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Project 2.8
Russian Human Radiobiological Tissue Repository: A Unique Resource for Studies of Plutonium-Exposed Workers

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Mission of the Biorepository

- The Russian Radiobiological Human Tissue Repository (RHTR) at the Southern Urals Biophysics Institute (SUBI) in Ozyorsk, Russia, was established in 1958 to maintain and expand a collection of biological samples that are critical to supporting the overall mission of deepening the understanding of the long term health consequences of chronic, low dose radiation exposures.
- The biorepository facilitates a wide range of basic and molecular epidemiology studies designed to assess the relationship between radiation exposure and multiple health outcomes and cellular responses.

CONTENTS & METHODS

Methods of Collection and Storage Overview of the Biorepository Materials

Bio-Banking Methods

- Two main groups have been enrolled:
  - **Workers of the Mayak Production Association (Mayak),** located in Ozyorsk, Russia
  - **Residents of Ozyorsk** who were never occupationally exposed to ionizing radiation (control group).
- Tissues and other biospecimens are collected with signed, informed consent of participants.
- All specimens are annotated with demographic, occupational, dosimetry, and medical information including pathology data.
- Protocols are in compliance with international Best Practices Guidelines (e.g. U. S. National Cancer Institute and International Society for Biological & Environmental Repositories).

Autopsy Tissue Bank

- Autopsy tissues and whole organs of nearly **1,200** subjects (both workers and controls)
- Tissues are obtained post-mortem; these materials are stored in liquid formalin or as formalin-fixed paraffin embedded (FFPE) blocks and slides.

Surgical Tissue Bank

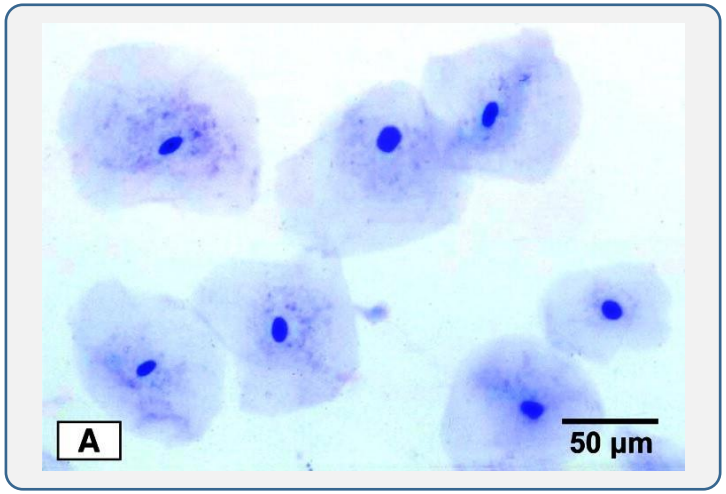
- Surgical and/or biopsy tissues from **>1000** subjects, including workers and controls
- Tissues are obtained in the operating rooms, mainly representing malignant diseases
- FFPE blocks, slides, and flash-frozen tissues (stored at -80^{0}C) are available

Blood Components Bank

- Whole blood and its components from nearly **7,530** subjects, including workers and controls
- Blood is processed < 2 hours after collection into individual aliquots of leukocytes, erythrocytes, lymphocytes, immortalized B-lymphocytes, plasma, and serum. All of these materials are in frozen storage at -80^{0}C.
- DNA is extracted from the white blood cells and stored.
- DNA from nearly **1,000** parental-offspring trios is available from families in which one or both parents is a Mayak worker, and from **~250** control families.

Other Tissues Bank

- Buccal epithelial cells, induced sputum, saliva, bone marrow, and lymphoid tissue have been collected and stored from ~ 1,800 individuals.



