential amino acids, 10mM HEPES, 100U/ml penicillin and 100ug/ml streptomycin.

10 Transfer 1 x 10^5 HER2 CAR T cells (with or without BATF3 overexpression) to a new 24-well plate with 2 x10^5 SKBR3 cells (1:2 E:T ratio) every 3 days (T cells are stimulated on days 3, 6, and 9).

11 T cells are removed from antigen stimulation for 2 days to recover after the final round of tumor cell stimulation before ATAC-seq on day 14 after transduction.

ATAC-seq

12 Sort a total of 5×10^4 transduced $CD8^+$ T cells for Omni ATAC-seq as previously described[75]. See the published Omni ATAC-seq reference protocol: https://doi.org/10.1038/protex.2017.096

13 Libraries were sequenced on an Illumina NextSeq 2000 with paired-end 50-bp reads. Read quality was assessed with FastQC and adapters were trimmed with Trimmomatic[72].

14 Trimmed reads were aligned to the Hg38 reference genome using Bowtie[76](v1.0.0) using parameters -v 2–best–strata -m 1.

15 Reads mapping to the ENCODE hg38 blacklisted regions were removed using bedtools2 (ref. [77]) intersect (v2.25.0). Duplicate reads were excluded using Picard MarkDuplicates (v1.130 (ref. [78])).

16 Count-per-million-normalized bigWig files were generated for visualization using deeptools bamCoverage[79] (v3.0.1).

17 Peak calling was performed using MACS2 narrowPeak[80] and filtered for $P_{adj} \leq 0.001$. Peak calls were merged across samples to make a union-peak set.

18 A count matrix containing the number of reads in peaks for each sample was generated using featureCounts[73] (subread v1.4.6) and used for differential analysis in DESeq2 (ref. [68]) (v.1.36).

19 ChIPSeeker[81] was used to annotate the genomic regions and retrieve the nearest gene around each peak.

20 HOMER (v4.11) package[82] was used to find transcription factor binding motifs that contributed to changes in chromatin accessibility with BATF3 OE compared to control cells.

20.1 We defined the set of target differentially accessible peaks using DESeq2 (Padj < 0.05) and a background set of nondynamic regions (p value > 0.2 and |log2(fold change)| < 0.2) with all sets having a sufficiently large number of sequences.

20.2 Next, for each set we extracted FASTA sequences from the human reference genome (GRCh38) and ran findMotif.pl to discover motifs and compute the enrichment over background. By default, this

function uses a hypergeometric distribution to score motifs to calculate enrichment p-values, controlling for differences in GC-content across target and background sets.