IL-15 DEPENDENT NK CELLS AND DENDRITIC CELLS CROSS-TALK IN HIV-1 ELITE CONTROLLERS

00311

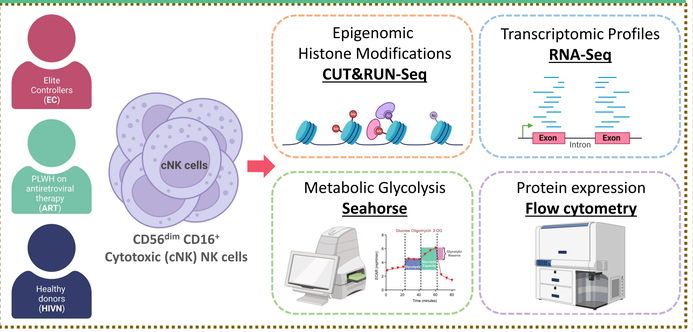
Ciputra A. Hartana[1], Ce Gao[1], Yelizaveta Rassadkina[1], Mathias Lichterfeld[1,2,3], Xu Yu[1,2]

[1]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, US, [2]Brigham and Women's Hospital, Boston, MA, US,
[3]Broad Institute of MIT and Harvard, Cambridge, MA, US

BACKGROUND

HIV-1-specific T cell responses are commonly regarded as the backbone of antiviral immunity in HIV-1 elite controllers (ECs), while the specific contribution of innate immune cells is less clear. Natural Killer (NK) cells are of particular interest, as they can mount direct cytotoxic effects against HIV-1-infected cells that do not depend on MHC class I restriction and are less prone to viral mutational escape.

METHODS



RESULTS

I. Distinct epigenomic profiles of CD56^{dim} CD16^{+} cNK cells from ECs were seen in the enrichment of activating histone marks.

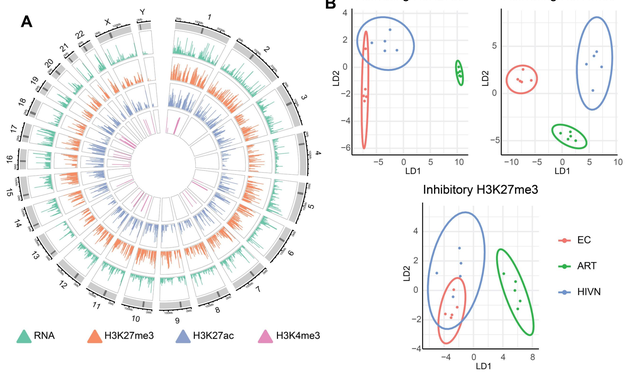


Figure 1: CD56^{dim} CD16^{+} cNK cells from ECs displayed distinct epigenomic profiles. (**A**) Circos plots reflecting the enrichment profiles of activating H3K27ac and H3K4me3 histone marks, as well as the inhibitory H3K27me3 mark from CUT&RUN-Seq in relation to the transcriptomes from RNA-Seq in all chromosomal locations in primary CD56^{dim} CD16^{+} cNK cells. (**B**) Linear discriminant analysis (LDA) of the epigenomic profiles of primary cNK cells in ECs (n = 5), ARTs (n = 5) and HIVNs (n = 5).

II. Differentially expressed genes in cNK cells from ECs were predicted for activation of pathways related to the crosstalk between dendritic cells and NK cells, NK cell signaling, metabolic regulation and IL-15-dependent immune activation.

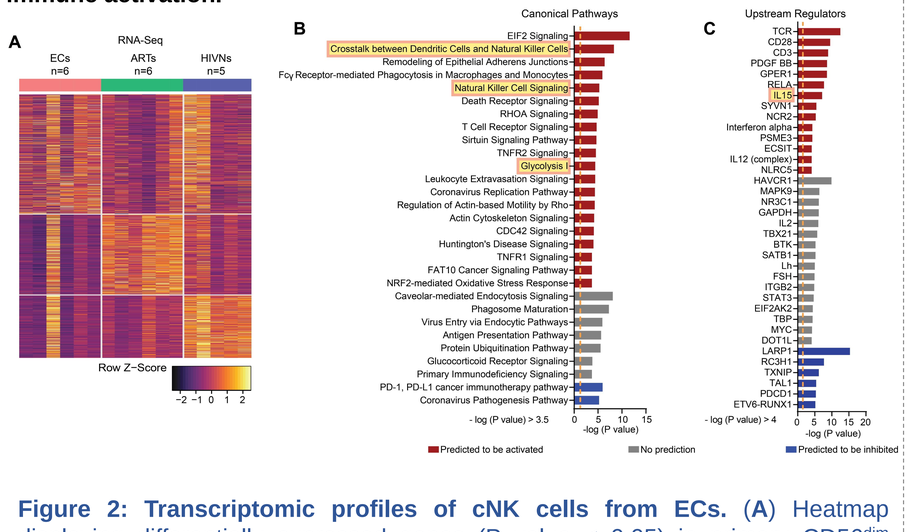


Figure 2: Transcriptomic profiles of cNK cells from ECs. (**A**) Heatmap displaying differentially expressed genes (P value < 0.05) in primary CD56^{dim} CD16^{+} cNK cells from ECs (n = 6), ARTs (n = 6) and HIVNs (n = 5) using RNAseq analysis. (**B-C**) Significant canonical pathways (B) and upstream regulators (C) predicted by Ingenuity Pathway Analysis (IPA) of differentially expressed genes from (A). Predicted up- and down-regulated pathways were marked in red and blue, respectively. Pathways with no predicted changes were marked in gray. Orange dashed lines marked –log (P value) = 1.3.

