HHS Public Access

Author manuscript

Science. Author manuscript; available in PMC 2019 January 12.

Published in final edited form as:

Science. 2018 January 12; 359(6372): 163–164. doi:10.1126/science.aar5541.

Silencing stemness in T cell differentiation:

Epigenetic repression is required for the generation of CD8$^+$ effector T cells

Amanda N. Henning[1,2], **Christopher A. Klebanoff**[3,4], and **Nicholas P. Restifo**[1,2]

[1]Center for Cell-Based Therapy, National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD 20892, USA.

[2]Center for Cancer Research, NCI, NIH, Bethesda, MD 20892, USA.

[3]Center for Cell Engineering and Department of Medicine, Memorial Sloan Kettering Cancer Center (MSKCC), New York, NY 10065, USA.

[4]Parker Institute for Cancer Immunotherapy, MSKCC, New York, NY 10065, USA.

Function al diversity in multicellular organisms is achieved through the differentiation of stem cells. During this process, stem cells must retain both the capacity for self-renewal and the ability to differentiate into highly specialized cell types to produce a diverse array of tissues, each with distinct functions and organization. This plasticity is achieved through alterations to the epigenome, heri-table and reversible modifications to DNA and histones that affect chromatin structure and gene transcription without altering the DNA sequence itself. Alterations to the epigenome enable cell type–specific transcriptional control that can change dynamically over the life of a cell. Such flexibility and responsiveness are instrumental in directing gene expression changes throughout cellular differentiation and lineage specification. The acquisition of more specialized functions during differentiation requires not only that the epigenome turn “on” genes involved in lineage commitment, it also necessitates that genes associated with stemness are simultaneously turned “off” (1). On page 177 of this issue, Pace *et al.* (2) demonstrate that this phenomenon exists in CD8$^+$ T cells, in which epigenetic repression of stemness-associated genes by the histone methyltransferase SUV39H1 is required for T cell effector differentiation. Understanding these mechanisms addresses important questions in immunology and is applicable to cancer immunotherapy.

The CD8$^+$ T lymphocyte compartment of the adaptive immune system has emerged as a model for developmental biology in adult mammalian cells owing to its remarkable degree of functional plasticity (3). CD8$^+$ T cells can rapidly differentiate from a quiescent, long-lived memory state into an effector state characterized by short-lived cytotoxicity toward cancer cells or cells infected with intracellular pathogens (4). Multiple differentiation models have been proposed to account for the observed changes in CD8$^+$ T cell subsets during an immune response. The linear differentiation model places effector T cells (T_{eff} cells) at the end of the differentiation process after the development of multiple intermediary memory T cell subsets (3). Specialized memory T cells, including the relatively rare T memory stem cells (T_{scm} cells) and the more common central memory T cells (T_{cm} cells), have characteristics associated with conventional stem cells. This includes enhanced self-renewal, which is essential for maintaining long-term immunological memory, and the ability to

reconstitute other $CD8^+$ T cell subsets, which maintains the functional diversity of the $CD8^+$ T cell compartment (5–7). T_{scm} cells have enhanced stem cell–like capabilities, whereas T_{cm} cells are poised to rapidly initiate an effector response. With further T cell activation, memory subsets can differentiate into T_{eff} cells followed by terminal differentiation, functional senescence, and ultimately apoptosis (cell death). An alternative model suggests that naïve T cells (T_n cells) differentiate into T_{eff} cells immediately after activation, with "dedifferentiation" into memory cells occurring after pathogen clearance (8). Because the dedifferentiation of lineage-restricted cells rarely occurs in nature outside of cancer formation (9), we and others (7) feel that the linear differentiation model is more consistent with typical patterns of cellular differentiation.

$CD8^+$ T cell subsets can be partitioned on the basis of distinct patterns of gene expression. Multiple subset-specific transcription factors regulate gene expression throughout differentiation (4). Although transcription factors are critical mediators of gene expression programs, their activity is largely dependent on epigenetic modifications, the profiles of which can also be used to distinguish T cell subsets (10). Indeed, activating epigenetic modifications are progressively gained at T_{eff} cell–associated gene loci after T cell activation (10, 11). Recently, characterization of repressive epigenetic modifications during differentiation, as described by Pace *et al.* and others (11–14), have highlighted the importance of epigenetic silencing for proper T_{eff} cell differentiation. Specifically, epigenetic silencing of stem cell- and T cell memory–associated genes in activated T cells permits efficient T_{eff} cell differentiation and function, such that elimination of this activity results in defective T_{eff} cells (2, 11–14).

