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The German Uranium Miners' Biobank—A Biobank for OMICs Radiation Research

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Simple Summary: The uranium miners of the former German mining company "Wismut" (SDAG/SAG Wismut), with about 400,000 employees, represent one of the largest populations of radiation-exposed miners worldwide. A comprehensive bio- and databank of uranium miners was established at the Federal Office for Radiation Protection and is now available for molecular epidemiological research to link radiation-induced perturbations of biological pathways or processes and putative adverse outcome(s), e.g., by OMICs profiling at different biological organization levels. The bio- and databank includes biomaterial and data from more than 1000 individuals. Occupational radiation exposure and dust exposure was assessed over the lifetime working period by a comprehensive job exposure matrix. Biomaterial is available from blood as well as from formalin-fixed lung tissue from different groups, including healthy miners and lung cancer cases. Various experimental data on the OMIC level are also available for subsamples. Materials and anonymized data from this highly informative radiation exposure biobank can be accessed upon request. This report presents the current status of the first worldwide systematic German Uranium Miners' Biobank.

Abstract: Systematic bio- and databanks are key prerequisites for modern radiation research to investigate radiation response mechanisms in the context of genetic, environmental and lifestyle-associated factors. This report presents the current status of the German Uranium Miners' Biobank. In 2008, the bio- and databank was established at the Federal Office for Radiation Protection, and the sampling of biological materials from former uranium miners with and without lung cancer was initiated. For this purpose, various biological specimens, such as DNA and RNA, were isolated from blood samples as well as from formalin-fixed paraffin-embedded lung tissue. High-quality biomaterials suitable for OMICs research and the associated data on occupational radiation and dust exposure, and medical and lifestyle data from over 1000 individuals have been stored so far. Various experimental data, e.g., genome-wide SNPs, whole genome transcriptomic and miRNA data, as well as individual chromosomal aberration data from subgroups of biobank samples, are already available upon request for in-depth research on radiation-induced long-term effects, individual radiation susceptibility to lung cancer and radon-induced fingerprints in lung cancer. This biobank is the first systematic uranium miners´ biobank worldwide that is suitable for OMICs research on radiation-exposed workers. It offers the opportunity to link radiation-induced perturbations of biological pathways or processes and putative adverse outcome(s) by OMICs profiling at different biological organization levels.

Keywords: biobank; radon; uranium; mining; occupational; exposure; radiation effects; health effects; high and low doses; long-term effects

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1. Introduction

Silver mining existed since the 12th century in the south of Eastern Germany in the Federal State of Saxony and Thuringia (Figure 1) [1,2]. After World War II, the mines were reopened and the Soviet-Stock Corporation was founded with the code name Wismut [2]. The Wismut mining company (SAG/SDAG Wismut) produced a total of 220,000 tons of uranium during its operation period from 1946 to 1990, making it the third largest uranium producer worldwide [2].

