THE RUSSIAN HUMAN RADIOBIOLOGICAL TISSUE REPOSITORY: A UNIQUE RESOURCE FOR STUDIES OF PLUTONIUM-EXPOSED WORKERS

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The Russian Radiobiological Human Tissue Repository (RHTR) at the Southern Urals Biophysics Institute in Ozyorsk, Russia, was established to collect and store biospecimens supporting research on health consequences of chronic, low-dose radiation exposures. The purpose of this paper is to describe the RHTR resources and the availability of high-quality biological specimens. RHTR has enrolled two groups of subjects from 1951 to the present time: exposed workers at the Mayak Production Association facilities and residents of Ozyorsk who were never occupationally exposed to ionizing radiation (controls). Biospecimens are collected with informed consent of participants and are annotated with demographic, occupational, dosimetry and medical information. To date, 900 individuals have provided surgical tissues and 1000 have provided autopsy tissues. Blood samples are also collected and stored. Familial DNA is available from parent–offspring triads. Biospecimens and annotated data are available to interested scientists worldwide, *via* the RHTR website.

INTRODUCTION

The health and safety of workers provide the most credible evidence of working conditions at any industrial facility. The current knowledge of the health effects of occupational exposures and understanding of the effectiveness of workplace exposure controls owe much to the medical scientists who dedicated their careers to improving and safeguarding the health of the affected workers. Several of these individuals deserve special mention. A.I. Burnasyan founded the system of industrial hygiene monitoring in the nuclear industry of the USSR. Among thousands of medical officers who contributed their knowledge and efforts to the protection of nuclear workers, G.D. Baysogolov and A.K. Guskova were founders of the National School of Radiation Medicine. They laid the foundation for Southern Urals Biophysics Institute (SUBI), which was founded in 1958, and the Radiobiological Human Tissue Repository (RHTR), which was envisioned as the main source of biological specimens that would further the understanding of the biological mechanisms that underlie radiation susceptibility and protection. K.N. Muksinova was the primary initiator and the head of this facility until 2008, after which E.N. Kirillova became the RHTR head and continues to lead the facility today.

Nearly two decades later, the RHTR, also known as the Mayak Worker Tissue Repository, now is a unique resource for studying effects of prolonged radiation exposure associated with occupational activities of the Mayak workers. This biorepository has been

supported with the financial and scientific support and collaboration of the US Department of Energy and the Russian Federal Medical Biological Agency within the framework of the bilateral US–Russian Agreement on International Cooperation for Minimization of the Effects of Prolonged Radiation Exposure, which is also known as the Joint Coordinating Committee for Radiation Effects Research (JCCRER) Agreement of 1994. Through this support, the RHTR has been provided with state-of-the-art equipment and scientific protocols, and the work has been performed using the most advanced materials and reagents. During the current period of funding, the priorities have been 2-fold, as described below (1) barcoding and annotating the extensive historical and currently active acquisition of biological specimens, in compliance with international standards of best practices; and (2) creating policies and procedures and a website to encourage scientists from around the world to request specimens and data to support research on the biological effects of ionizing radiation exposure. The purpose of this paper, therefore, is to describe the resources of the RHTR and the availability of its high-quality biological specimens to support the research of biomedical scientists.

METHODS

Main objective

The main objective of the RHTR is the collection and storage of biological materials from individuals exposed in the course of their occupational activity, in

support of investigations on the effects of chronic, low-dose exposure including organ, tissue, cellular and molecular endpoints. Such uses of the annotated biological materials will significantly extend the capabilities of researchers in different countries focused in the disciplines of radiation medicine and biology. At the present time, this objective is further expanded to include increasing the awareness of the world's scientists of this unique resource, thereby encouraging the expanded use of the stored materials.

History of the biospecimen collection

In the early years of the RHTR, there was a single biobank of autopsy tissues stored in liquid formalin, together with formalin-fixed paraffin-embedded (FFPE) tissue blocks and slides. An inventory of the blocks, slides and autopsy tissues was maintained for many years by the staff of the SUBI Pathologoanatomy Laboratory (headed by V.K. Lemberg). Malignant neoplasms constituted the major component of the tissue collection at that time, but significant collections of non-malignant tissues were also represented, including bones, heart, blood vessels, muscle and brain tissue. As the proportion of malignant, acute and chronic conditions grew, the biobank grew to include the surgical and biopsy tissues collected at the time of surgical interventions and diagnostic biopsy. The autopsy and surgical tissues may be used in studies on radiation carcinogenesis, radiation non-tumor pathology, gerontology, plutonium metabolism and distribution in the organs of deposition.