QC & QA

Methods of Quality Control
Quality Assurance Testing

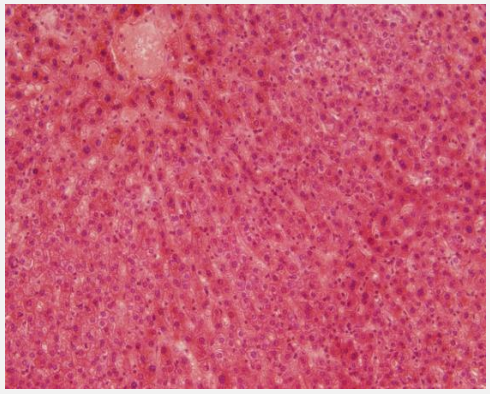
Quality Assurance

- The pH of the storage solution is checked annually: in 2017, for example, **1,040** liters of fresh formalin were used for replenishing **130** storage containers of autopsy tissues.
- Excellent integrity of tissues in FFPE blocks stored for 8 to 50 years was demonstrated by histological evaluation by an independent pathologist at Georgetown University, who examined slides of the liver for 77 subjects and of 54 subjects with breast cancer.
- DNA obtained from the liver cancer slides revealed that 80% of these fixed tissues yielded genetic material of sufficient quality and quantity for molecular-genetic studies.

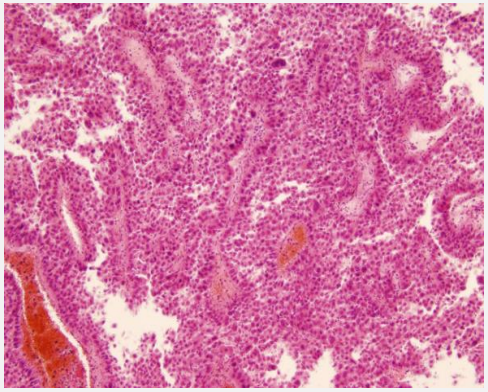
Histological Quality

- There was 98% agreement between the diagnosis of the outside reviewer and the original diagnosis obtained at SUBI for the liver cancer specimens.
- 90% agreement was observed from breast cancer specimens (attributable to the lack of routine immuno-histochemistry evaluation at SUBI).

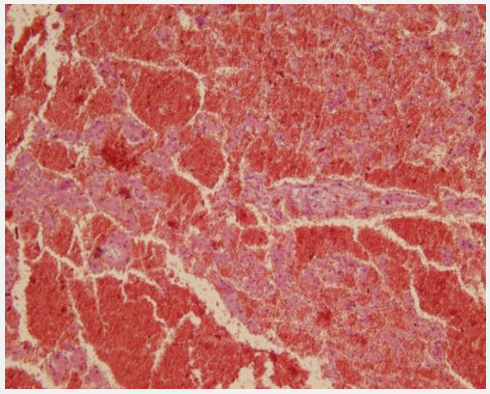
Normal Liver



Hepatocellular Carcinoma



Angiosarcoma of the Liver



Quality of Stored Blood

- **Viability of leukocytes** was evaluated in frozen sera (3-54 months in storage) from 32 subjects by trypan blue staining: light microscopy showed 99.3% of the cultures had viable cells.
- **Capability to thaw and grow lymphocytes** was analyzed in specimens (stored frozen for 18-24 mos.) from 4 subjects: all 4 cultures revealed normally dividing cells at the metaphase.
- **Integrity of cell surface proteins in lymphocytes** was analyzed in specimens (stored frozen for 12 mos.) from 9 subjects: all cells expressed CD3, CD3+CD4, and CD3+CD8 markers.
- **Ability to measure serum proteins** was evaluated in frozen sera (2-7 years in storage) from 59 individuals: commercially available ELISA test kits showed high expression levels for the interleukins IL-15, IL-17, and IL-18, the protein transforming growth factor (TGF)-β1, and the receptor HER-2/neu.

