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Epigenetic regulation of telomerase expression in HIV-1-specific CD8+ T cells

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Abstract

Telomerase activity in HIV-1-specific CD8+ T cells from controllers contributes to the maintenance of highly-functional cytotoxic T cell responses against HIV-1. Here, we show that high expression of telomerase in controllers is associated with hypermethylation at the distal and hypomethylation at the proximal hTERT promoter, while HIV-1-specific CD8+ T cells from progressors showed an inverse pattern with distal promoter hypomethylation and proximal promoter hypermethylation. These data indicate distinct epigenetic signatures in HIV-1-specific T cells in progressors and controllers.

Keywords

HIV-1; T cell immunity; HIV-1 controllers

Infection with HIV-1 induces a strong T cell mediated immune response, which may importantly influence the spontaneous disease progression of HIV-1 infection [1]. In contrast to other viral diseases, however, HIV-1-specific T cells fail to control HIV-1 viremia, and chronic infection with persisting moderate to high level antigenemia evolves in the majority of patients. Under these conditions of ongoing viral replication, HIV-1-specific CD8+ T cells develop a state of functional exhaustion characterized by cellular hypoproliferation, although cells remain metabolically active and are capable of executing specific effector functions such as antigen-specific interferon- γ secretion [2, 3]. This cellular condition is associated with erosion of telomeres, the distal segments of chromosomal DNA that shorten during each cell division, and the average length of telomeres in HIV-1-specific CD8+ T cells from progressors indeed approximates the level of terminal cellular senescence defined by the Hayflick limit [4]. Telomere shortening can be antagonized by telomerase, an enzyme that is highly selectively expressed in stem cells, tumor cells and activated lymphocytes. The activity of this enzyme in T cells appears to critically contribute

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Author contribution:

Research idea, study concept and writing of manuscript: XGY and ML

Performance of PCR assays and data analysis: KW, KS, JB

Contribution of PBMC samples: FP, BDW.

Discussion and critical review of manuscript: ESR, BDW.

to the preservation of pathogen-specific cellular immunity by protecting T cells against exhaustion and senescence [5], and is importantly involved in the maintenance of polyfunctional HIV-1-specific T cells in controllers [4, 6]. However, mechanisms that govern the higher activity of telomerase in HIV-specific T cells from controllers are unclear.

To analyze molecular pathways regulating telomerase activity in HIV-1-specific T cells, we used quantitative RT-PCR to analyze the mRNA expression intensity of hTERT, the catalytic subunit of telomerase, in sorted tet⁺ HIV-1-specific CD8⁺ T cells from HIV-1 controllers (n=8, viral load: 50 copies/ml (50–470), CD4 count: 682/ul (361–900), epitopes (see [4] for sequences): B8-EI8, B8-FL8, A2-SL9, A2-IV9) and progressors (n=8, viral load: 166,550 copies/ml (40,000–380,000), CD4 count: 423/ul (301–598), epitopes: B8-EI8, A2-SL9, A2-IV9, B27-KK10); autologous EBV-/CMV-specific CD8⁺ T cells (recognizing the A2-NV9 and B8-FL8 epitopes [4]) were analyzed for reference purposes. Overall, we found that hTERT expression was significantly stronger expressed in HIV-1-specific CD8⁺ T cells from controllers and CMV-/EBV-specific CD8⁺ T cells from either patient population, as opposed to HIV-1-specific CD8⁺ T cell populations from progressors (Figure 1A). These data suggest that differences in telomerase activity of HIV-1-specific CD8⁺ T cells from controllers and progressors [4] are regulated by transcriptional control of hTERT expression.

