High-resolution Antibody Profiling of KSHV-infected Individuals Presenting With and Without Kaposi Sarcoma Reveals Distinct Viral-Exposure Signatures

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Introduction

Kaposi sarcoma-associated herpesvirus (KSHV) is a gamma herpesvirus and the etiologic agent of Kaposi sarcoma (KS), an AIDS-defining cancer affecting immunocompromised individuals. Sub-Saharan Africa (SSA) exhibits a particularly high KSHV prevalence and KS incidence. Immune suppression in addition to KSHV infection is thought to drive KS development [1]. Prior co-infections have been shown to influence disease pathogenesis or lack thereof [2]. Prior exposure history can be determined through the elucidation of the humoral antibody (Ab) responses against these infectious agents. Detailed analysis and quantification of these Ab responses will be vital to discern host-pathogen interactions, define prior infection and identify potential prognostic biomarkers, and contribute to developing strategies to prevent KS development. Here, we utilized VirScan [3], a high-throughput phage display library of 56-mer peptide tiles containing 28 amino acid overlaps, spanning the proteomes of all viral pathogens with known human tropism, coupled with high-throughput phage immunoprecipitation sequencing (PhIP-seq) [4]. We identified significantly enriched Ab responses (i.e., reactive peptides) of each individual against 556 viral organisms and quantified the breadth and magnitude of responses on a protein or organism-level to derive unique viral exposure signatures in KS vs ASY controls.
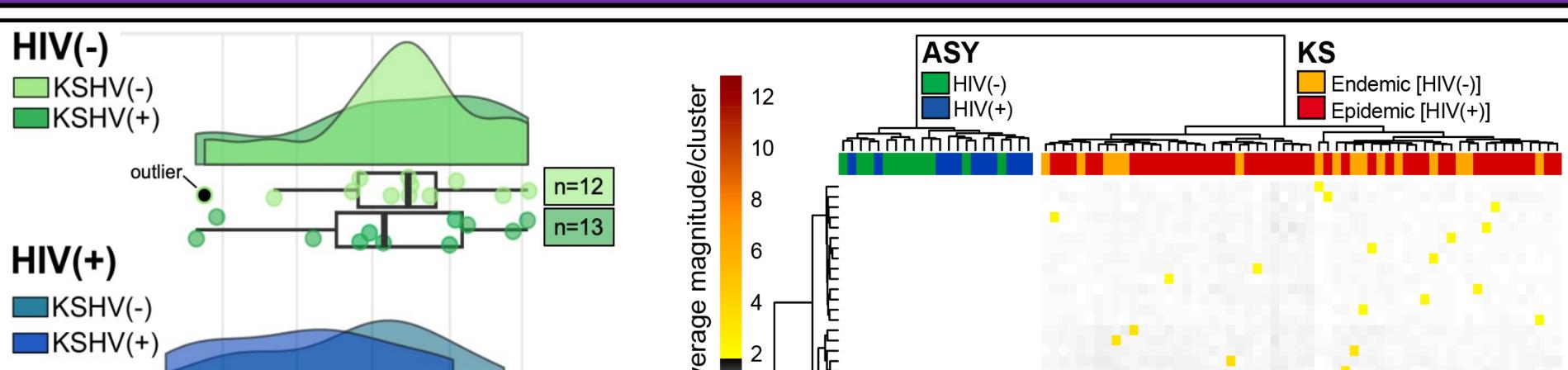
Methods

Kaposi Sarcoma n = 59 — EnKS (n=16), EpKS (n=43)
Asymptomatic n = 22 — HIV⁻ (n=11), HIV⁺ (n=11)
Sample Preparation — ① Isolate Plasma ② Quantify Total IgG

Phage Immunoprecipitation and Sequencing (PhIP-Seq)
① Phage IP ② PCR ③ Sequencing & Analysis

Quantification of the antibody response
Percent Reactivity — For peptide i — 60% Reactive
Breadth & Relative Breadth — ΣProtein A 3/7, ΣProtein B 2/3
Magnitude

Results

Figure 1. **Distribution of viral richness among KSHV, HIV, or co-infected individuals compared to uninfected controls.** Viral richness is defined as the number of unique viral organisms in an individuals' Ab repertoire. Each dot refers to one individual. The box-whisker plots display each data point on the inter-quartile range. Half violin plots show where densities within a group are clustered. Our data indicates that viral richness is lower in individuals with KSHV or HIV infection compared to seronegative controls, with increased intra-patient variability. Co-infection of KSHV and reduces the median viral richness to a greater extent.

