26. HIV-1 Mediated Insertional Mutagenesis Increase the Persistence of Infected T Cells in Patients Under ART by Triggering Their Differentiation Into Long Lived T-Regulatory and T-Central Memory Cells

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It has been recently suggested that HIV-1 by integrating near cancer-associated genes could promote the expansion and persistence of infected cells in patients under Anti Retroviral Therapy (ART). However, the molecular mechanism/s of insertional mutagenesis used and the physiological impact on the cells harboring these integrations are completely unknown.

Here, we found that in the peripheral blood mononuclear cells from 54 HIV-1 infected patients under ART, BACH2 and STAT5B were targeted by a significant number of integrations compared to other lentiviral integration datasets ($p<0.0001$) and with the same orientation of gene transcription. Furthermore, we observed that in the blood of 34% of HIV-1 patients under ART (30/87) there are cell clones expressing aberrant chimeric transcripts containing viral sequences fused to the first protein coding exon of BACH2 or STAT5B and predicted to encode for unaltered full-length proteins. However, it is unlikely that the ectopic expression of these transcription factors in T cells per se would trigger cell transformation. Indeed, accordingly with their roles in the regulation of T-cell identity and homeostasis, forced expression of STAT5B and BACH2 in naive CD4+ T-cells significantly skewed their differentiation towards functional T-regulatory cells with a marked immunosuppressive potential. Moreover, STAT5B ectopic expression promoted the IL-2 independent proliferation of T-regulatory cells that was however blocked by TGFB treatment, indicating that these cells are able to respond to external differentiating cues.

Importantly, tracking the expression of HIV-1/STAT5B transcripts in T cell subpopulations and monocytes purified from the blood of patients under ART, we found that, in all patients tested (N=6), chimeric mRNAs were present only in T-regulatory and T-central memory cells but not in CD8+ T-cells, T naïve, T stem cell memory, T effector memory nor monocytes.

Our findings provide novel evidence that HIV-1 takes advantage of insertional mutagenesis to favor its persistence in the host by activating STAT5B and possibly BACH2. However, the selective advantage conferred by these integrations does not involve T-cell transformation but rather their differentiation into functional T-regulatory and T-central memory cells which are long lived, potentially able to diminish the immune surveillance against infected cells thus favoring the escape from the immune system and long-term viral persistence.

Hence, new targeted therapies aimed at interfering with BACH2 and STAT5B regulated pathways could provide the general means for the immunological re-sensitization towards HIV-1 infected cells to reduce long lived cellular reservoirs and the eradication of the viral infection in HIV-1 patients.

27. Aberrant Expression of the Stem Cell microRNA-126 Induces B Cell Malignancy

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MicroRNAs are essential regulators of normal and malignant hematopoiesis. miRNAs are relevant for gene therapy, since they can be exploited to fine-tune the expression profile of vector constructs or to alter viral tropism (Gentner&Naldini, 2012). We exploited the hematopoietic stem cell (HSC) specific expression of miR-126 to de-target transgene expression from primitive compartment (Gentner et al, 2010; Chiriaco et al, 2014; Escobar et al, 2014) and described the function of miR-126 in HSC where it regulates the balance between quiescence and self-renewal (Lechman et al, 2012). We here report a novel role for miR-126 in the induction and maintenance of high-grade B cell malignancies. By ectopically expressing miR-126 in transplanted BM cells, we observed that up to 60% of mice (n=71) developed B cell malignancies. LV insertion site (IS) analysis revealed that all tumors were monoclonal. We then tracked back leukemic clone to different hematopoietic lineages prospectively purified from the mice 2-6 months before disease onset. IS sharing between normal lineages and leukemic clone suggests stem or multipotent progenitor cell as origin for most tumors. Importantly, we show that miR-126 is the direct cause of genesis and maintenance of leukemia, since leukemogenesis is abolished when miRNA expression is inhibited by doxycycline (doxy) using a tetracycline-repressible miR-126 cassette, and established symptomatic leukemia completely regresses when miR-126 is switched off by doxy through induction of apoptosis. Transcriptional profiling indicated that miR-126 regulates multiple genes in p53 pathway both in murine blasts and in normal human CD34+ cells. Previous work suggested expression of miR-126 in acute lymphoblastic leukemia (ALL) and germinal center lymphoma. To further establish the relevance of miR-126 in human disease, we measured miR-126 expression in blasts from 16 adult patients with ALL. miR-126 was highly expressed in most studied ALL cases (Phil+: n=11, Phil-: n=5), at similar levels as CD34+ cells. We then down-regulated miR-126 in primary blasts from human B-ALL patients (n=5), and we observed increased apoptosis and impaired engraftment in xenograft models after primary and secondary transplantation (miR-126/KD: n=32 mice; Ctrl: n=37 mice), demonstrating the relevance of miR-126 in human B-ALL. In conclusion, we present a novel spontaneous mouse model for high grade B cell malignancies which are addicted to miR-126 expression, provide insight into the dynamic process of leukemogenesis by clonal IS tracking and unveil key tumor signaling pathways controlled by miR-126. Down-regulation of miR-126 could be exploited as therapeutic strategy in ALL, since it would deplete leukemic cells while expanding normal HSC, two ways to restore normal hematopoieis.