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Molecular profiling of breast and lung cancer in women with HIV reveals high tumor mutational burden

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Abstract

Objective: This study compared the mutation profile and tumor mutational burden (TMB) in women living with HIV (WLWH) diagnosed with lung adenocarcinoma (n=8) or breast ductal neoplasm (n=13) that were enrolled into the Women's Interagency and HIV Study (WIHS).

Design: Previous studies tend to focus on single-institutions based on sample availability, while this study is based on a representative, multi-center cohort that represents the racial and ethnic composition of women with HIV in the United States

Methods: The study sequenced the complete human exome of n = 26 cancer samples from HIV+ women, using Ion torrent next generation sequencing. The study cohort was compared to a HIV- cohort obtained from the Genomic Data Commons Data Portal of the NCI.

HIV+ and HIV- cohorts were compared using ion torrent next-generation sequencing.

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Author contributions

CC and CR designed and performed experiments, analyzed data, wrote manuscript. JL contributed to the sequencing analysis. DPD and AA designed experiments, analyzed data, and contributed to the final version of the manuscript. HS, CL, NH, AF, IO, MF, ES provided = funding and samples. All authors approved the final manuscript.

Conflict of interest statement

The authors have declared that no conflict of interest exists.

Results: There were no differences in known cancer mutations between breast cancer and lung cancer that developed in WLWH and those that developed in HIV seronegative (HIV-) women; however, WLWH presented a significantly higher tumor mutational burden (TMB) in comparison to HIV- patients. 75% of lung cancers and 61% of breast cancers were defined as TMB-high (more than 10 mutation/mb of DNA).

Conclusions: This study affirms the recommendation that WLWH be included in clinical trials of novel treatments for these cancers. While these data are preliminary, the high TMB in WLHV suggests, paradoxically, that this immune challenged population may benefit greatly from immune checkpoint inhibitor therapies.

Keywords

High-Throughput Nucleotide Sequencing; Women; Exome; Mutation; Breast; Lung

Introduction

Infection with human immunodeficiency virus (HIV) increases the risk of cancer in people living with HIV (PLWH). The oncogenic properties of HIV go beyond systemic immunodeficiency due to CD4 depletion during the end stage of HIV disease. They may be attributable to viral proteins directly, or to events over the long pre-clinical course of infection. Little is known how cancers that develops in PLWH differ from cancer that develops in persons who were not exposed to HIV-related immune suppression and persistent low-level inflammation. Consequently, HIV was classified an oncogenic virus [1]. Using whole exome sequencing, this study ascertained the mutational status of lung and breast cancers that developed in a well-characterized cohort of women living with HIV (WLWH) and compared it to the general population. We hypothesized that incident cancers in HIV infected persons would exhibit genomic patterns that could inform cancer therapy in this population.

The introduction, early and lifelong application of effective combination antiviral therapy (cART) reduced the risk of severe immune deficiency and progression to Acquired Immune Deficiency Syndrome (AIDS), which was defined as a combination of opportunistic infections and viral associated cancers, such as Kaposi Sarcoma and Lymphoma. In low- and middle-income countries (LMIC) with less than optimal access to cART and a less than ideal public health structure, most cancers that develop in PLWH are still those associated with viral infections and loss of immune function [2]. In countries with ready access to cART non-infection associated cancers, such as breast cancer and lung cancer are on the rise and are predicted to become the most prevalent cancer types in PLWH [3]. This rise in breast and lung cancer is primarily attributable to the increased median age of PLWH [4, 5]. Lung cancer is emerging as one the leading causes of deaths among PLWH, as it already is in the general population. The increased incidence of lung cancer has been attributed to the more frequent smoking in PLWH [6–10]. However, some studies found that lung cancer incidence remained significantly elevated in PLWH, even after adjustment for smoking habits [11]. Adenocarcinoma, the most common type of lung cancer in PLWH, are not strongly linked to smoking. These epidemiological observations raise the possibility that HIV-infection may be an additional risk factor for lung cancer [12, 13].

Another common cancer in PLWH is breast cancer. Here, the epidemiological data are less clear. Breast cancer is the most common cancer in women world-wide. WLWH do not have a higher risk of developing breast cancer than HIV seronegative (HIV-) women [14]; however, this may be explained by competing comorbidities in HIV+ women prior to the introduction of cART. The proportion of HIV+ women with breast cancer is increasing over time. Importantly, HIV+ women with breast cancer have a 2-fold lower survival relative to cases in the general population, despite -- presumably -- equal access to cancer treatment and cART. This survival difference motivated this study of common genomic alterations that define breast cancer in HIV+ women as compared to HIV- women.

The assertion of a direct role for HIV in oncogenesis, beyond modulating the immune system, is controversial. No HIV-specific mechanisms driving increased lung and breast cancer risk in PLWH has been identified. Few genomic studies of lung and breast cancer in WLWH have been reported [15, 16]. They tended to be single institution-based and relied on convenience samples or case reports. By comparison, this study is based on a multi-center cohort of women with HIV in the United States. The Women's Interagency and HIV Study (WIHS) is a large, prospective cohort study designed to investigate the consequences of HIV disease in women.