HIV(-): KSHV(-) n=12, KSHV(+) n=13
HIV(+): KSHV(-) n=11, KSHV(+) n=11

Figure 2. **Unsupervised clustering of differential Ab repertoire based on magnitude of responses can differentiate between KS and ASY.** Prior to analysis, we removed private epitopes. Remaining peptide targets of the Ab repertoire were filtered such that they were either consistently targeted (>25% prevalence) or never targeted by either KS (yellow/red) or ASY (green/blue), considering HIV co-infection in each group. To maximize inter-group variability, an optimized k-means algorithm was used to generate 86 clusters of peptides (rows). To analyze co-infection profiles that differentiate symptomatic disease from asymptomatic infection, the red clades representing groups of Ab responses unique to KS or ASY were extracted. For hierarchical clustering of each individual (column) and peptide clusters (row), weighted version of Manhattan distance (Canberra) with Ward method was used. Overall, we extracted ~1300 KS and ~580 ASY differentially reactive peptides that derive from ~75 viral species. We utilized these peptides to characterize and compare co-infection profiles of each group.
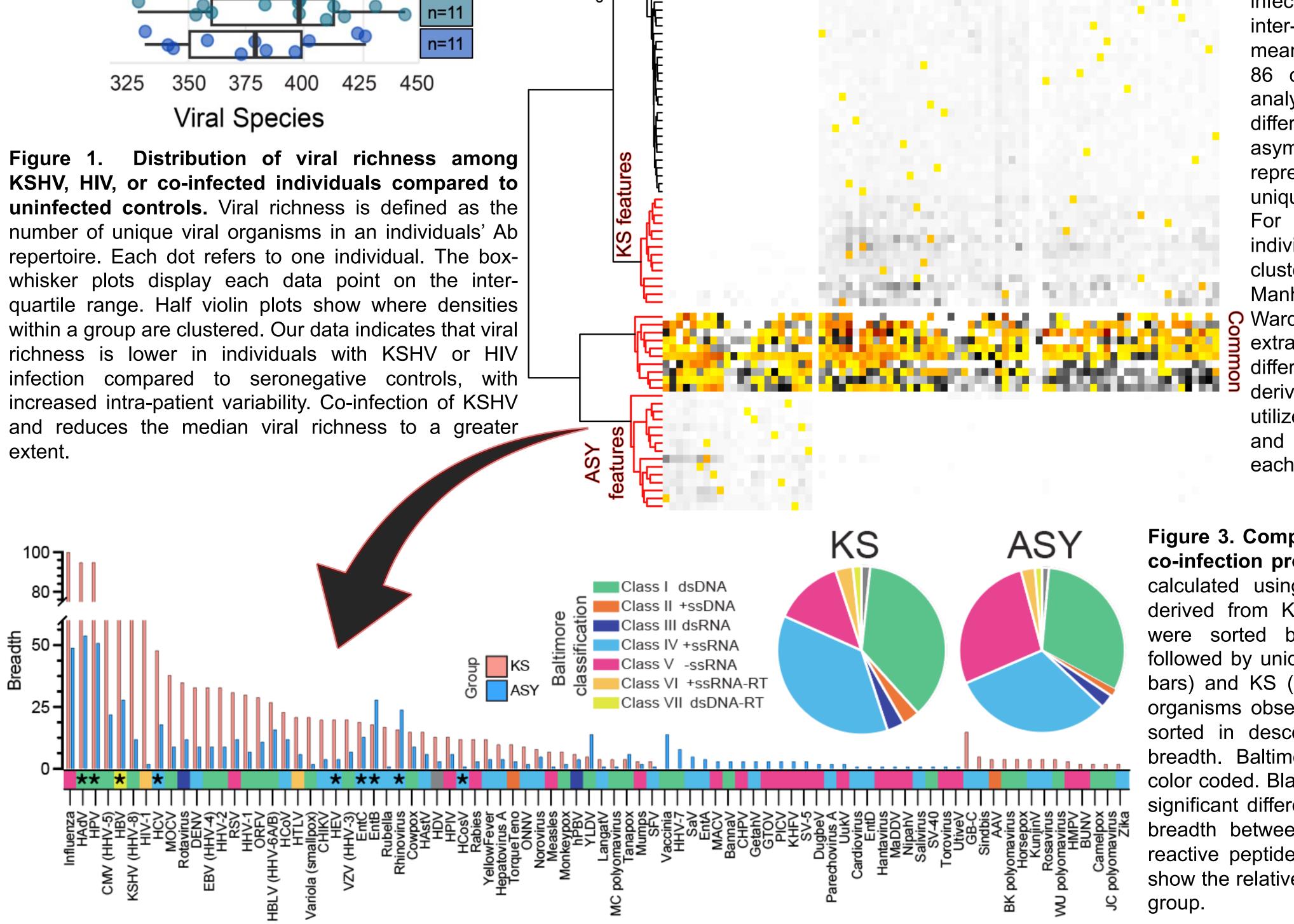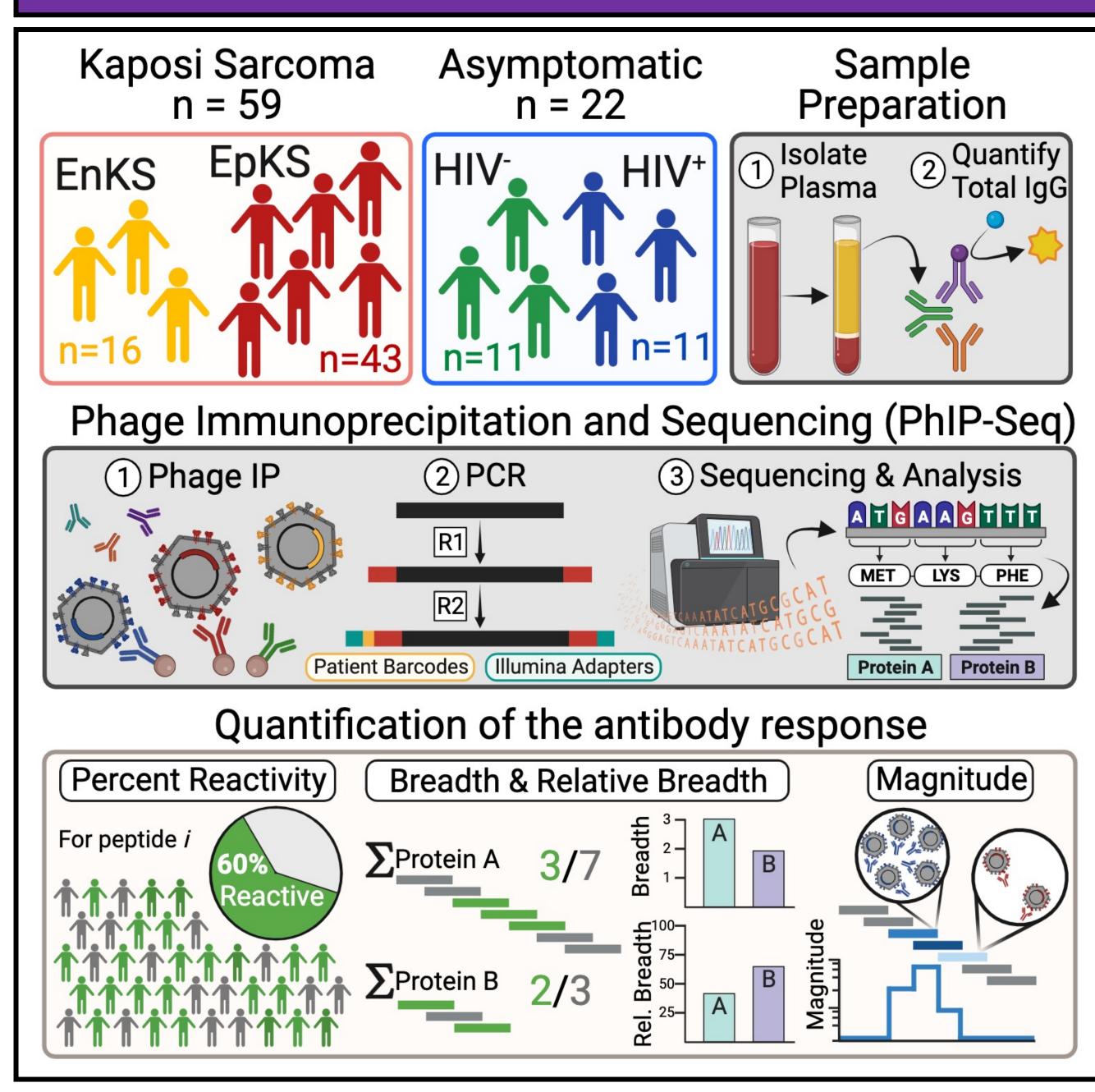