Since 2001, the biobank has been extended in scope and complexity to include blood samples, extracted genomic DNA, sputum, buccal epithelial cells and other types of biological samples. The biobank of blood and its components collected from the Mayak workers of different age groups, e.g. samples of the whole blood, leukocytes, erythrocytes, lymphocytes, immortalized B-lymphocytes, plasma, serum and DNA, should be of great interest for researchers. Such samples are especially valuable for research in biological dosimetry, radiation cytogenetics, immunogenetics, radiobiology and radiation immunology.

Study population

The RHTR obtains and stores annotated biospecimens not only from individuals occupationally exposed to radiation but also from other workers of the facility, who were not occupationally exposed to radiation, and persons from the Ozyorsk general population matched by age and sex (control group). At the present, 6986 workers and 1041 controls have contributed 283 790 biospecimens to the facility.

Informed consent

Collection, storage and transfer of biospecimens and their annotated data on the participants comply with the basic principles of human rights protection for research subjects specified in the Fundamentals of Legislation of the Russian Federation on Health Protection of Citizens No. 5487-1 (including amendments in 1993, 1998, 1999 and 2000). Principles of protecting rights and safeguarding the identifiable data are set forth in additional laws, e.g. Law on Information, Information Technologies and Information Protection No. 149-FZ and Law on Personal Data No. 152-FZ. Compliance with the basic principles of human rights protection as research subjects in the RHTR operation is monitored at the local level by the SUBI Institutional Review Board (IRB) that has been recognized by the USA as the IRB of Record, under the Federal-wide Assurance number 00006759, as of April 2004. All subjects who donated biospecimens provided written informed consent. Only de-identified information is provided to researchers.

Storage conditions and quality assurance

Biological samples are stored in a variety of conditions, depending on the material. Autopsy tissues are stored in formalin solution at room temperature: the pH of the solution is routinely measured and the solution is replaced with fresh formalin when the testing reveals $\text{pH} < 7.2$. Autopsy and surgical tissues may also be stored in the form of FFPE blocks and slides (stored at room temperature), and as frozen tissue (stored at -80°C). Blood samples, components derived from whole blood, extracted DNA, buccal epithelial cells and sputum samples are stored at -80°C . The temperature of each freezer is monitored daily and recorded meticulously in temperature log books. All of these operations are governed by written Standard Operating Procedures that comply with Best Practices Guidelines developed by the International Society for Biological and Environmental Repositories⁽¹⁾ and the US National Cancer Institute⁽²⁾. For assessment of the integrity of stored blood cells, DNA and serum at different time periods after frozen storage, special tests are performed, which confirm the viability of cells and their retained functional capacities. The adequacy of these specimens for molecular-biological studies is assessed based on the following standard tests: leukocyte viability test, blast-cell transformation capability of lymphocytes, colony forming capability of immortalized B-lymphocytes, concentration of immunoglobulin in blood plasma and agarose gel electrophoretic separation of PCR products from DNA specimens.

Database

The RHTR database, maintained on an SQL file server and programmed in MySeq and C-Sharp,

contains information on the stored biospecimens and their physical locations: this is enabled by barcode labels that link the item to the relational database. Thus, every item in storage is linked *via* a unique identification number to the registrant's sociodemographic data, exposure history and dosimetry data (including external doses, plutonium body burden, plutonium burden in main organs of deposition, other hazardous factors prior to employment at Mayak), and summary data from medical records, including past diseases, such as site-specific cancer, methods of treatment, medications and dosage, radiation therapy courses and doses, and the cause of death for those who died. The database has been maintained with the highest standards of completeness and data integrity. Interested researchers may request de-identified data along with biospecimens to support specific research projects, and the website (www.rhtr.subi.su) lists the information and types of specimens that are available, in real time (see below).