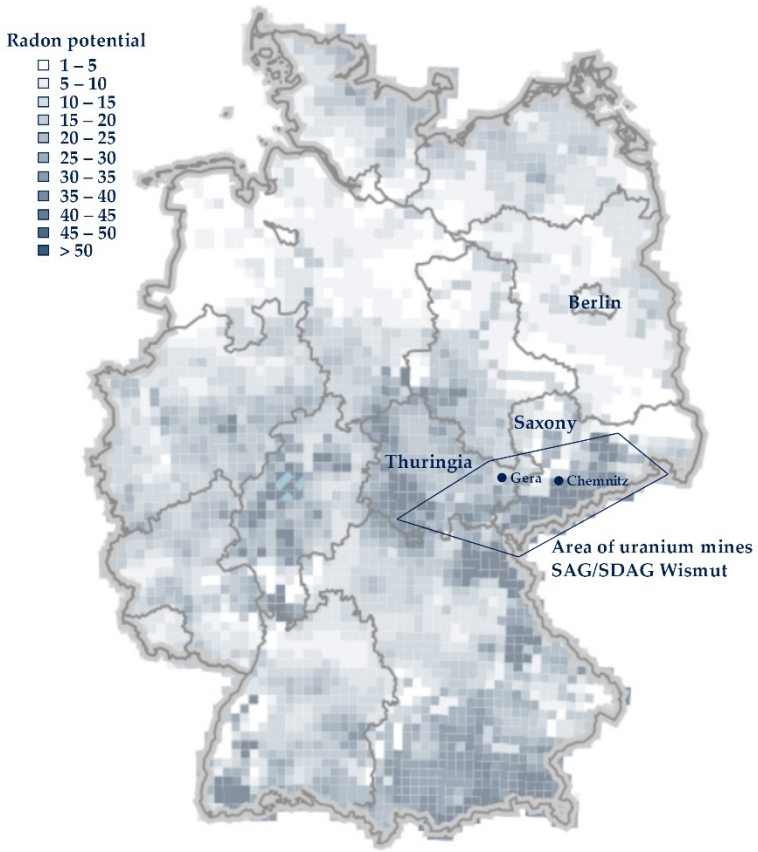

Figure 1. Localization (black pentagon) of the former SAG/SDAG Wismut uranium mines within Germany. Most of the mines are located near Gera (Thuringia) and Chemnitz (Saxony). The underlying radon potential (dimensionless variable) 1 m underground is indicated by the color code in the map [3]. The area of the uranium mines has naturally high radon concentrations in the soil and air. Figure 1 was modified from the map generated by (https://www.imis.bfs.de/geoportal/ accessed 9 November 2021).

With about 400,000 employees, the German uranium miners are one of the largest populations of radiation-exposed miners worldwide [1]. During their employment, the workers were mainly exposed to short-lived nuclides from radon and radon progeny (RnP), long-lived nuclides (LRN) from uranium (^{235}U, ^{238}U) and external gamma radiation. In addition to the radiogenic exposure, further noxae were fine dust, silica dust and arsenic-containing dust, depending on the composition of the rocks. In the early years (1946–1958), exposure to radiation and dust was particularly high for underground workers, that is, before artificial ventilation was available and when dry drilling instead of wet drilling was performed [1].

After the end of active mining in 1990, the Wismut cohort study was set up in 1993, including 64,000 miners. The study is conducted at the Federal Office for Radiation Protection (BfS) and is still ongoing [4]. Mortality data are regularly evaluated every 5 years [5]. A comprehensive job exposure matrix (JEM) was established to assess individual

radiogenic exposure and exposure to fine dust, silica dust and arsenic dust inhalation [6–9]. Annual and cumulative exposures have been determined in working level months (WLM) for RnP, in kBqh/m^3 for LRN and in mSv for external gamma radiation. The absorbed doses in milligray (mGy) in the red bone marrow from each radiation source (RnP, radon gas and LRN) and absorbed doses arising from alpha and low-linear energy transfer (LET) from all radiation sources were calculated with software [10,11] based on biokinetic and dosimetric models.

Systematic biobanking of occupationally exposed workers, especially from uranium mines, has not been performed in the past. However, it is a key prerequisite for modern radiation research to investigate radiation mechanisms in the context of genetic, environmental and lifestyle-associated factors in the etiology of radon-induced lung cancer and other health effects such as leukemia or other complex diseases. Therefore, the BfS initiated sampling of biological materials of former Wismut workers with and without lung cancer in 2008. This report presents the current state of the German Uranium Miners' Biobank (GUMB) located at the BfS.

2. Materials and Methods

The GUMB is composed of biomaterial and data obtained from 3 groups: (1) the Wismut biosample bank from the blood of healthy miners (WiProBa) (2) Wismut lung cancer biosamples from formalin-fixed paraffin-embedded (FFPE) lung tissue material of the pathological archive (LuCa) and (3) Wismut lung cancer biosamples of DNA (lung cancer) from blood (Indoor Radon study [12,13]).