III. Increased activating epigenetic features in the IL2-Rβ gene in cNK cells from ECs may poise these cells to improved responses to paracrine IL-15 stimulation from myeloid dendritic cells (mDCs); showing evidence for an improved cross-talk between mDCs and NK cells from ECs.

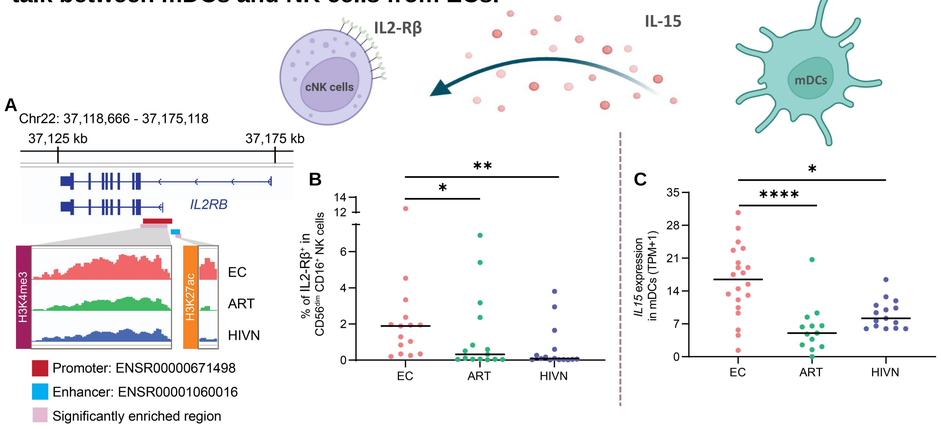


Figure 3: CD56^{dim} CD16^{+} cNK cells from ECs were supported by mDC – NK cell crosstalk via IL-15 - IL2-Rβ axis. (A) CUT&RUN-Seq reads for activating H3K27ac and H3K4me3 marks at the *IL2RB* gene locus in primary cNK cells from a representative EC, ART and HIVN. Purple areas mark regions with significantly increased histone H3K27ac or H3K4me3 reads. Predicted promoter and enhancer regions from Ensembl database are highlighted in red and blue, respectively. (**B**) The frequencies of IL2-Rβ^{+} cNK cells were compared among ECs (n = 15), ARTs (n = 15) and HIVNs (n = 15). (**C**) The mRNA expression (TPM+1) of *IL15* in mDCs was compared among ECs (n = 20), ARTs (n = 13) and HIVNs (n = 15). Kruskal-Wallis test was used as the statistical test. *P < 0.05, **P < 0.01, ****P < 0.0001

IV. Upon IL-15 stimulation to cNK cells, an upregulation of the anti-apoptotic molecule BCL-2 was prominently seen, especially in activated CD69^{+} cNK cells from ECs.

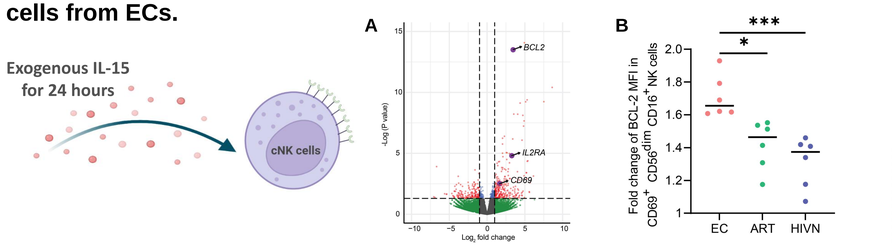


Figure 4: Upregulation of BCL-2 in cNK cells from ECs after IL-15 stimulation. (**A**) Volcano plot showing differentially expressed genes in IL-15-stimulated vs. unstimulated NK cells. Red dots represent data with –log (P value) > 1.3 and log2 fold change > 1 or <-1. (**B**) The Mean Fluorescent Intensity (MFI) of BCL-2 in CD69^{+} cNK cells were compared among ECs (n = 6), ARTs (n = 6) and HIVNs (n = 6). The MFIs were shown as fold changes after 10 ng/ml IL-15 stimulation, relative to unstimulated controls. Kruskal-Wallis test was used for statistical analysis. *P < 0.05, ***P < 0.001

V. IL-15-stimulated NK cells from ECs displayed an elevated level of glycolysis.

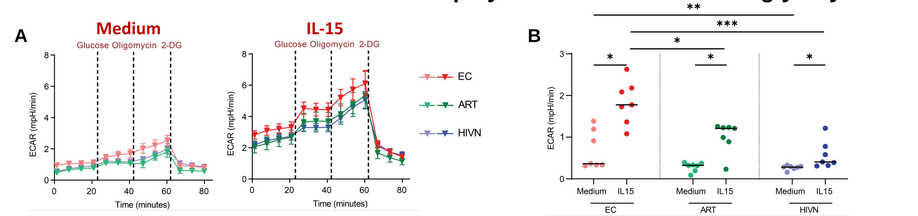


Figure 5: Increased glycolysis in NK cells from ECs. (**A**) Extracellular Acidification Rate (ECAR) of NK cells from ECs (n = 7), ARTs (n = 7) and HIVNs (n = 7) with or without 10ng/ml IL-15 stimulation for 24 hours was measured at indicated timepoints in response to glucose, oligomycin and 2-DG, using a Seahorse XFe96 Analyzer. (**B**) Glycolysis of NK cells from (A) were compared. Paired t-test and one-way ANOVA test were used to compare ECAR among cohorts. *P < 0.05, **P < 0.01, ***P < 0.001

CONCLUSIONS

Our data suggested distinguished features of cNK cells from ECs, consistent with trained innate immunity, which are supported by NK cell – mDC cross-talk.