Sensitive detection of oncoviruses integrated into a comprehensive tumor immuno-genomics platform

#3788

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Introduction

HPV, HBV, HCV and EBV viruses are causally linked to over 11% of cancers worldwide while KSHV, HTLV and MCV are linked to an additional 1%. As use of immunotherapy expands to a broader variety of cancers, it is important to understand how these oncoviruses may be impacting the overall immune response in patients as part of the tumor and tumor microenvironment. However, typical cancer diagnostic and genomic biomarker assays do not include oncovirus detection.

Methods

To enable detection of these oncoviruses, we have developed ImmunoID NeXT, a novel augmented exome and transcriptome based platform that comprehensively characterizes tumor and tumor microenvironment from a single sample. As part of the platform assay design we developed targeting for the following oncoviruses: HPV, HBV, HCV, EBV, KSHV and HTLV. Furthermore we developed analytics for detecting these viruses from both the DNA and RNA data. For all the experiments reported here we extracted DNA and RNA from the samples, made libraries and sequenced them on a NovaSeq to 30G. High quality read counts were computed for all targeted oncoviruses.

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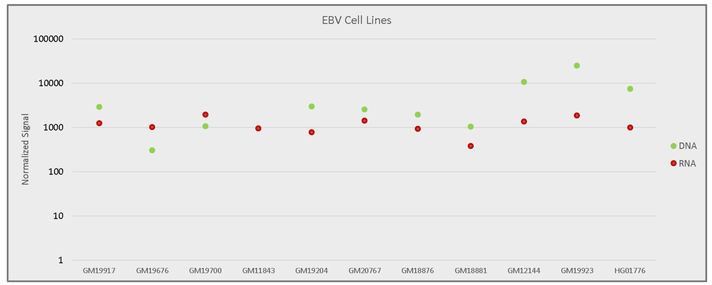
Results

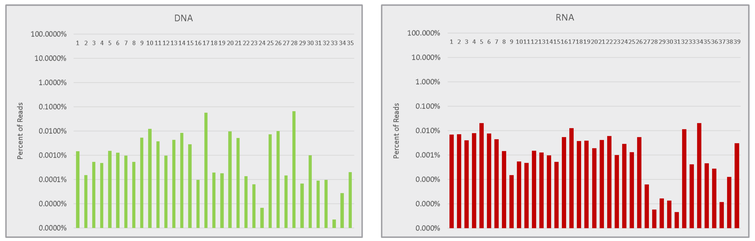
EBV Cell Lines

To test the ability of the platform to detect oncoviruses, we identified a set of 11 EBV cell lines from Coriell in which EBV was used as a transformant. We detected EBV in all the Coriell cell lines in both DNA and RNA indicating strong sensitivity of the platform. Wide dynamic range suggests quantification may be possible as well. In the DNA and RNA there were no detections of any other oncovirus indicating high specificity.



Oncoviruses Fraction

Much less than 1% of the overall exome/transcriptome reads map to oncoviruses in all positive cases tested. DNA and RNA had similar low fractions.



Mixed Oncoviral Cell Lines

We obtained 22 cell lines from ATCC containing HPV16, HPV18, HPV45, HPV68, HBV, EBV, KSHV, HTLV1 and HTLV2 in which the oncoviruses were known to be in the tumors from which the cell lines were created. In the ATCC samples we detected 23 out of 23 oncoviruses expected in both the DNA and RNA. We detected all the different types of oncoviruses that we targeted except for HCV because it wasn't in any sample. In all but one case the signals were strong.