2.1. Wismut Biosample Bank from Blood: WiProBa

Blood was taken between 2009–2011 from adult volunteers predominantly without cancer history after they received written and oral information on the study's purpose by a medical doctor in the context of regular medical checkups. The sampling was organized and coordinated by the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA). Sampling preferences were high-exposure individuals (WLM > 850), and age- and smoking-matched controls (WLM $\leq$ 50) selected and coded by Zentrale Beratungsstelle Wismut (ZeBWis).

2.1.1. Biological Samples

Biological samples are stored in the "Radiation Biology Section" of the BfS. The biobank is located in a restricted place and separated from personal data.

According to previously tested standard operating procedures (SOPs), blood samples (65–80 mL) were taken by physicians, including 3 EDTA tubes (Becton Dickenson, Heidelberg, Germany) for DNA and peripheral blood leukocyte isolation, 4 PAXgene tubes (Becton Dickenson, Heidelberg, Germany) for RNA isolation, 2 Li-Heparin tubes (Becton Dickenson, Heidelberg, Germany) for peripheral blood leukocyte isolation and 1 BD^{TM} P100 tube (Becton Dickenson, Heidelberg, Germany) for plasma isolation. Samples were stored at room temperature and shipped on the same day to different institutes to perform subsequent isolation of the different biological materials or for permanent storage. The SOPs were previously described in detail by Pesch et al. [14] and also partly by Gueguen et al. [15].

2.1.2. DNA Extraction

DNA was extracted by the salting out procedure and stored in a TE buffer (Tris/HCl (pH 8.0) 10 mM; EDTA 1 mM) at −20 °C and transferred to −80 °C for long-term storage at the BfS.

2.1.3. PAXgene Tube Sampling for RNA Extraction

For the first 24 h, tubes were placed in an upright position and frozen at −20 °C then transferred to permanent storage at –80 °C. RNA for all samples was obtained via RNA

extraction with a Blood RNA KIT (Qiagen, Hilden, Germany). From 200 samples, RNA was extracted by the Qiagen PAXgene Blood miRNA kit according to the manufacturer's description, which isolated total RNA and miRNA at the same time. All samples were stored at −80 °C.

2.1.4. Plasma Biobanking

Plasma tubes (BD^{TM} P100; Becton Dickenson, Heidelberg, Germany) were centrifuged at 2500× *g* for 15 min at 4 °C directly after arrival in the lab. Aliquots of 300 µL were prepared in cryotubes shock-frozen in liquid nitrogen; half of them were stored at −80 °C and the other half in liquid nitrogen at −196 °C.

2.1.5. Isolation of Peripheral Blood Leukocytes

PBL were isolated by Ficoll gradient centrifugation in special Leucosep^{TM} tubes (Greiner Bio-One, Kremsmünster, Austria). PBL were washed once with PBS and, after centrifugation, cell samples were frozen in aliquots of 3–5 × 10^6 cells. Cells were preserved in a cryomedium containing 10% DMSO, 20% FCS and RPMI containing penicillin/streptomycin. After freezing stepwise to −80 °C, they were transferred for long-term storage in liquid nitrogen at −196 °C.

2.1.6. Individual Data

Anonymized individual data on radiogenic exposure and silica/arsenic dust exposure were obtained from the scientific JEM provided by Zentrale Beratungsstelle Wismut (ZeBWis) [6–9]. With respect to radiation, workers were mainly exposed to short-lived radon progeny RnP, LRN from uranium decay and gamma radiation. The JEM used was identical to the one described in detail for the Wismut cohort study [4,9,16]. Data on smoking, medication and health as well as radiogenic exposures other than from occupational exposure, e.g., medical diagnostic or treatment, were obtained by a personal questionnaire. Exposure and epidemiological data are stored in a separate database on the BfS server in the "Radiation Epidemiology and Risk Assessment Section" of the BfS.

2.1.7. Protection of Personal Data

Experimental data obtained from analyses of the biological samples were and will also be stored for future investigations in the STORE databank [17] on the BfS server. Access to these data is restricted (physically and by databank protection through passwords). The biobank and database are stored at different sections of the BfS. Individual exposure and health data have different codes from the biosamples, and both have different codes from the result files. The rules defined by the EU General Data Protection Regulation (GDPR) have been strictly applied. No access to personal data is given. The only link to personal data is the signed consent sheet.

