Nucleolar Access Is Variable in Leukocytes Depending on Cellular Migration & Adhesion

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Background
The nucleolus is a prominent structure within eukaryotic nuclei. The multifunctional structure is formed from ribosomal DNA (rDNA) regions of acrocentric chromosomes 13, 14, 15, 21 and 22 in humans. In addition to ribosome biogenesis, the nucleolus has been shown to be important in mRNA splicing, DNA damage responses, and RNA metabolism among other cellular functions. Nucleoli provide a direct link between transcription and translation, making it a critical structure for protein expression. Several viruses have been shown to alter nucleolar function by targeting viral proteins to this structure. HIV-1 early-expressed proteins Tat and Rev both have highly basic nucleolar localization signals (NoLS) which may cause HIV-induced alterations of mRNA splicing, cytoplasmic transport, and translation. During our studies on HIV-1 and immunometabolism we noticed differences in nucleolar availability by highly-basic NoLS-containing peptides between adherent versus migrating leukocytes. This may suggest viral proteins like HIV-1 Tat and Rev may not be able to localize to the nucleoli in migrating leukocytes like memory CD4+ T cells, a known HIV-1 latent reservoir.

Methods
• To investigate chemotactic versus adherent behavior of leukocytes, primary human peripheral blood mononuclear cells (PBMC) were cultured on fibronectin-coated microscope dishes with 20IU/mL IL-2 in serum-containing RPMI for 4–7 days prior to imaging. Cellular morphology was imaged using SEM at the GWNIC
• Nucleolar presence in both adherent and chemotactic leukocytes was confirmed using brightfield time-lapse imaging on an incubated stage.
• Nucleolar accessibility of adherent versus chemotactic cells was assessed using a deca-arginine peptide conjugated to FITC (R10-FITC), a fluorescent peptide that has previously been shown to localize to the nucleoli.
• Nucleolar structure of PBMC was done using transmission electron microscopy using standard heavy metal staining. Live cell confocal, scanning and transmission electron microscopy was done at the GWNIC

Results
Under confocal microscopy, large prominent nucleoli could be seen in both adherent and chemotactic leukocytes. Despite clear presence of nucleoli in both cell types, only nucleoli from adherent cells were labelled with the R10-FITC peptide, suggesting differences in the activity or accessibility of the nucleolus in chemotactic cells.

Conclusions & Discussion
• NoLS-containing peptides are highly basic and their positive charges have been shown to bind to the high amount of negatively charged RNA found within the nucleoli
• The inability of R10-FITC to localize to the nucleoli of chemotactic cells may suggest decreased presence of rRNA and ribosome biogenesis within the nucleoli
• Changes in nucleolar activity between migrating and adherent leukocytes may be directly linked to ribosome number and protein production within these two states
  • When cell is adherent, the nucleolus is available and cell undergoes anabolism
    • Pentose phosphate pathway produces complex sugars for DNA/RNA synthesis & fatty acid synthesis
    • When cell is migrating, the nucleolus is not available and cell undergoes catabolism
      • During migration cell needs ATP, so cell undergoes oxidative phosphorylation in aerobic conditions
    • Viral interference of nucleolar availability through proteins like HIV-1 Tat and Rev may not only change mRNA splicing and protein translation in the infected cell, but also may change the ability of the cell to migrate or adhere to its environment.
    • HIV virus may only be able to successfully replicate when cell is adherent because nucleolus is available for protein production

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