RESULTS

Biorepository inventory

The RHTR is the custodian of archived biospecimens at SUBI and, at the same time, constantly acquires new donations of biospecimens from workers and controls. Its growing collection of specimens is annually enhanced with samples of blood and its components from ≥ 400 subjects, autopsy tissue samples from ~ 15 subjects, and biopsy and surgical tissue samples from 40 to 50 subjects. As new specimens are acquired, they are immediately bar-coded. This allows precise tracking of the location of each specimen in the collection and its linkage to the database of occupational, medical and sociodemographic variables.

The RHTR collections are divided into several distinct sub-banks of biospecimens. The following figures were reported from the database as of 1 April 2016.

- The *Autopsy Tissue bank* contains biospecimens from 997 individuals (940 workers and 57 controls) obtained post-mortem; these materials are stored in liquid formalin or alcohol, or as FFPE blocks and slides.
- The *Surgical/Biopsy Tissue Bank* contains biospecimens from 929 individuals (491 workers and 438 controls) obtained in the surgical theaters, mainly representing malignant diseases. Both FFPE blocks, slides and flash-frozen tissues, are available.
- The *Repository of Blood and Blood Components* is the most complex of the RHTR's collections. A total of 6507 individuals (5868 workers and 639 controls) have contributed specimens of whole blood, which were processed within 2

hours of collection into individual aliquots of leukocytes, erythrocytes, lymphocytes, immortalized B-lymphocytes, plasma and serum. DNA is routinely extracted from the white blood cells and stored in separate aliquots. All of these materials are in frozen storage at -80°C . DNA from 312 parental-offspring trios is also available from 237 families in which one or both parents is a Mayak worker.

- The *Repository of Other Tissues* is a diverse collection of materials from 1811 subjects, including buccal epithelial cells, induced sputum, saliva, bone marrow and lymphoid tissue.

Quality assurance results and examples of scientific research supported by the RHTR

The RHTR carries out routine quality assurance evaluations of its stored biospecimens on an annual basis. At the same time, biospecimens that have been disbursed to outside scientists and used in approved research investigations also provide feedback on the quality of the stored materials. Selected examples are provided below.

During 2015, 140 l of fresh formalin were used for replenishing 687 storage containers of autopsy tissues. These tissues were used in variety of dosimetry studies by researchers in Ozyorsk and elsewhere in Russia. Excellent integrity of tissues stored in FFPE blocks for 8–50 years was demonstrated by histological evaluation by an independent pathologist at Georgetown University, who recently examined slides from 77 patients with liver cancer and 54 with breast cancer. This review revealed 98% agreement between the diagnosis of the outside reviewer and the original diagnosis obtained at SUBI for the liver cancer specimens (Figure 1); 90% agreement was observed from breast cancer specimens (this somewhat lower level of agreement was attributable to the lack of routine immune-histochemistry evaluation at SUBI). FFPE blocks were also used successfully in micro-dosimetric studies and autoradiography studies, including samples of lung tumor tissues stored in FFPE blocks that were successfully used in oncogenetic studies. 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.

Routine evaluations of the quality of frozen blood components have yielded excellent results, demonstrating their suitability for supporting molecular biology studies. The viability of leukocytes from a random sample of 32 subjects was analyzed by trypan blue staining after 3–54 months of being processed and frozen at -80°C : light microscopy revealed that 99.3% of the cultures yielded viable cells. The blast transformation capability of lymphocytes was analyzed in specimens obtained from four

Figure 1. Representative histological image (20x): (a) healthy liver tissue section. Arrow points to autoradiograph of alpha radiation tracks from absorbed plutonium particles, (b) specimen of hepatocellular carcinoma showing 70% tumor, grade 4, cirrhosis of adjacent liver and portal lymphoid aggregates. Arrow points to the tumor, (c) cholangiocarcinoma specimen showing 60% tumor, grade 1, portal lymphoid aggregates and lobular hepatitis. Arrow points to the tumor and (d) angiosarcoma of the liver showing 75% tumor, low-grade and well-differentiated pattern. Arrow points to the tumor.