2.2. Sampling of DNA and RNA from the Tissue of Lung Cancer Cases in the Pathological Archive: LuCa

In 1999, the histopathological archive of the former Institute of Pathology of the health service of the Wismut company was transferred to the Institute of Prevention and Occupational Medicine (IPA). It consisted of biological material (slides, FFPE tissue blocks and whole lungs) from 28,975 autopsy cases collected from 1957 to 1994. From these 28,975 cases, 17,466 (60%) were identified as Wismut workers [2,18].

FFPE tissue blocks from male workers who died from 1986 onwards of lung cancer were chosen [2,18]. From 3 µm reference sections, pathologists defined tumor subtypes (adenocarcinoma, squamous cell carcinoma, small cell lung cancer) and classified them as tumor and non-tumor tissue. Only tissue blocks containing sufficient quality, defined by the quantity of non-necrotic areas, have been chosen for isolation of DNA and RNA. From 15 sections of 10 µm, DNA or RNA was isolated.

2.2.1. DNA Isolation from FFPE Tissue

Thirteen sections of 10 µm from tumor- and non-tumor FFPE material were used for DNA isolation. In the first step, slides were deparaffinized through incubation at 65 °C for 30 min, then by chemical treatment in Roti-Histol (Roth, Karlsruhe, Germany) for 10 min (twice), followed by a gradient of ethanol concentrations (96%, 80%, 70%, 60% 1 min each) and finally 1 min in distilled water. Deparaffinized tissue was transferred into reaction tubes (Eppendorf, Hamburg, Germany) and subsequently evaporated by vacuum centrifugation for 5 min to eliminate any liquids. According to the manufacturer's instruction (QIAamp® DNA FFPE Tissue Kit, Qiagen), DNA was isolated using a QiaCube (Qiagen, Hilden, Germany). Due to the long fixation times of the Wismut samples with formalin, proteins were heavily crosslinked. The protocol was therefore modified by doubling the amount of proteinase K and by incubation for at least 16 h. DNA was eluted in 100 µL of the ATE buffer supplied by the manufacturer. DNA concentration and purity were determined by NanoDrop ND-1000 (NanoDrop Technologies Inc., Wilmington, DE, USA). DNA was stored in two aliquots at −80 °C.

2.2.2. RNA Isolation from FFPE Tissue

RNA was isolated from 2 FFPE sections of 10 µm from tumor and non-tumor tissue. The tissue was deparaffinized as described above. RNA isolation was performed according to the instructions of the RecoverAll Total Nucleic Acid Isolation Kit for FFPE (Life Technologies, Vilnius, Lithuania). RNA was eluted in 60 µL of an elution solution. Concentration was determined by NanoDrop ND-1000 (NanoDrop Technologies Inc., Wilmington, DE, USA) and quality was determined by Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany), according to the manufacturer's instructions, RNA was defined by the RNA integrity number (RIN). RNA was stored at −80 °C.

2.3. Indoor Radon (Wismut Cases): Lung Cancer

DNA samples of lung cancer cases of the German case–control study on lung cancer (1990–1995) were kindly provided by the former Institute of Epidemiology (Prof. Dr. Wichmann), Helmholtz Center, Munich [12,13]. Here, genomic DNA was extracted manually from 10 mL of whole EDTA–blood samples using the Puregenec DNA extraction kit (Gentra Systems, Minneapolis, MN, USA). DNA samples were stored at the BfS.

3. Results

3.1. WiProBa

Study participants were recruited from six medical centers during regular medical checkups of former workers. Checkups are performed yearly for workers with exposures higher than 50 WLM and every 3 years for workers with less than 50 WLM. The response rate was dependent on the study centers and varied between 13% and 73%. The average age of individuals varied between 70 and 90 years (Figure 2). Of these, 63% claimed to have stopped smoking, 5% were still smoking and 32% were never smokers (Figure 3).