Since epigenetic methylation of the CpG islands in the hTERT promoter can critically regulate hTERT expression in cancer cells [7, 8], we subsequently tested whether the differential mRNA expression of hTERT in HIV-1-specific CD8⁺ T cells from HIV-1 progressors and controllers is associated with opposing patterns of hTERT promoter methylation. Briefly, bisulfite sequencing of the hTERT promoter was performed after amplification with nested PCR [9], and the resulting PCR products were cloned and sequenced by T7 or T3 primers as described before [10]. Overall, we found that the cytosine residues in the distal segments of the promoter region (–700bp to –400 bp) were strongly methylated (median of 50%, range 0–100%), while methylation was substantially less pronounced (median of 0%, range 0–100%) in the proximal regions of this gene sequence (–400bp to +100bp). Interestingly, within the distal promoter segment, lowest levels of methylation were observed in HIV-1-specific CD8⁺ T cells from progressors, in comparison to HIV-1-specific CD8⁺ T cells from controllers or CMV-/EBV-specific CD8⁺ T cells from either patient cohort (Figure 1B). In the proximal segments of the hTERT promoter, an opposite pattern was observed, with significantly less methylated cytosine residues in HIV-1-specific CD8⁺ T cells from controllers compared to HIV-1-specific CD8⁺ T cells from progressors, but no significant methylation difference between HIV-1-specific CD8⁺ T cells from controllers and CMV-/EBV-specific CD8⁺ T cells from either cell population. When analyzing the methylation status at a resolution of each individual cytosine residue, we found that methylation within the hTERT promoter in HIV-1-specific CD8⁺ T cells controllers clustered at selected sites involving cytosine residues 3–7, 10–14, 16, 18, 20, 23–24 and 28 of the distal promoter segment. Notably, these hypermethylated regions are entirely unmethylated in normal tissue cells without detectable telomerase expression and contain several binding sites for inhibitory transcription factors, such as MZF-2 and IK1 [7, 11].

Overall, these data reveal distal hypomethylation and proximal hypermethylation at the hTERT promoter in HIV-1-specific CD8⁺ T cells from progressors, while an inverse pattern was observed in HIV-1-specific CD8⁺ T cells from controllers and CMV-/EBV-specific CD8⁺ T cells from either patient cohort. Most surprisingly, we observed that the higher hTERT expression in HIV-1-specific CD8⁺ T cells from controllers was associated with distal hTERT promoter hypermethylation, which appears contradictory to current views of gene expression silencing by DNA cytosine methylation, and to the general notion of more pronounced DNA methylation during cellular aging and senescence [12]. Yet, a paradoxical

hTERT hypermethylation in a regulatory region (500–700bp upstream) of the hTERT promoter has previously been reported in cancer cells with high constitutive hTERT expression [7, 13], and it appears that antigen-specific T cells may in part imitate this regulatory pattern to promote long-lasting telomerase activity. Indeed, transcription factor binding to this distal region of the hTERT promoter has a negative regulatory impact on gene expression [14, 15], and the observed DNA hypermethylation in cells from controllers may promote hTERT transcription by blocking binding of inhibitory transcription factors. In contrast, the proximal segments of the hTERT promoter seem to have an activating role for regulating hTERT gene expression, and binding of activating transcription factors to this region might be facilitated by DNA hypomethylation [8].

Over the recent years, multiple genetic characteristics of HIV-1 controllers or progressors have been identified by large genome-wide association studies [16]. In contrast, epigenetic features, which as more flexible regulators of gene expression are likely to have a significant impact on the evolution of the host's immune activity against HIV-1, have received less attention. This study provides initial insight into the epigenetic regulation of a functional and long-lasting T cell mediated immune response against HIV-1 in controllers, and will be important for a more thorough understanding of molecular mechanisms guiding T cell proliferation, exhaustion and senescence.

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Figure 1. Distinct patterns of hTERT promoter methylation in HIV-1-specific CD8+T cells from controllers and progressors

(A): mRNA expression of hTERT in HIV-, CMV- or EBV-specific CD8+ T cells from HIV-1 progressors and controllers. Data were normalized to the house keeping gene β -actin; significance levels were calculated by Mann-Whitney U test. (B): Box and Whisker plots summarizing the degree of methylation of cytosine residues in the proximal (n=28 CpGs) and distal (n=70 CpGs) hTERT promoter in HIV-1-specific CD8+ T cell populations and corresponding CMV-/EBV-specific CD8+ T cell populations from controllers and progressors. Box and Whisker plots reflect the median and the 10th, 25th, 50th and 90th percentiles; outliers are depicted by individual data points. Significance was tested using Mann Whitney U test.