Detected in DNA	Detected in RNA	Virus	Tissue	Notes
EBV	EBV	EBV	Hodgkin's lymphoma	Per ATCC : "The cells are EBNA positive"
EBV	EBV	EBV	Burkitt's lymphoma	Per ATCC : "Each cell an average of 60 EBV genome copies... derivative of Raji (ATCC CCL-86)"
EBV	EBV	EBV	Burkitt's lymphoma	Per ATCC : "The cells are EBNA positive"
EBV	EBV	EBV	Burkitt's lymphoma	Per ATCC : "The cells carry and produce EBV. Genes expressed EBV HLA A32, B17, Bw37."
EBV	EBV	EBV	Burkitt's lymphoma	Per ATCC : "The cells contain the Epstein-Barr virus (EBV) genome."
HPV16	HPV16	HPV-16 & 18	Cervix; from met site : small intestine	Per ATCC : "HPV-16, about 600 copies per cell) as well as sequences related to HPV-18."
HPV18	HPV18	HPV-18	Prostate	Transformed by lipofection-mediated with HPV-18 DNA
KSHV, EBV	KSHV, EBV	KSHV & EBV	Lymphoma	Per ATCC : "The cells contain two viral genomes: EBV and KSHV"
KSHV, EBV	KSHV, EBV	KSHV & EBV	Lymphoma	Per ATCC : "The cells contain two viral genomes: EBV and KSHV"
HBV		HBV	Liver, hepatocellular carcinoma	Per ATCC : "HBV DNA was detected by Southern blot. HBV RNA was not expressed."
HBV		HBV	Liver, hepatocellular carcinoma	Per ATCC : "HBV DNA was detected by Southern blot. HBV RNA was not expressed."
KSHV	KSHV	KSHV	Lymphoma	Per ATCC : "BC-3 has an intact KSHV genome of about 170 kb"
KSHV	KSHV	KSHV	Body cavity based lymphoma	Per ATCC : "positive for the KSHV genome at 150 copies/cell.
HPV16	HPV16	HPV-16	Tongue cancer	100–150 copies of HPV-16 / cell according to https://web.expasy.org/cellosaurus/CVCL_1899
HLTV2	HTLV2	HTLV-2	Hairy cell leukemia	Per ATCC : "contain a replication competent HTLV-II and two defective HTLV-II genomes."
HTLV1	HTLV1	HTLV-1	Cutaneous T cell lymphoma	Per ATCC : "The cells produce Human T cell Leukemia virus (HTLV-I)"
HBV	HBV	HBV	Liver, hepatocellular carcinoma	Per ATCC : "HBV DNA was detected by Southern blot. HBV RNA was not expressed."
HBV	HBV	HBV	Liver, hepatocellular carcinoma	Per ATCC : "This line contains an integrated hepatitis B virus genome."
HPV68	HPV68	HPV-18/68	Cervix; from met site : omentum	Per ATCC : "hyperdiploid to hypohexaploid … greater homology to HPV-68 than HPV-18."
HPV45	HPV45	HPV-18/45	Cervix; from met site : lymph node	Per ATCC : "hypodiploid … HPV-18, also partial HPV-45. HPV-45 E6/E7 region poly(A)+ RNA."
HPV16	HPV16	HPV-16	Cervix; carcinoma	Per ATCC : "1–2 copies per cell … hypertriploid"
HPV16	HPV16	HPV-16	Bone marrow/stroma	Transformed with LXSN16E6E7, an artificial retrovirus containing the HPV E6 & E7 genes

Tumor Samples

We looked for oncoviruses in a series of tumor samples of unknown status that we had run for other experiments. There were 44 melanoma and 18 colon tumors. These cancer types are not expected to be associated with oncoviruses. We did not observe any oncoviral detections further attesting to the high specificity of the assay. We also tested 6 head and neck samples that were p16 negative and also negative by our assay. We are in the process of obtaining positive tumor samples for additional testing.

Conclusion

Oncovirus detection integrated into an augmented exome and transcriptome assay is sensitive and specific for cell line data. We are planning additional studies on tumor samples to continue to characterize the performance of our platform. Oncoviral characterization of the tumor as part of a comprehensive tumor immunogenomics platform can serve and an important additional biomarker for understanding immunotherapy response.

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