USING NEXT GENERATION SEQUENCING TO DETECT GENES RELATED TO CANCER IN WOMEN WITH HIV

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BACKGROUND

- Next Generation Sequencing (NGS) is a tool used in the clinical setting to detect HIV resistance mutations at a frequency down to 1% of the viral population[1].
- DNA sequences not aligning to the viral genome were being detected and may indicate upregulated host transcripts circulating in the patient plasma.
- HIV proviral DNA has been shown to preferentially integrate into genes that are actively expressed, and people infected with HIV have an increased risk for cancer compared to the general population[2-3].

Objective

- Upregulated plasma RNA relevant to cancer progression may be detected in patient plasma by NGS testing.
- Consideration of HIV resistance testing by NGS as a screen for the potential of cancer development in patients with HIV.

METHODS

Viral RNA from peripheral blood plasma
- 11 random HIV+ females
- Sequenced using Ion Torrent Sequencer

BLAST unaligned sequences
- Analyzed for presence of human genes (Figure 1)
- Settings set to use standard nucleotide BLAST with Human Genomic + transcript databases
- Optimized for highly similar sequences (megablast)
- Only sequences with 95% homology or greater to genes related to cellular proliferation and disease processes considered

Patient medical record reviewed
- Cancer diagnoses, cancer type, test orders relevant to suspicion of cancer, general demographics, appointments made with oncology
- Matched with information from BLAST data

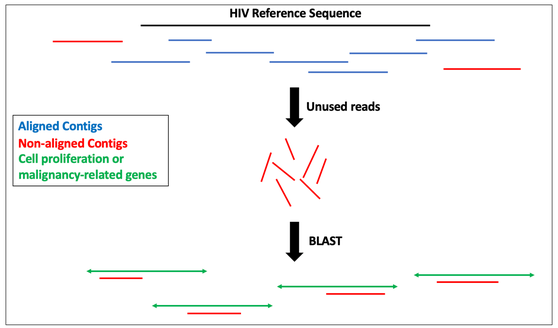

Figure 1. Method of Identifying Contigs to Analyze Through BLAST. RNA sequences from HIV positive females are identified using Next Generation Sequencing and aligned to HIV genome while unaligned contigs are separated out. These unaligned contigs are compiled and entered into Basic Local Alignment Search Tool (BLAST) to identify presence of sequences that match with human genes associated with cell proliferation or malignancies.

RESULTS

- BLAST results of patients' plasma RNA transcripts identified presence of human genes with known roles in cellular proliferation in all 11 patients as well as sequences related to malignancies.
- Sequences from one patient included genes related to lung cancer and tumorigenesis and was found to have been diagnosed with adenocarcinoma of the lung (Patient 5, Table1).
- While other patients had sequences for tumorigenic genes, the manifestations were less direct (Table 2).

Patient	Age Yrs	Years Since HIV Diagnosis	CD4 count at Time of NGS (mm^3)	Viral load at Time of NGS (RNA copies/mL)	Cancer History (yrs from HIV diagnosis to pathology result)	Number of Genes Identified by Cancer Type
1	50-59	7	862	unk	abnormal breast ultrasound (5), colon polyps, mass on back	
2	40-49	12	370	3070	cervical cancer (8)	
3	20-29	5	174	126	CIN 1(4)	
4	30-39	22	46	27322	NI	
5	50-59	8	122	75692	adenocarcinoma lung (7)	
6	40-49	4	52	1756138	NI	
7	30-39	3	1515	21643	NI	
8	20-29	7	26	1192	NI	
9	50-59	1	212	86028	skin cancer, basal cell carcinoma (1)	
10	50-59	25	229	<20	Abnormal pap smear (23), likely cystic lesion on neck (23)	
11	50-59	30	386	89	NI	

Key: Brain, Breast, Leukemia, Liver, Lung, Multiple

Table 1: Patient Demographics. CD4+ count and viral load values are those with the closest proximity to the date of the blood draw for NGS. UNK – unknown viral load. This information was matched with results from BLAST to identify any correlation between genes detected and the development of malignancies. The genes detected have reported roles in cell cycle regulation and/or tumorigenesis based on literature review. Empty boxes indicated no known history. NI- History not identified.

Table 2. Genes Identified in Alphabetical Order

- Acetylhydrolase 1b regulatory subunit 1
- Adenosine deaminase RNA specific
- ADP dependent glucokinase
- Amyloid beta precursor protein binding protein 2
- CD58
- Centrosomal protein 85 like
- Chromosome 6 ORF 106
- C1q/tumor necrosis factor-related protein-1
- DDB1 and CUL4 associated factor 12
- Deleted In Lymphocytic Leukemia 1
- Dual specificity phosphatase 5 pseudogene 1
- Ectopic viral integration site 5
- Ecdysoneless cell cycle regulator
- Fc fragment of IgM receptor
- GFAP
- Heat shock protein family A (Hsp70) member 9
- Homo sapiens P2Y receptor family member 8
- Interleukin 3 receptor subunit alpha
- IQ motif and Sec7 domain 1
- Leukocyte immunoglobulin like receptor A3
- LINE1 type transposase domain containing 1
- Malic enzyme 3
- Metastasis associated lung adenocarcinoma transcript 1
- Myosin X
- Negative regulator of ubiquitin like proteins 1
- Nuclear factor erythroid 2 like 1
- Phospholipase C like 2
- Platelet activating factor
- POU class 4 homeobox 1
- RINGO/Speedy E
- Rho GTPase activating protein 30
- Rho GTPase activating protein 39
- Secreted protein acidic and cysteine rich
- Sirtuin 5
- Vav guanine nucleotide exchange factor 2
- Zinc Finger MIZ-Type Containing 1
- Zinc finger protein 875

CONCLUSIONS

- A significant number of human genes were detected in the non-aligning sequences from viral NGS related to cell cycle and malignancies.
- It is difficult to say whether the findings of the cell cycle and malignancy-related genes within the contigs indicate upregulation of these transcripts or if it is a coincidental finding of a neighboring gene.
- Zinc Finger MIZ-Type Containing 1 gene was found in 6 of the patients and is reported upregulated in multiple cancer types.
- Further analysis is required to determine if NGS testing can be utilized to identify human gene sequences that are captured in the assay process when testing for HIV antiretroviral therapy resistance. The significance of the presence of these particular genes in the sequence pool has yet to be borne out.

Citations

1. Armstrong WS, Guarner J, Kraft CS, Caliendo AM. Human Immunodeficiency Virus. Microbiol Spectr. 2016;4(4):53-68. doi:10.1128/microbiolspec.DMIH2-0024-2015
2. Hughes SH, Coffin JM. What Integration Sites Tell Us about HIV Persistence. Cell Host Microbe. 2016;19(5):588-598. doi:10.1016/j.chom.2016.04.010
3. Wagner TA, McLaughlin S, Garg K, et al. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. Science (80-). 2014;345(6196):570-573. doi:10.1126/science.1256304