Case Study Supporting the Use of Biorepository Materials for Molecular Sciences

Whole Exome Sequencing of Tumor DNA from Mayak Workers

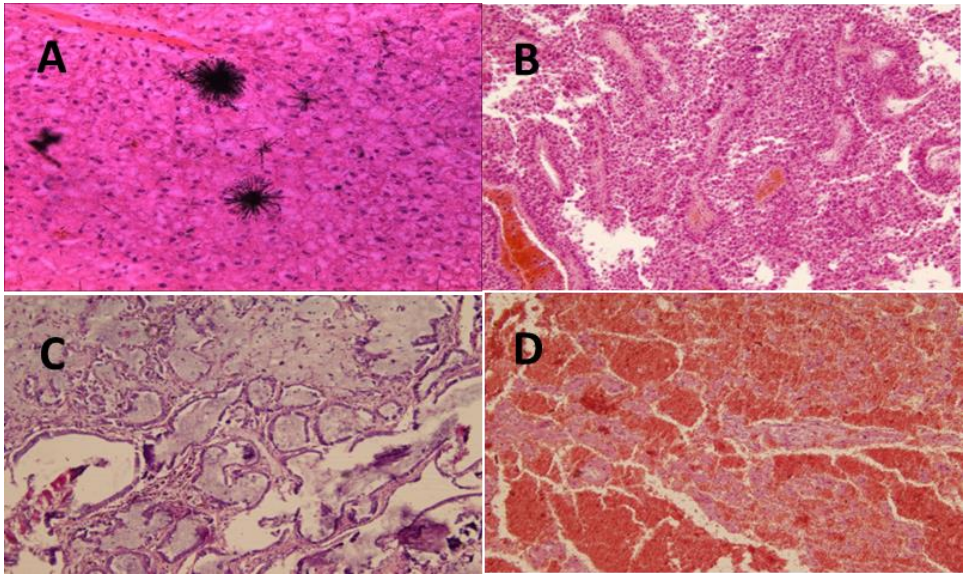
Goal of DNA sequencing of liver tumors

- The Mayak worker cohort and the Russian Radiobiological Human Tissue Repository (RHTR) represents a unique opportunity to collect critical data about the influence of radiation-induced DNA mutations in primary liver cancer.

- The goal of this study was to use **whole exome sequencing** to characterize somatic DNA mutations in primary liver tumors from this cohort to identify driver mutations and investigate the contribution of ionizing radiation to the mutational landscape of liver cancer.

Study methods

- For this pilot NGS study we selected 7 cases stored in RHTR
  - **3 HCC (hepatocellular carcinoma)**
  - **2 CCA (cholangiocarcinoma)**
  - **2 ASL (angiosarcoma of liver)**
- We assessed DNA mutations in >20,000 genes using whole-exome sequencing

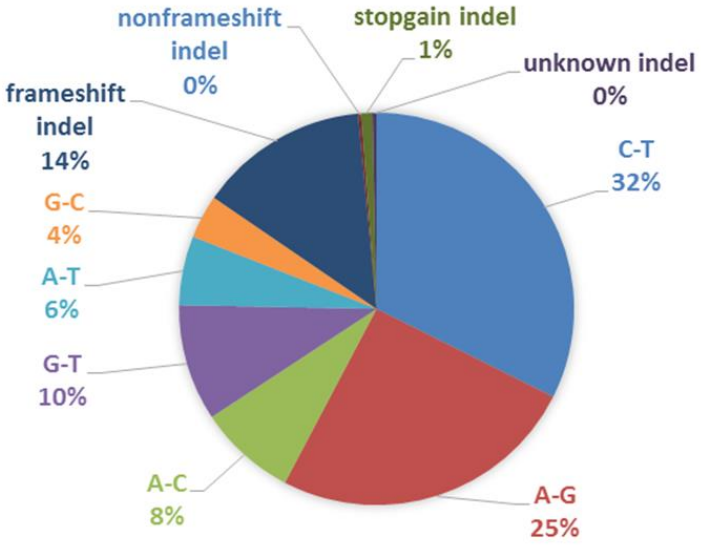


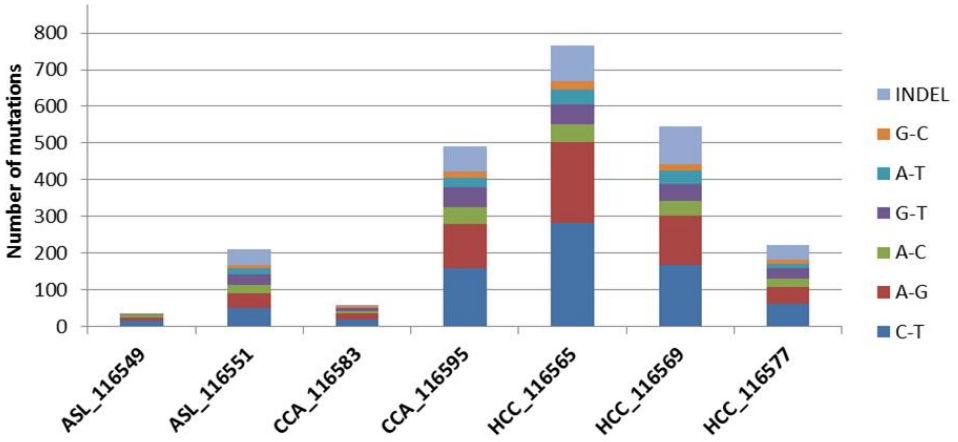
- **A.** healthy liver tissue section with autoradiograph of alpha radiation tracks from inhaled plutonium **B.** Specimen of HCC. **C.** Specimen of CCA. **D.** Specimen of ASL.