Figure 3. **Comparison of KS and ASY specific co-infection profiles.** Species-level breadth was calculated using differentially reactive peptides derived from KS and ASY groups. Organisms were sorted based on common recognition, followed by unique recognition among ASY (blue bars) and KS (red bars), respectively. Common organisms observed in both groups were further sorted in descending order of KS differential-breadth. Baltimore Class of each organism is color coded. Black asterisks represent statistically significant differences in organism/genotype-level breadth between KS and ASY, considering all reactive peptides in each group. The pie charts show the relative proportion of each class within a group.

Baltimore classification: Class I dsDNA, Class II +ssDNA, Class III dsRNA, Class IV +ssRNA, Class V -ssRNA, Class VI +ssRNA-RT, Class VII dsDNA-RT

Conclusion

- Individuals infected with HIV or KSHV alone exhibit a decreased general viral richness compared to those who are uninfected, and co-infection exacerbates this effect.
- Most non-ubiquitous viruses recognized by Ab repertoires of KS and ASY cohorts agree with consensus seroprevalence studies of viral species in SSA.
- **>1,000 differential epitopes derived from ~75 potential viral organisms distinguished KS and ASY.**
- More KS-distinct epitopes (~1300) were extracted compared to ASY-distinct epitopes (~580), however, ASY showed greater viral richness.
- KS patients exhibited a significantly higher oncogenic viruses such as Hepatitis spp. (B,C,E) and multiple HPV genotypes.
- ASY individuals exhibited significantly higher breadth of Ab recognition against specific respiratory infections such as Adenoviruses, Enteroviruses and Rhinoviruses. ASY also displayed a greater proportion of Ab recognition against Baltimore Class V viral organisms, while KS displayed higher proportion of Class I recognition.
- To validate the predictive, prognostic, and therapeutic value of the discovery of these differential epitopes, a longitudinal study with larger cohorts is warranted.

Acknowledgements

References

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