IDENTIFICATION OF PROVIRAL INTEGRATIONS WITHIN HIV-ASSOCIATED B-CELL LYMPHOMAS

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BACKGROUND

Defective HIV proviruses within a few specific genes have been associated with T cell clonal expansion and persistence during ART. **Recently, proviruses integrated in *STAT3* and *LCK* have been implicated in insertional mutagenesis driving rare cases of T cell lymphoma[1]**. However, B cell lymphomas are much more common HIV–associated cancers, and a case report by *Katano et al.* identified an **HIV provirus within *STAT3* in a B cell lymphoma** in an individual with AIDS[2]. HIV is not typically characterized as infecting B cells, but HIV positive individuals are at a great risk of developing non-Hodgkin's lymphomas of B cell origin despite being on ART[3]. **We hypothesize that site-specific HIV proviral integrations can lead to B cell transformation and may be a contributing cause to HIV-associated B cell lymphomas.**

METHODS

Whole genome amplified **DNA samples extracted from formalin-fixed paraffin embedded (FFPE) HIV-associated B cell lymphoma** samples were obtained from the AIDS and Cancer Specimen Resource (ACSR) and assessed for HIV provirus content by PCR targeting the U5 region of the LTR. Proviruses were quantified relative to *CCR5,* a human reference gene. Integration site analysis was performed on two DNA samples that showed significant quantities of HIV LTRs per cell.

RESULTS

An initial PCR screen identified 2 out of 110 samples as positive for HIV provirus (not shown). qPCR of the 110 samples demonstrated that the two positive samples were **highly positive (between 0.3-5.0 copies/cell)** for HIV proviruses, with many more containing significant, but lower, proviral DNA levels (Fig 1). The two initial highly positive samples were subjected to integration site analysis for detection characterization of the location of clonal proviruses. **Both samples contained many uniquely detected integration sites.** Sample TMA-8 contained 1930 unique integration sites and TMA-71 contained 3696 unique sites (Fig 2). In TMA-71, at least one clonal population was identified of interest within Chromosome 6, as indicated by an arrow in Fig 2. **The provirus was integrated in the opposite orientation of the B cell lymphoma-associated gene, *SGK1*, but it comprised only a small percentage (1.2%) of total integration sites detected** (Fig 3).

Detection of high levels of HIV provirus within a small fraction of HIV-associated B cell lymphoma DNA.

Large number of lymphoma samples have HIV provirus in at least 1% of cells

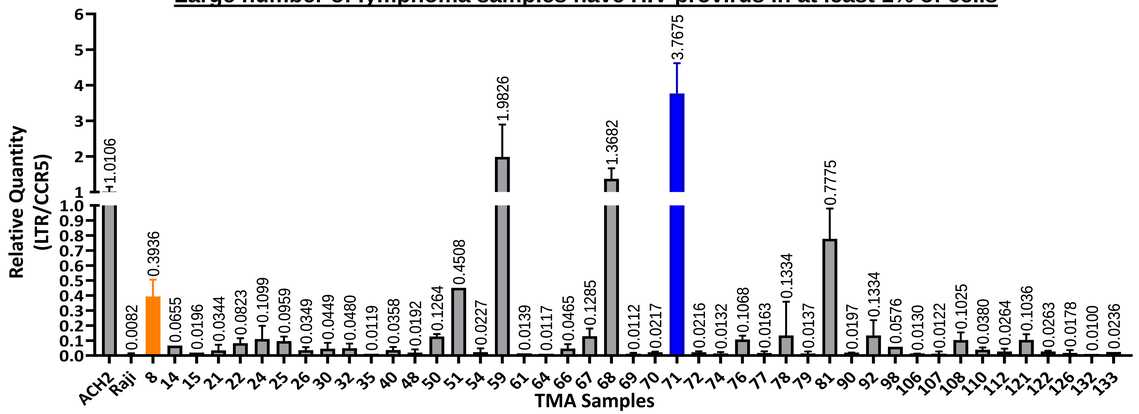


Figure 1: qPCR of remaining lymphoma DNA samples quantifying HIV provirus (LTR) relative to reference gene (*CCR5)*. ACH2 DNA used as positive control. Raji DNA used as negative control. Samples with LTR/*CCR5* ratio less than 0.01 removed from graph. Samples that showed sequences similar to ACH2 control were left out of graph.