Mutational landscape of liver tumors

- DNA mutations in primary liver tumors after occupational exposure to ionizing radiation: comparing tumor DNA to adjacent, nontumor tissue DNA from the same specimen

- 2,389 functional somatic mutations among seven tumors (average 341 mutations/tumor; range 35-766) for an average of 5.17 nonsynonymous mutations per Mb sequenced (66 Mb of sequence interrogated)

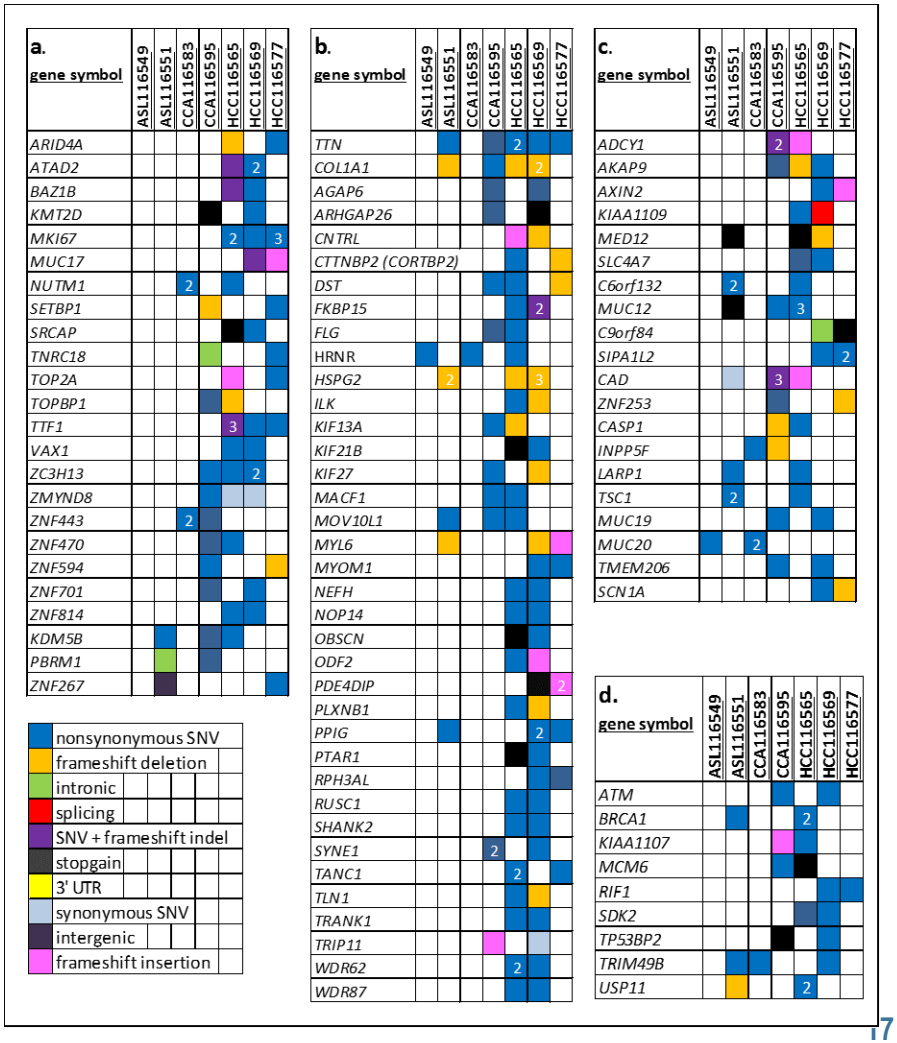




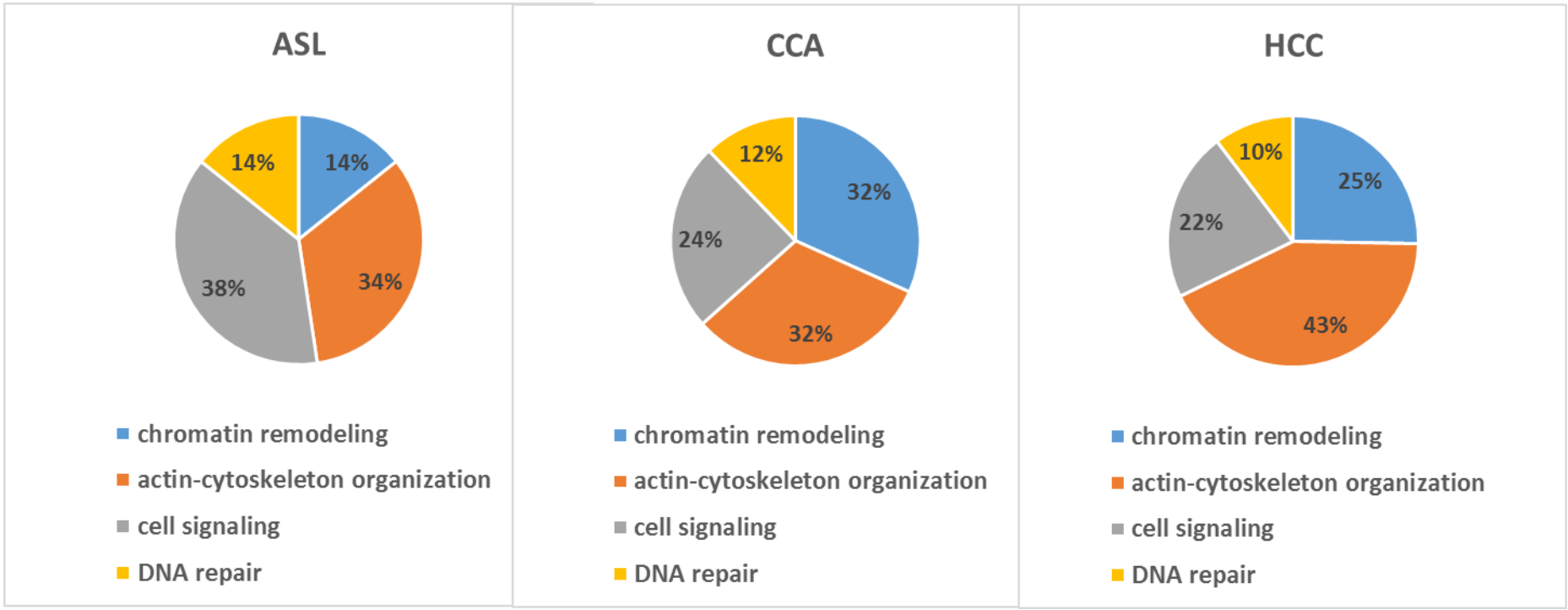
Recurrent mutations

Recurrent mutations across multiple tumors and tumor types reveal abrogation of critical gene pathways underlying liver tumors arising after exposure to ionizing radiation:

- ***Chromatin remodeling***
- ***Actin-cytoskeleton organization***
- ***Signaling pathways***
- ***DNA repair pathways***



Identification of genetic pathways



Frequency of genetic pathways dysregulated in different primary liver tumors

Future directions of liver cancer work

- These preliminary data confirm that samples from the biorepository are **high quality** and can generate **high quality molecular data** using state of the art DNA sequencing technologies.
- 15 additional samples have been now sequenced, and work is underway to conduct bioinformatics analysis and submit another paper for publication.
- These data will deepen the understanding of the potential molecular mechanisms underlying radiation-induced malignancies of the liver.

RHTR Web Site

- The RHTR web site (www.rhtr.subi.su) provides an interface, available in Russian and English, for interested scientists to view meta data of the biorepository.
- The full catalog of available specimens may be searched for tissue types and organ sites of interest, with the numbers of available specimens meeting these criteria. The meta data accounts for both incoming (new) and outgoing (depleted) biospecimens, and are updated twice annually.
- Users can select the types of subjects they are interested in studying (exposed and unexposed workers and controls), their **radiation dose ranges**, **types of cancer**, **gender**, **age range**, and other characteristics. These meta data contain no individually identifiable information on the subjects, thus preserving their anonymity and privacy.
- Users who wish to apply for biospecimens **complete an online application** that is sent to the RHTR director.

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