From a total of 419 individuals, blood samples were collected. From 23 out of the 419 individuals, blood samples were collected at two independent time points within 2 years. To consider the relevant factors for future biomarker analyses, information about previous acute or chronic diseases, cancer and cancer treatment, current medication, exposure to ionizing radiation or smoking were collected in form of a questionnaire. A medical doctor of the study center assisted subjects to fill out the questionnaire and complete the medical history accurately. Individuals reported to have had diseases such as heart attack, high blood pressure, tuberculosis, asthma, emphysema, chronical bronchitis, silicosis, asbestosis, diabetes, renal diseases, rheumatoid arthritis, multiple sclerosis, allergies and cancer. Seventy-four individuals reported cancer of the following types: prostate, kidney, urinary bladder, skin, blood system, colon, lung and stomach. Twelve individuals received radiation therapy and 13 received chemotherapy. Besides the radiogenic occupational data

(Figure 4A), data on occupational exposure to silica, fine and fine arsenic dust are also available for each individual.

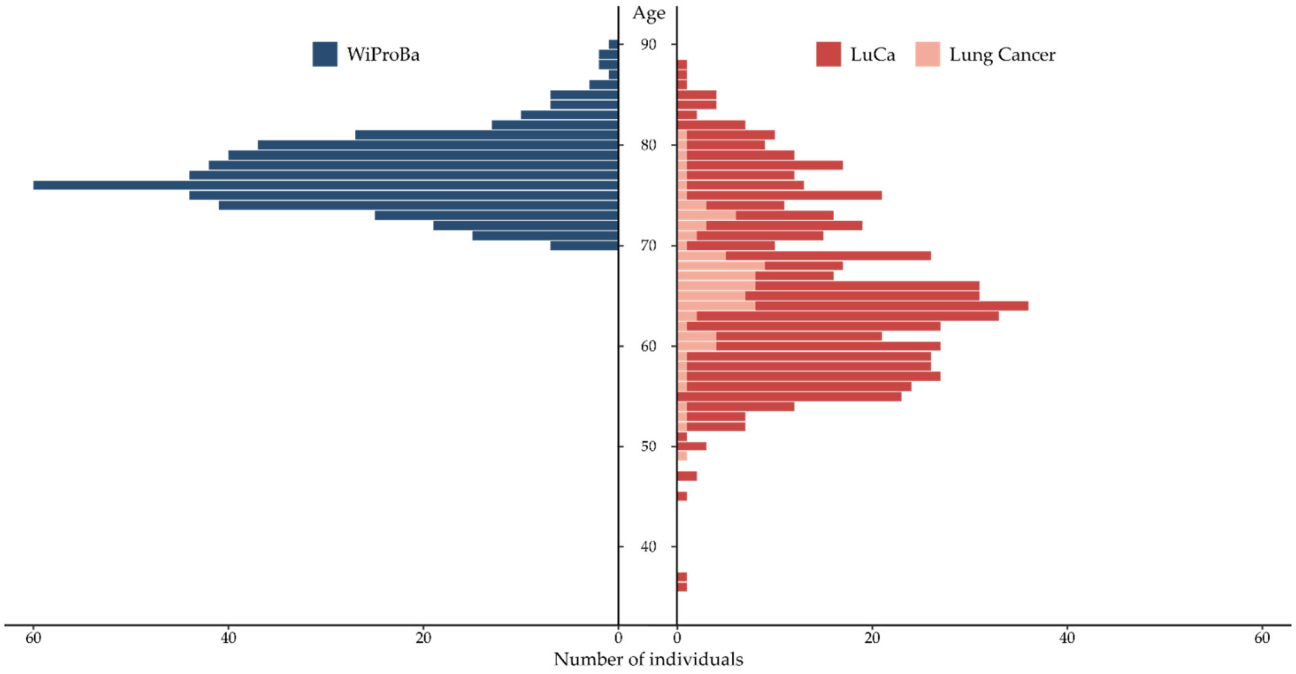

Figure 2. Age structure of the three subgroups: WiProBa (blue), LuCa (red) and lung cancer (light red).

