39. Genetic Mutation in Immune Cells

(Emory University)

Asset Overview

Product Type	Cell Therapy
Disease Area	Oncology
Indication	Cancer
Current Stage	Lead Optimization
Target	DNA methyltransferase 3a
MoA	• Once Deleting, changing, or inserting nucleotides within the DNMT3A gene in human CD8 T cells, the DNMT3TA gene product does not function for methylation. • Modification of the DNMT3A gene provides an improvement in antigen-specific T cells functions and/or an enhancement of the longevity of the cells
Brief Description	• Conditional deletion of the de novo methyltransferase Dnmt3a at an early stage of effector differentiation resulted in reduced methylation and faster re-expression of naive-associated genes, thereby accelerating the development of memory cells. • Longitudinal phenotypic and epigenetic characterization of the memory-precursor effector subset of virus-specific CD8 T cells transferred into antigen-free mice revealed that differentiation to memory cells was coupled to erasure of de novo methylation programs and re-expression of naïve associated genes. • Epigenetic repression of naive-associated genes in effector CD8 T cells can be reversed in cells that develop into long-lived memory CD8 T cells while key effector genes remain demethylated, demonstrating that memory T cells arise from a subset of fate-permissive effector T cells.
Intellectual Property	US20210275592A1
Publication	Effector CD8 T cells dedifferentiate into long-lived memory cells. Nature, (2017)
Inventors	Rafi Ahmed, Benjamin YOUNGBLOOD

Highlights

- MP CD8 T cells acquire genome-wide effector-associated DNA methylation programs.
- Dnmt3a-mediated de novo DNA methylation regulates the kinetics of gene re-expression during the effector-to-memory CD8 T cell transition.
- Effector CD8 T cells erase Dnmt3a-mediated DNA methylation programs during their development into memory CD8 T cells.
- Conditional deletion of Dnmt3a in activated CD8 T cells inhibits effector-associated de novo DNA methylation but does not impair maintenance methylation.

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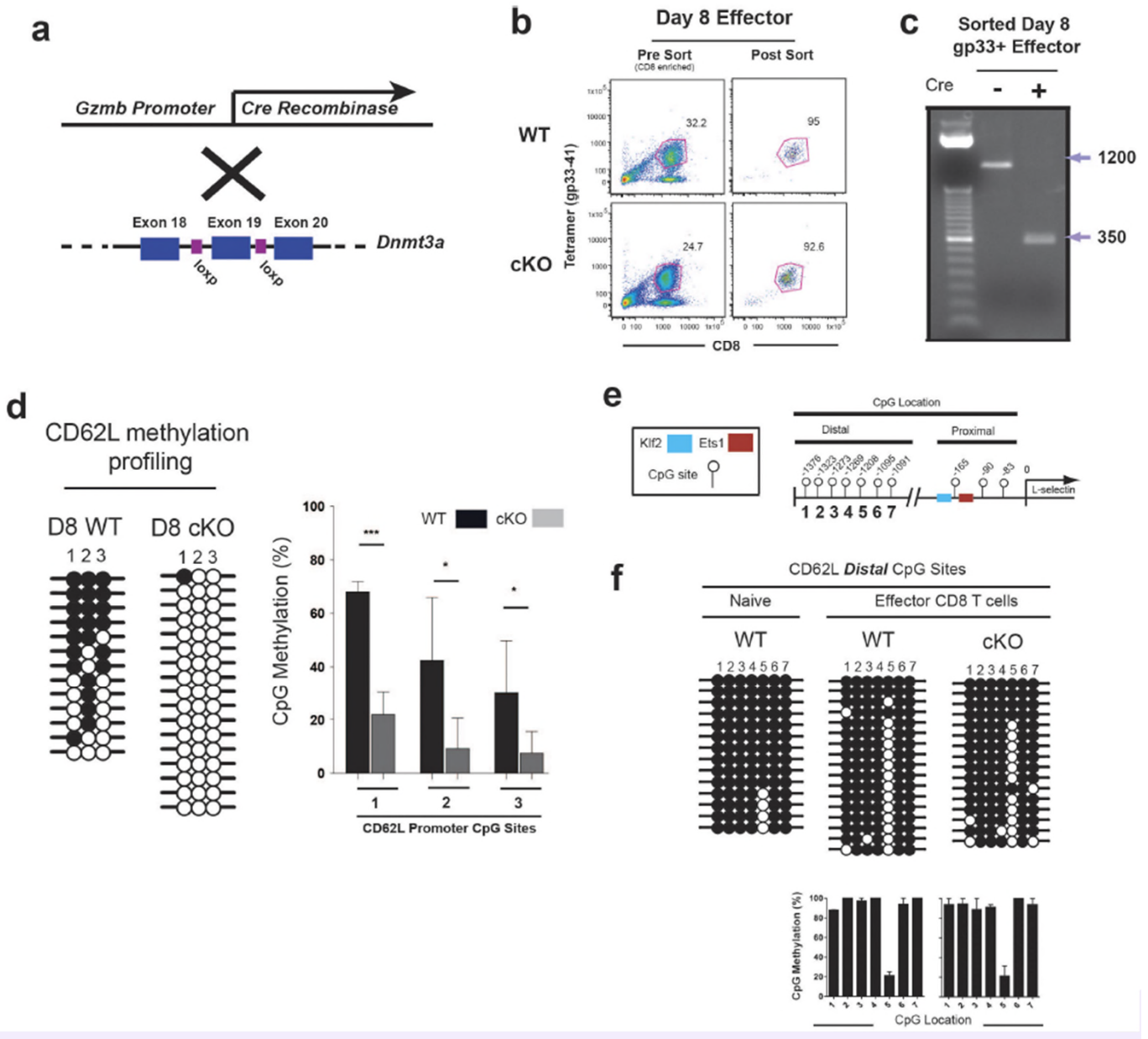
Key Data

Effects of conditional deletion of Dnmt3a in activated CD8 T cells



a, Cre recombinase expression is driven by the *Gzmb* promoter to initiate recombination of *Dnmt3a* exon 19 following T cell activation. **b**, Representative FACS analysis of virus-specific CD8 T cells sorted 8 days after acute viral infection of wild-type and *Dnmt3a* cKO mice. **c**, Recombination of genomic DNA from FACS-purified *Dnmt3a* cKO virus-specific CD8 T cells was assessed by PCR using primers that anneal to DNA outside the floxed target region. The larger PCR amplicon corresponds to the intact locus and the smaller PCR product is the amplicon of the recombined locus. **d**, Representative and graphical summary of *Sell* promoter methylation in wild-type and *Dnmt3a* cKO cells. Mean and s.d. were calculated from bisulfite sequencing analysis of six individually sorted populations. **e**, Diagram of *Sell* promoter CpG location proximal and distal to the TSS. **f**, Representative DNA methylation analysis of CpG sites distal to the *Sell* promoter regions in day 8 wild-type and *Dnmt3a* cKO antigenspecific effector CD8 T cells. Graphical summary of the average *Sell* distal CpG methylation in wild-type and *Dnmt3a* cKO cells calculated from bisulfite sequencing analysis of four individually sorted populations.