Exome sequencing of primary liver tumors from workers occupationally exposed to ionizing radiation reveals novel candidates for driver genes

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Objective

To use biospecimens from a unique radiobiological repository to identify the mutational landscape of liver tumors from workers occupationally exposed to ionizing radiation.

Introduction

The Mayak Production Association, established in 1948, was the first facility built by the Soviet Union to manufacture nuclear weapons. From 1948-1982, Mayak workers were occupationally exposed to ionizing radiation from inhaled plutonium (isotope ²³⁹Pu), and many subsequently developed cancers of the lung, liver and bone, the organs of primary deposition after exposure. Once plutonium is inhaled and deposited in the liver, it serves as a constant source of alpha radiation, thus contributing to increased liver cancer risk by causing DNA mutations in the cells of exposed individuals. Epidemiological studies of the Mayak worker cohort estimate the excess relative risk (ERR) of liver cancer among Mayak workers at 2.6 and 29 for males and females, respectively. In this study we used whole exome sequencing (WES) to characterize somatic mutations in 3 histologically distinct primary liver tumors (angiosarcoma of the liver (ASL), cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC)) from Mayak worker subjects occupationally exposed to ionizing radiation (IR), both to identify mutations in candidate driver genes and investigate the contribution of IR to the mutational landscape of liver cancer.

The Russian Radiobiological Human Tissue Repository (RHTR) in Ozyorsk, Russia, which collects and stores biospecimens from the Mayak worker cohort, offers a unique opportunity to evaluate human health effects of chronic, low-dose plutonium exposure due to its size (over 280,000 biospecimens from over 25,000 workers employed from 1948-1982), 25% female population, and extensive monitoring. In this pilot study, we used WES of over 21,000 coding genes to characterize somatic mutations in biobanked liver cancers from the Mayak worker cohort. This pilot study identifies several novel candidate driver mutations that are likely to be caused by ionizing radiation exposure, and provides the first data on the mutational signatures of ionizing radiation exposure in liver cancer. These data may assist in developing non-invasive biomarkers for exposed subjects potentially at risk for liver cancers.

Methods

Liver tumor samples (n = 7) and adjacent non-tumor tissue from Mayak workers from 3 distinct histological subtypes of liver cancers: hepatocellular carcinoma (HCC; n=3), cholangiocarcinoma (CCA; n=2) and the extremely rare angiosarcoma of the liver (ASL; n=2) were collected at autopsy between 1978-1990 and stored in the RHTR. Tumor samples were processed into formalin-fixed paraffin-embedded (FFPE) tissue on slides, and immunohistochemistry and pathological review confirmed the diagnosis (Figure 1). Demographic data including radiation dose levels were recorded (Table 1).

For each subject, total DNA was extracted from FFPE sections using a custom phase separation method (available upon request), and the DNA quality and quantity was estimated with UV-VIS using the NanoDrop spectrophotometer, and fluorometry using the Qubit 2.0 Fluorometer. Indexed, paired-end sequencing libraries were prepared using the Agilent SureSelectXT Human All Exon V6+COSMIC kit. Sequencing was performed on the Illumina HiSeq 4000 platform using PE 150 bp reads. FASTQ raw data were aligned to the human reference genome b37 using BWA-mem v0.7.12. Somatic variants were called using GATK v3.3-0, MuTect v1.1.7 and VarScan v2.4.1, functionally annotated using ANNOVAR v2015-06-17, and the effect on protein function of each variant was predicted using SIFT (Sorting Intolerant From Tolerant), Poly-Phen-2, and the ENSEMBL Variant Effect Predictor.

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How to Request Biospecimens

The Russian Radiobiological Human Tissue Repository (RHTR) website (www.rhtr.subi.su) provides descriptions of the stored biospecimens. After completing the online application, a Tissue Review Committee will review it and decide on approval. Approved users bear only shipping costs.

Results

Mutation spectrum identified from primary tumors: To comprehensively characterize somatic mutations in primary liver tumors from subjects occupationally exposed to ionizing radiation, we analyzed WES data from 14 tissue samples of paired primary liver tumor and adjacent non-tumor tissue from seven subjects. The average sequencing depth of coverage was 780x for tumor samples and 690x for paired adjacent non-tumor samples. The mean number of nonsynonymous mutations per subject was 380.4 (range 24-857) (Figure 2).

Genetic alterations in primary liver tumors: Angiosarcoma of the liver (ASL): DNA sequence analysis identified novel candidate driver genes in ASL in the following pathways: angiogenesis (*HSPG2* and *FLT3*), PIK3CA/AKT/mTOR signaling (*LARP1*, *TSC1* and *YWHAE*), actomyosin dynamics (*COL1A1* and *GMIP*), Wnt/ β -catenin signaling (*MED12*), DNA damage response (*BRCA1*), and immune response (*PAGE1*) (Figure 3).

Cholangiocarcinoma (CCA): In addition to confirming driver genes of CCA (*PBRM1*), we identified additional potential driver genes involved in chromatin remodeling (*NUTM1* and *PRDM16*), cAMP signaling (*PDE4DIP*), and PIK3CA/AKT/mTOR signaling (*INPP5F*).

Hepatocellular carcinoma (HCC): In HCC tumors in this cohort we validated known HCC driver genes from different etiologies (*TSC1*, *PDE4DIP*, *SRCAP*, and *PTPRB*), and identified putative driver genes involved in Wnt/ β -catenin signaling (*AXIN2* and *MED12*), chromatin remodeling (*BCOR*, *TTF1*, *NUTM1*, *PRDM16*, and *ARID4A*), actomyosin dynamics (*COL1A1*, *SYNE1*, *GMIP*, and *KIF21B*), PIK3CA/AKT/mTOR signaling (*HSP90AB1*), cAMP signaling (*PDE4DIP*) and angiogenesis (*HSPG2*).