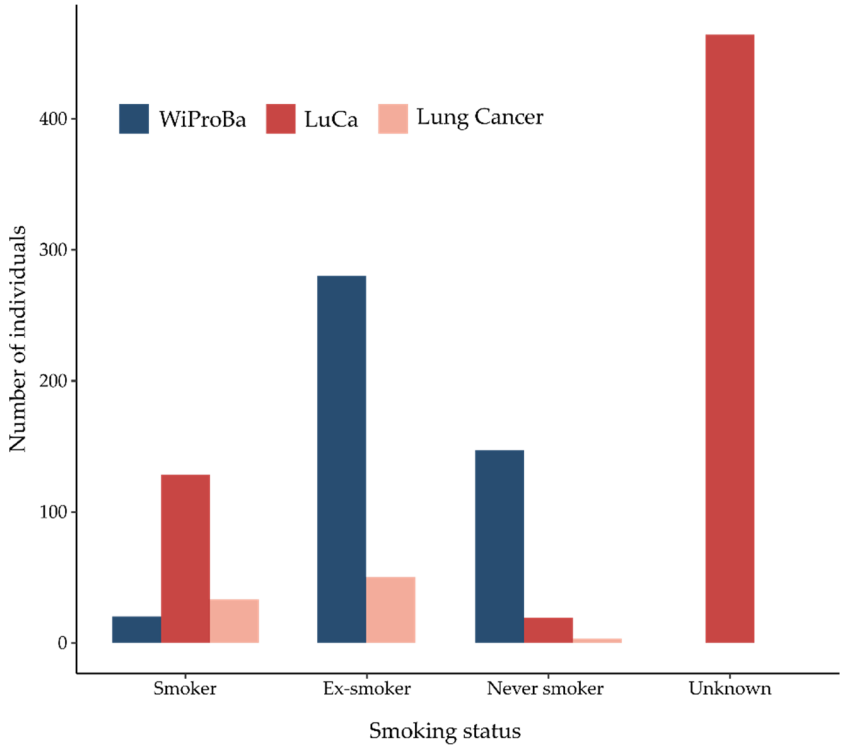

Figure 3. Smoking status in the three subgroups: WiProBa, LuCa and lung cancer. The smoking status was unknown for more than two-thirds of the individuals in the LuCa subgroup.

In addition to occupational radiation exposure, therapeutic radiation exposure and diagnostic radiation exposure in the last 12 months, such as CT, cardiac catheter or X-ray examination, was recorded in the questionnaire. With respect to occupational exposure,

the aim was to recruit preferentially highly (>850 WLM) exposed workers and, as a proper comparator, workers who were less exposed (≤50 WLM) but matched according to age and smoking. If the individuals from WiProBa were grouped in two radon categories that were matched according to smoking and age: moderate and high exposure (>50 WLM) versus low exposure (≤50 WLM), a high frequency of silicosis (16.9% versus 0.7%) and chronical bronchitis (16.5% versus 6.1%) was present among former miners in the more exposed group. This is most likely a result of silica dust and the high correlation between occupational silica dust and radon exposure [19,20].