subjects whose specimens had been in frozen storage from 18 to 24 months: all four cultures were examined under light microscopy and revealed normally dividing lymphocytes at the metaphase phase, as shown in Figure 2. The integrity of lymphocytes obtained from nine subjects and stored for 1 year in the form of suspension in a dimethyl sulfoxide-containing culture medium at -150°C was evaluated by their capability of expressing membrane proteins. The phenotyping of these stored lymphocytes was performed using a flow cytofluorimeter manufactured by Beckman Coulter, USA, with monoclonal antibodies; the experiment revealed that the cell membrane markers expressing CD3, CD3 + CD4 and CD3 + CD8 were all expressed at high levels. Finally, a sample of frozen sera from 59 individuals was evaluated to see if regulatory proteins could be detected by standard, commercially available enzyme-linked immunosorbent assay test kits after 2–7 years in frozen storage; the results revealed high expression levels for the interleukins IL-15, IL-17 and IL-18; the protein transforming growth factor (TGF)- $\beta 1$ and the receptor HER-2/neu.

Procedures for requesting biospecimens

The RHTR website (www.rhtr.subi.su) provides a user-friendly interface, available in Russian and English, for interested scientists to view metadata of the biorepository in real time. This means that the full catalog of available specimens may be searched for tissue types and organ sites of interest, with the resulting numbers of available specimens meeting these criteria reflecting the most current information in the website, including the capacity to account for both incoming (new) and outgoing (depleted) biospecimens. 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 and age range, and other

Figure 2. Metaphase slide prepared from 3-d culture of FGA-stimulated lymphocytes that had been stored for 24 months at -80°C .

Figure 3. Application and approval process for requesting biospecimens from the Russian Radiobiological Human Tissue Repository, Standard Operating Procedure #006, version 1.1. Processes are sequentially listed on the left and required paperwork is shown on the right.

characteristics. The metadata contains 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. The application is reviewed for completeness, and the director may contact the applicant to clear up any

questions about the biospecimen request. The completed application is then sent to a Tissue Review Committee, consisting of two scientists from Russia and two from the USA, which reviews the application for scientific merit and recommends either approval or disapproval to the RHTR director. If approved, the applicant will bear the costs of

shipping and export license fees, but there are no charges for the specimens themselves. Details of the application process are shown in Figure 3.

DISCUSSION AND CONCLUSION

The RHTR is the only biorepository of its kind in Russia, and one of the only biorepositories in the world with a website that features real-time metadata searching. Its unique banks of biomaterials span the full range of tissue types and storage media needed for scientific research in the current molecular era. The RHTR collects, processes and stores blood specimens from Mayak workers and residents of Ozyorsk who were never exposed to occupational radiation (controls); in addition, organs and tissues are collected at surgery and biopsy procedures and at autopsies. Uniquely, the collection includes genomic DNA from parental–offspring trios, which can support advanced genomic studies. The annotation of the biospecimens is complete and comprehensive, including verification of clinical diagnosis and pathology, and demographic, occupational, dosimetry and detailed medical information for all the registrants.

Ongoing specimen quality testing is another notable feature of the RHTR. This is enabled by the fact that the director and other scientists responsible for the laboratory activities are all accomplished scientists from diverse fields including molecular biology, genetics and medical pathology. Thus, they are well qualified to design the experiments on tissue quality and cellular viability, critically evaluate the results and adjust standard operating procedures in light of the results. Their participation in the worldwide discourse of scientific research is evident on the basis of manuscripts submitted to international peer-reviewed scientific journals (Russian and international), and invited presentations at scientific conferences and meetings where they interact with colleagues from other institutions and keep abreast of the latest developments and advances in science. RHTR scientists are also committed to informing the world's scientific community about the unique aspects of the biospecimens stored at the RHTR.

Quality control and quality assurance procedures underlie all of the work of the RHTR. Standard operating procedure books define all aspects of the work and are constantly updated as the latest improvements and technologies are implemented. The RHTR's electronic database, currently in an SQL server environment, features barcoding and scanning of the collections, allowing for precise control and tracking of the inventory and the annotation of every single item in the biobanks. The database further supports the maintenance of the website by providing de-identified metadata to prospective users, who are able to peruse the online catalog of specimens, select variables and dose ranges of interest, and apply to receive the materials at no cost other than shipping fees. Thus, the RHTR looks forward to supporting the research of an ever-widening cadre of international scientists interested in the important medical and biological consequences of occupational exposure to ionizing radiation.

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