Investigations into the repressive chromatin landscape of $CD8^+$ T cells have focused on DNA methylation and trimethylation (me3) of specific lysine residues (K) on the histone H3 (specifically, H3K27me3 and H3K9me3). The epigenetic "writer" proteins responsible for adding these modifications include DNA methyltransferase 3A (DNMT3A), an enzyme responsible for de novo DNA methylation, and the histone methyltransferase enzymes enhancer of zeste homolog 2 (EZH2) and SUV39H1 (10). In mice, conditional ablation of *Dnmt3a* (12) and *Ezh2* (13) in T cells and germline ablation of *Suv39h1* (2) result in an altered phenotypic composition of antigen-specific $CD8^+$ T cells after viral infection: Both the proportion and number of responding T_{eff} cells are reduced and the frequency of memory T cells are increased. In vitro experiments using *Ezh2*-deficient T cells suggest selective apoptosis within the T_{eff} cell population (14), which accounts for the equal numbers of antigen-specific memory cell subsets as well as the impaired functional efficacy of CD8+ T cells after secondary viral challenge observed in *Ezh2*- and *Suv39h1*-deficient mice (2, 13). Preserved memory T cell formation is consistent with the linear differentiation model that places memory cell development before differentiation into T_{eff} cells. By contrast, in a model that predicts that memory T cells originate from T_{eff} cells, one would expect numbers of memory T cells to decrease as well as T_{eff} cells.

Transcriptional and epigenetic profiling of *Dnmt3a*-, *Ezh2*-, and *Suv39h1*-deficient T_{eff} cells illustrates a common defect that is responsible for impaired T_{eff} cell differentiation. Genes encoding master regulators of the stem and memory cell state fail to ac quire repressive epigenetic modifications, leading to aberrant gene expression and differentiation (2, 12, 13).

Therefore, epigenetic repression of essential stem and memory genes is required for full T_{eff} cell differentiation (see the figure). That T_{eff} cell differentiation is still possible with loss of any one of these epigenetic writers illustrates the functional redundancy in silencing stem and memory genes, stressing the importance of this mechanism. This mirrors the epigenetic silencing of developmental and pluripotency genes during differentiation of human embryonic stem cells (1) and further highlights transcriptional silencing of stem cell–associated genes as a hallmark of cellular differentiation.

Understanding the mechanisms of epigenetic regulation of T_{eff} cell differentiation has considerable implications for multiple fields, including cancer immunotherapy. Less differentiated T cell subsets, such as T_{scm} and T_{cm} cells, have enhanced proliferative potential and greater antitumor activity when transferred into both mice and humans compared with the more differentiated T effector memory cell (T_{em} cell) and T_{eff} cell subsets. This is likely due to their stem cell–like properties (4, 6). Because the majority of cells currently used for T cell–based cancer immunotherapy are T_{eff} cells, the epigenetic silencing of stem and memory genes in these cells poses a considerable therapeutic roadblock. To reacquire therapeutically beneficial stem cell–like properties, T_{eff} cells would need to be epigenetically reprogrammed. This can be experimentally accomplished, albeit inefficiently (15). A greater understanding of the $CD8^+$ T cell epigenome may therefore provide essential clues for how to unlock the potential of highly differentiated, tumor-antigen-specific T cells infiltrating tumors (4). Epi-genetic modifying drugs may reverse the repression of stem and memory genes in differentiated T cells and improve T cell-based immunotherapies.

REFERENCES

1. Hawkins RD et al., Cell Stem Cell 6, 479 (2010). [PubMed: 20452322]
2. Pace L et al., Science 359, 177 (2018). [PubMed: 29326266]
3. Restifo NP, Gattinoni L, Curr. Opin. Immunol 25, 556 (2013). [PubMed: 24148236]
4. Gattinoni L et al., Nat. Rev. Cancer 12, 671 (2012). [PubMed: 22996603]
5. Gattinoni L et al., Nat. Med 17, 1290 (2011). [PubMed: 21926977]
6. Klebanoff CA et al., J. Clin. Invest 126, 318 (2016). [PubMed: 26657860]
7. Graef P et al., Immunity 41, 116 (2014). [PubMed: 25035956]
8. Youngblood B et al., Nature 10.1038/nature25144 (2017).
9. Friedmann-Morvinski D, Verma IM, EMBO Rep. 15, 244 (2014). [PubMed: 24531722]
10. Phan AT et al., Immunity 46, 714 (2017). [PubMed: 28514673]
11. Crompton JG et al., Cell Mol. Immunol 13, 502 (2016). [PubMed: 25914936]
12. Ladle BH et al., Proc. Natl. Acad. Sci. U.S.A. 113, 10631 (2016). [PubMed: 27582468]
13. Gray SM et al., Immunity 46, 596 (2017). [PubMed: 28410989]
14. Kakaradov B et al., Nat. Immunol 18, 422 (2017). [PubMed: 28218746]
15. Takahashi K, Yamanaka S, Cell 126, 663 (2006). [PubMed: 16904174]