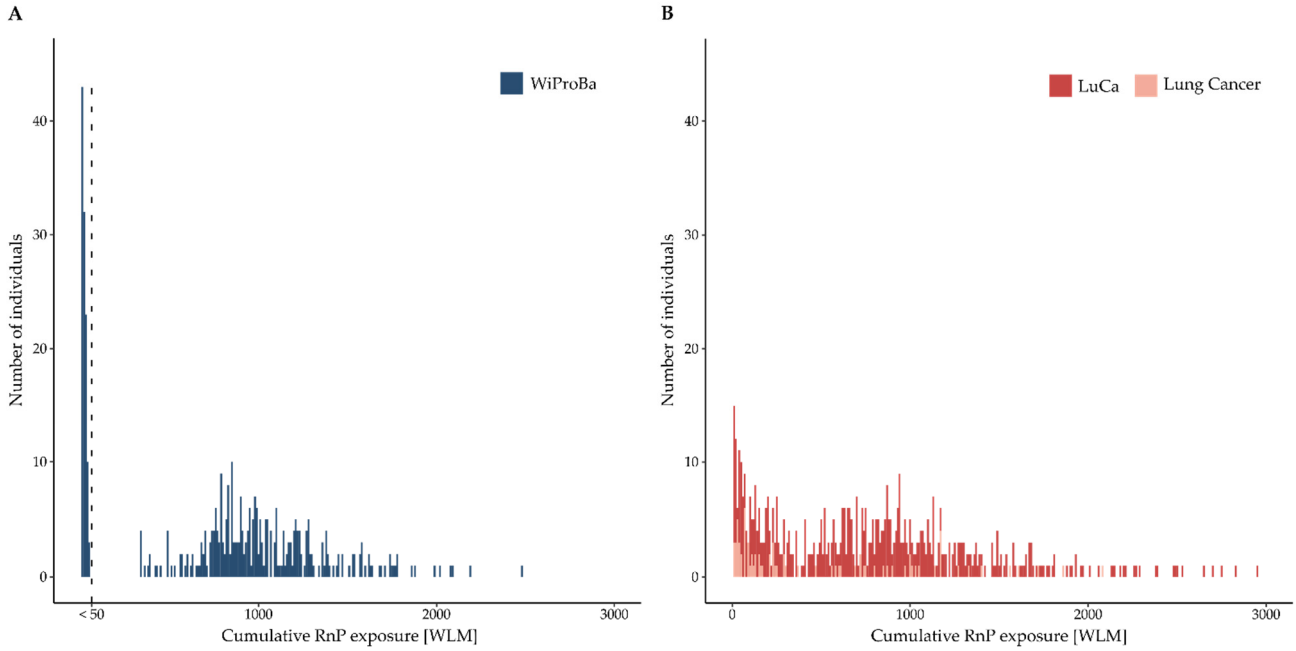

Figure 4. Cumulative radon and radon progeny (RnP) exposure of individuals in the WiProBa (**A**), LuCa and lung cancer (**B**) subgroups. The dotted line (in Figure 4A) marks a WLM exposure of 50. In addition to the moderately and highly exposed miners (>50 WLM), a control group of less exposed (≤50 WLM) miners were recruited and matched according to age and smoking.

Occupational exposure data were available for 412 of 419 individuals. After a pilot sampling phase, in which various transport options for sensitive material such as RNA and protein were tested (on ice, direct centrifugation, normal regular mail service at room temperature), samples were shipped by mail to different laboratories (IPA, BfS, Protagen AG and the Helmholtz Center Munich (HMGU)). Each center focused on special processing steps to isolate DNA, RNA and lymphocytes for cryopreservation or storage of PAXgene samples and processing of plasma samples. PAXgene samples and plasma samples stabilized with proteinase inhibitors were stored at –20 °C (PAXgene) and –80 °C or in liquid nitrogen (plasma) for future gene and protein expression analyses. A quality check specifically for sensitive material such as RNA and proteins revealed that the isolated RNA and proteins are of good quality even if the material was sent by the usual postal route [21]. The total RNA amount from a PAXgene sample was, on average, 5 µg with 70 ng/µL. Quality defined by the RNA integrity value was above 7.0 for most samples, even if the samples were isolated from PAXgene samples 2 years after sampling. A quality check on several molecular levels (DNA methylation of *ATM* and *LINE1*, *ATM* SNP typing, *ATM* mRNA, miRNA and protein degradation) demonstrated that most of the samples were of good quality for further methylation, expression or genotyping analyses. Thus, the applied SOPs were well suited to set up a high-quality biobank to allow modern high-throughput molecular genetic or biochemical analyses of the acquired biological materials.