Within the sample size limitations of this study, no evidence for specific genomic differences between breast cancer and lung cancer were discovered; however, tumor mutational burden (TMB) was far greater in PLWH than matched controls. It remains unclear why both cancer risk and cancer survival is worse among PLWH than for the general population. This study supports the recent recommendation that PLWH should be included in all clinical trials of novel treatment approaches for these two cancers and receive the same consistent clinical treatment according to the same standards of care as HIV negative persons.

Methods

Samples.

The Women's Interagency HIV Study (WIHS) is a cohort of women with and at risk for HIV infection [17]. Seven WIHS sites (New York, Chicago, Washington D.C., San Francisco, Chapel Hill, Atlanta and Miami) contributed 26 tissue blocks plus matching PBMC from breast and lung cancer cases between 2000–2017 after obtaining participant consent. Specimens were de-identified replacing the patient information with a WIHS ID number. The study was IRB approved at each participating WIHS site. Lung (n=219) and breast (n=719) cancer sequencing data from HIV negative individuals were obtained from Genomic Data Commons Data Portal.

DNA extraction.

Tumor DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen Inc.) per manufacturer protocol with 3 hrs tissue digestion. PBMC DNA was extracted using a MagNA Pure Compact Instrument and Nucleic Acid Isolation Kit I -Large Volume (Roche Inc.).

Exome analysis.

Barcoded libraries were prepared from 100 ng DNA with an Ion AmpliSeq Exome RDY library preparation kit (Thermo Inc.) quantitated by Qubit dsDNA HS assay, sized with an Agilent Bioanalyzer 2100 high-sensitivity DNA assay (Agilent Technologies), and pooled to 80 pM final concentration before sequencing on the Ion Chef and S5 sequencer (Thermo Inc.). Base calling, quality filtering, and demultiplexing were performed on the instrument with default parameters. Reads >50 bp were mapped to the human genome (NCBI build hg38_2016) using CLC Genomics Workbench 9 (Qiagen Inc) and low frequency variant detection tool with the parameters: minimum frequency = 10%, and minimum coverage = 100x. Only non-synonymous variants with a minimum average quality score of ≥19 and a forward/ reverse balance of 0.5 were included. Tumor variants were filtered against germline variants obtained from PBMC for each patient.

Statistical analysis.

Results are reported as mean ± standard deviation (SD). The unpaired 2-tailed t-test with Welch correction was used to compare groups.

Results

The study sequenced the human exomes of initially n = 26 tumor biopsies from HIV+ women provided as formalin-fixed paraffin-embedded (FFPE) blocks or slides, as well as matched PBMC samples. Two samples were excluded due to the slides being stained, two samples were excluded due to low DNA yield and one additional sample was excluded due to low library quality. A total of 21 samples, 8 lung and 13 breast cancers, and matched normal PBMC, were included in the final analysis. All samples were primary tumors and confirmed by pathology. The clinical information for the study cohort is provided in Table 1. The participants were between 41–73 years old, with an average of 54.5 and 56.8 years for the lung and breast cohort respectively. Most patients were either white or African American. Only one person identified as Hispanic. For the lung cohort, 87.5% were adenocarcinomas and 12.5% were squamous cell carcinomas. For the breast cancer cohort 100% were ductal carcinomas, 38.5% were classified as in situ and 61.5% as invasive.

All samples were subjected to targeted amplification and next generation sequencing. The reads were aligned to the human genome (NCBI build hg38_2016) and single nucleotide variants (SNVs) identified. A median of 42,106,610±13,447,761 reads were obtained for each tumor sample, translating to 88.07%±0.03% median coverage at 10x. A median of 33,157,434±11,861,795 reads was obtained for each PBMC sample, translating to 93.08% ±0.04% median coverage at 10x. Any tumor SNVs that were also present in the matched PBMC samples were removed. To focus the data set further, all non-synonymous SNVs were removed as well.

The study cohort was compared to a HIV- cohort obtained from the Genomic Data Commons Data Portal of the NCI. This cohort contained n=719 breast and n = 219 lung cancer samples. The HIV seronegative cohort was filtered by the following parameters sex: female, age: 41–74 years, sample type: primary tumor, subtype: breast ductal and lobular

neoplasms and lung adenocarcinomas, respectively. The HIV- group in lung cancer was further divided into smokers (>1 cigarette/day) and non-smokers (<1 cigarette/day).

The most mutated gene in lung and breast samples was for TP53. There were no significant differences between the HIV+ and HIV- cohorts, regarding the frequencies of key oncogenes and tumor suppressor genes.

Increased TMB in breast and lung cancer in PLWH

The term TMB describes the total number of mutations in DNA. It has emerged as a useful clinical predictor in certain cancer types and therapy types, such a checkpoint inhibitor therapy [18, 19]. For lung cancer the TMB for the HIV+ cohort (mean = 53.13/MB; range: 146.13–1.07/MB) was significantly higher compared to HIV- both non-smoker (mean = 14.09/MB; range: 77.97–0.03/MB) and smoker (mean =15.23/MB; range: 54.40–0.43/MB). (Figure 1). However, TMB did not differ significantly between of non-smokers and smokers in the HIV- cohort. For breast cancer the same phenomenon is observed, where the HIV+ cohort (mean = 82.46/MB; range: 673.7–0.40/MB) has a significantly higher TMB in comparison to the HIV- cohort (mean = 4.38/MB; range: 264.93–0.23/MB).