Sample ID	Diagnosis	Accumulated dose due to external exposure (Gy)	Absorbed dose for liver (Gy)	Pu body burden (kBq)	Absorbed dose for bone marrow due to Pu (Gy)	Absorbed dose for liver due to Pu (Gy)	SEX	AGE	Smoking	Smoking interval (year)	Alcohol consumption	Occupation	% Tumor	% Benign	Grade	% Necrosis
116549	ASL	2.2	1.9	1.1	3.6	2.5	M	54	Yes	36	Yes	Metalworker	40%	60%	LOW	0%
116551	ASL	1.0	0.9	32.9	1.3	3.7	F	69	No	-	No	Engineer-Electrician	20%	80%	LOW	30%
116583	CCA	2.0	1.7	2.2	4.4	3.1	M	65	Yes	45	Yes	Builder	75%	25%	2	20%
116595	CCA	2.4	-	-	-	-	M	59	Yes	-	Yes	Builder	70%	30%	3	30%
116577	HCC	2.8	2.5	99.2	-	-	M	51	No	-	Yes	Operator	70%	30%	4	10%
116565	HCC	0.8	0.7	2.9	2.1	2.1	M	62	Yes	42	Yes	Operator	70%	30%	4	10%
116569	HCC	0.8	-	-	-	-	M	54	No	-	Yes	Builder	60%	40%	4	10%

Figure 1. Representative histological images (A) ASL_116549: consistent with angiosarcoma. (B) ASL_116551: consistent with angiosarcoma. (C) HCC_116565: consistent with hepatocellular carcinoma. (D) HCC_116569: consistent with hepatocellular carcinoma. (E) HCC_116577: consistent with a well-differentiated hepatocellular carcinoma. (F) CCA_116583: consistent with cholangiocarcinoma. (G) CCA_116595: consistent with cholangiocarcinoma.

Figure 2. Somatic mutation spectrum in primary liver tumors Summary of tumors sequenced and the frequencies of mutations seen. (A) Frequencies of specific types of substitutions by mutation class for each individual tumor (B) Frequencies of specific types of substitutions by translation effect for each individual tumor (C) Frequencies of insertions, deletions and SNVs. (D) Pie charts showing the distribution of mutations by type of alteration and translation effect (E) Co-occurrence of mutations in each tumor sequenced. Recurrently mutated genes are shown on the y-axis and subject IDs on the x-axis. The matrix in the center of the figure represents individual mutations in each tumor. Genes in bold are previously reported in the literature for HCC, CCA and/or ASL; genes underlined are determined as significantly mutated, putative driver genes in previous reports. For each panel the alteration types were represented by different colors, respectively.

Pathway	Genes (ASL)	Genes (CCA)	Genes (HCC)
PIK3CA/AKT/mTOR	LARP1 , TSC1 , YWHAE	INPP5F , RPTOR	TSC1 , PIK3R1 , HSP90AB1 , PRK2
MAPK/ERK	MAPK1 , PPP2R5D		FGFR1 , FGFR4 , ELK4 , PPP2R1A
Actomyosin dynamics	COL1A1 , GMIP , NAV3	SYNE1 , DNAH12 , ARAF1 , CEP170	COL1A1 , SYNE1 , GMIP , KIF21B , DST , KIBP2 , CEP170 , MYO11 , CYLC1 , TUN1 , TUN2 , MYH4 , MYH9 , ARL2 , FES , PHO4
cAMP signaling	GNAI2 , MYL6	PDE4DIP , ADCY1	PDE4DIP , GNAS , GNAI2 , MYL6 , NPY1R , ADCY1
Wnt/ β -catenin	MED12		APC , AXIN2 , MED12 , BCCL2
DNA damage response	BRCA1 , BAG6 , ABL1	ATM , BRCA2 , BAG6 , TOPBP1	BRCA1 , SLX4 , RBB1 , TOPBP1 , FANCA
Chromatin remodeling	SMARCB1	PBRM1 , SMARCC1 , BRPF3 , TERT , NUTM1 , PRDM16	SRCAP , BCOR , TTF1 , ARID4A , ARID4B , NUTM1 , PRDM16 , CHD3 , CHD7 , HOACA , KMT3B , SETBP1
Angiogenesis	HSPG2 , FLT3		PTPRB , KDR , HSPG2
TGF- β signaling	ACV92A , SMAD3		COL1A1 , TGFBRI
Cell adhesion and migration	FN1		FN1 , SORL2 , LAMA2 , DSGAM

Figure 3. Classification of select genomic alterations identified by roles in cell signaling pathways. (A) Genes in bold are previously reported in the literature for HCC, CCA and/or ASL; genes underlined are determined as significantly mutated, putative driver genes in previous reports; asterisk indicate recurrently mutated genes (B) Pie charts depicting the frequency of the number of genes mutated in a given biological pathway for each tumor type.

Conclusions and Future Work

- This pilot study is the first report of WES analysis of histologically distinct liver tumors from a cohort occupationally exposed to ionizing radiation.
- In addition to well-known cancer genes, sequence analysis identified several novel candidate driver genes involved in angiogenesis, PIK3CA/AKT/mTOR signaling, actomyosin dynamics, Wnt/ β -catenin signaling, DNA damage response, chromatin remodeling, cAMP signaling, and immune responses.
- This study demonstrates the great potential of the biospecimens housed in the **Russian Radiobiological Human Tissue Repository (RHTR)** to be used for future experiments using cutting edge genomic technologies to answer seminal questions on the genetic landscape of ionizing radiation-induced tumorigenesis.