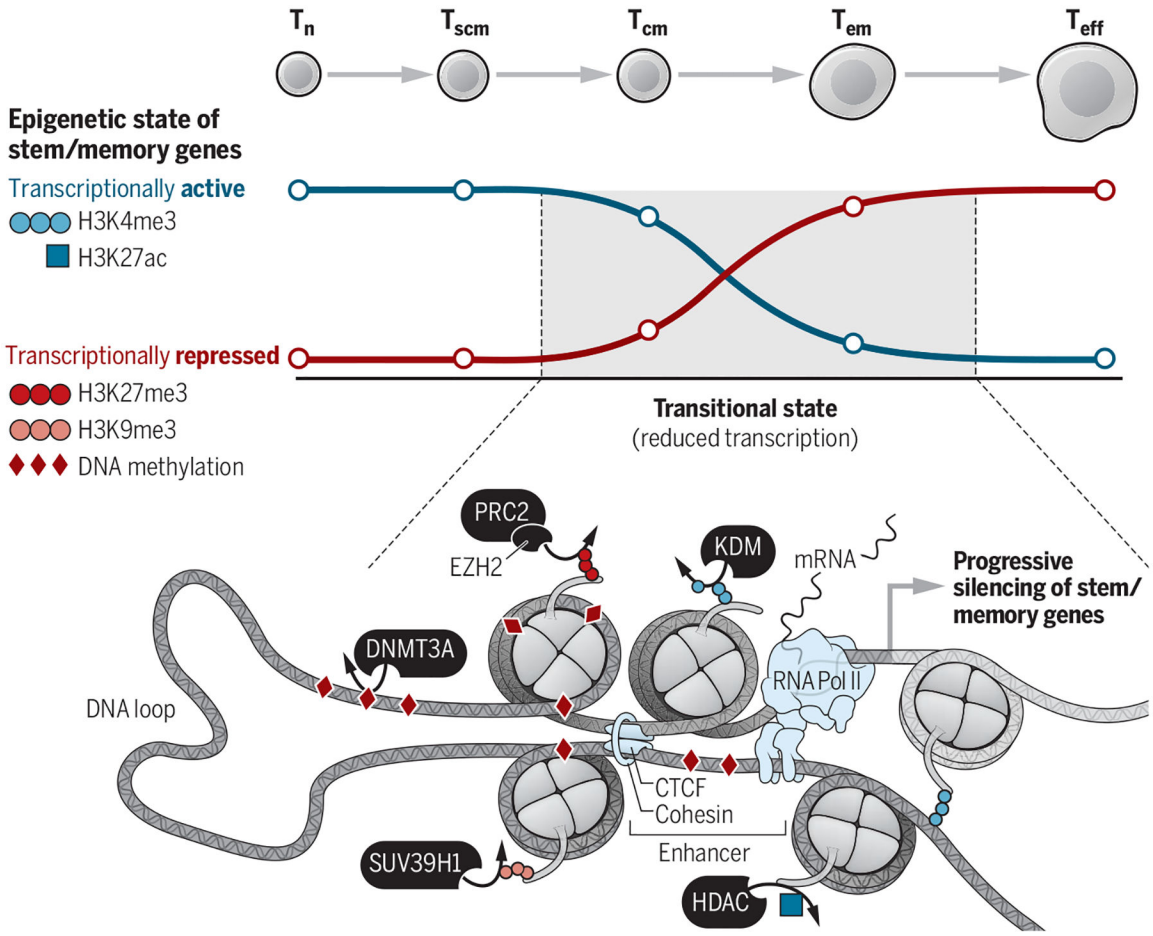

Figure. Shutting down stem and memory genes in CD8$^+$ T cells

As cells differentiate, stem and memory genes pass through transitional epigenetic states, in which epigenetic modifications associated with transcriptional activation, including H3K4me3 and H3K27ac, are lost via lysine demethylases (KDMs) and histone deacetylases (HDACs). Conversely, repressive modifications such as DNA methylation, H3K27me3, and H3K9me3 are gained because of epigenetic writers, including DNMT3A, EZH2 as part of the Polycomb repressive complex 2 (PRC2), and SUV39H1. Not shown but occurring simultaneously is the acquisition of activating epigenetic modifications at effector-associated genes during T cell differentiation.