Diverse integration sites found in the two initially screened samples

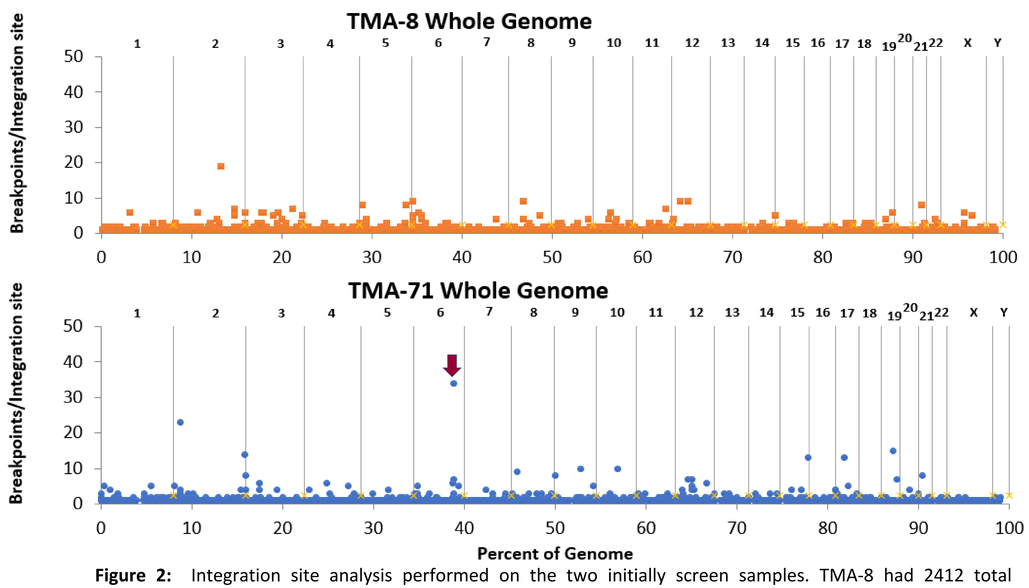


Figure 2: Integration site analysis performed on the two initially screen samples. TMA-8 had 2412 total integrations sites and 1930 unique sites. TMA-71 had 4219 total integration sites and 3696 unique sites. Arrow indicated site enlarged in Figure 3.

CONCLUSIONS

We have demonstrated the presence of high-levels of HIV provirus within a small fraction of HIV-associated B cell lymphoma. Although further studies will be required to confirm the precise cell type that contains them, B cells can be targets for HIV infection based on prior in vitro studies. HIV-driven B cell lymphoma may still be a possibility with detection of HIV integration within *SGK1*, a gene associate with the *STAT3* protein, found in a small percentage of one sample. *SGK1* mutations around the identified integration site have been associated with diffuse large B cell lymphomas[4]. **Further work needs to be done to determine to what extent B cells become infected with HIV and to what extent its integration can lead to B cell lymphoma development.**

ADDITIONAL KEY INFORMATION

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- AIDS and Cancer Specimen Resource provided the B cell lymphoma DNA used in this study.
- HIV Dynamics and Replication Program at the National Cancer Institute provided ACH2 DNA used in this study.

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Sample TMA-71 demonstrated a small percentage of clonal integration within *SGK1*, a DLBCL associated gene

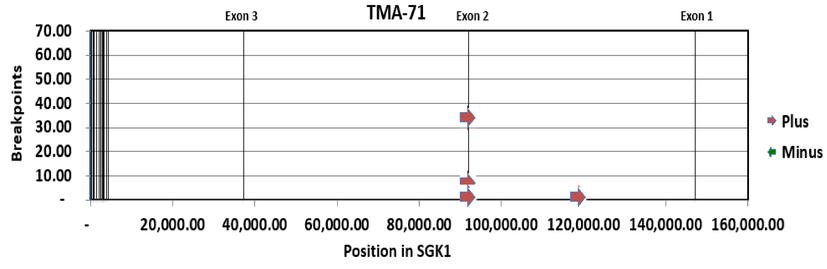


Figure 3: Integration site analysis performed on sample TMA-71. Number of breakpoints represents number of clonal integration sites. *SGK1*, is shown as it was the gene with the largest number of breakpoints out of a total of 3,696 unique integration sites.