Discussion

Cancer has become the leading cause of mortality and morbidity in PLWH (reviewed in [20]). Recent improvements to cART, such as long-lasting injectables, promise further advancements in the lifelong suppression of HIV viral loads; however, HIV infection rates are no longer declining in many countries, and a HIV cure or an HIV vaccine remains elusive. Hence, one can project an increase in the number of patients living with HIV and cancer [21].

This study tried to address a fundamental question in the field. Do cancers that develop in PLWH differ from those that develop in the general population, and should we make cancer treatment recommendations specific to PLWH?

In our cohort of HIV+ women with lung adenocarcinoma or breast ductal cancer, we observed no significant differences in the frequency of the most common mutated oncogenes; however, this study observed a significantly higher TMB in the HIV+ vs. the matched HIV- cohort. 75% of lung cancer cases and 61.5% of breast cancer cases were TMB-high, while the matched HIV- cohort were 52.4% and 31% for lung and breast cancer, respectively. The main limitation of this study is the small sample size, as both HIV infection and cancer are relatively rare events in the US.

The incidences of breast and lung cancer have been rising in PLWH and are projected to become the leading cause of mortality for this population. As TMB-high cancers are more susceptible to immune checkpoint inhibitor therapies-, the results from this study reemphasize the notion that HIV+ cancer patients on successful cART should not a priori be excluded from immune therapies. This conjecture is supported by recent successful clinical trials with pembrolizumab and nivolumab in HIV+ KS, HIV+ lung cancer and in Merkel Cell Carcinoma[22, 23].

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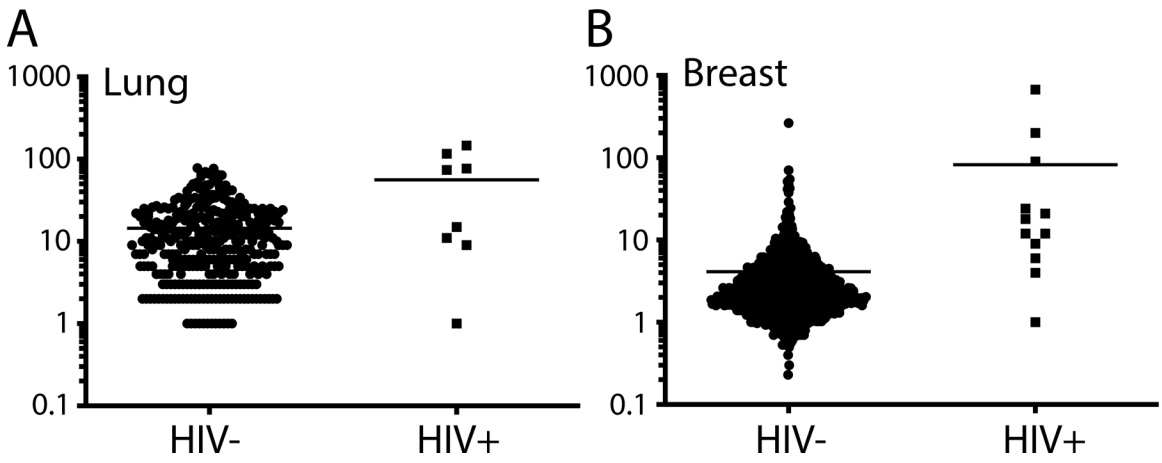

Figure 1: Tumor mutational burden of HIV+ vs. HIV- patients.

Dot plot showing the total number of mutations per Mb observed in whole-exome sequencing of HIV+ patients included in this study and HIV- patient obtained from the TCGA database for (A) breast and (B) lung malignancies. Each dot represents a tumor.

Table 1:

Patient characteristics

	Lung		Breast	
Characteristics	**HIV− (N=219)**	**HIV+ (N=8)**	**HIV− (N=719)**	**HIV+ (N=13)**
Age mean (range)	61.6 (41–73)	54.5 (41–73)	57.0 (41–73)	56.8 (41–73)
Ethnicity				
White	160 (73.1%)	5 (38.5%)	512 (71.2%)	2 (25.0%)
African American	24 (11.0%)	5 (38.5%)	115 (16.0%)	6 (85.7%)
Other	35 (16.0%)	3 (23.1%)	92 (12.8%)	0 (0.0%)
Race				
Hispanic	3 (1.4%)	1 (7.69%)	48 (6.7%)	1 (12.5%)
Non-Hispanic	716 (99.6%)	12 (92.3%)	671 (93.3%)	7 (87.5%)
Subtype				
Adenocarcinoma	219 (100%)	7 (87.5 %)	NA	NA
Ductal Carcinoma	NA	NA	719 (100%)	13 (100%)
Other	NA	1 (12.5%)	NA	NA