3.2. Lung Cancer Cases

3.2.1. LuCa

From 2010 to 2018, the BfS began to isolate DNA and RNA from FFPE tissue blocks of more than 600 cases, from tumor tissue as well as normal tissue. DNA from 617 tumor and 612 non-tumor tissue blocks was isolated, as well as RNA from 612 tumor blocks and 607 non-tumor blocks. Exposure data (Figure 4B) deduced from the working history as described above for the WiProBa, demographic data (Figure 2) and data on tumor type (Figure 5) defined by two independent pathologists are available. The genomic DNA is highly fragmented (Figure 6) but sufficiently suitable for performing PCR analyses of short fragments, e.g., for validation of candidate SNPs predisposing towards radiation-induced lung cancer. On average 14.52 µg (0.29–59.46) of DNA was isolated, generally more than twice the amount from tumor material than from non-tumor tissue. DNA purity was determined by OD260/OD280 and was 1.8 on average, and did not differ significantly between tumor and normal tissue. Isolated RNA was 4.62 µg on average (0.07–36.69) and, as for the DNA, almost double the amount was isolated from tumors than from normal tissue. The average RNA integrity number was 1.9. The material was stored at –80 °C. Currently, the material is under investigation via highly sophisticated methods such as comparative genomic hybridization and next-generation sequencing (NGS).

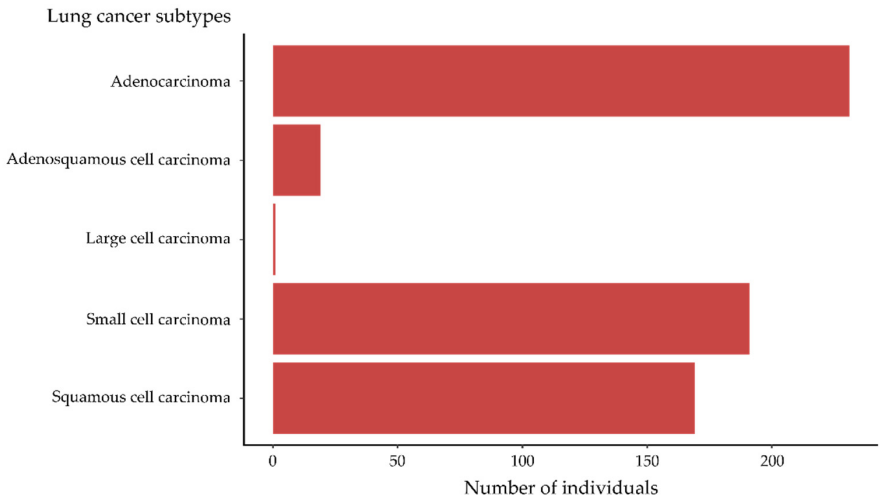

Figure 5. Tumor subtypes in LuCa.

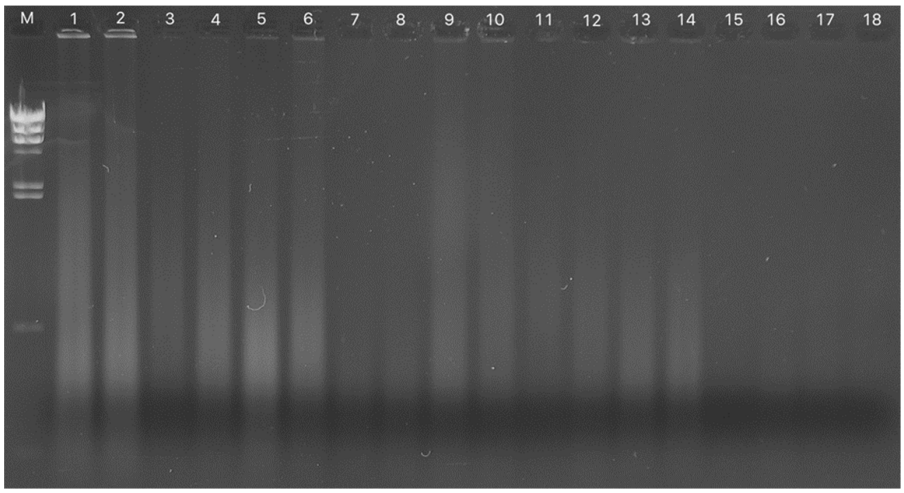

Figure 6. Lanes 1–18: Representative examples of DNA samples isolated from FFPE material. Lane M: λ x HindIII marker. DNA is stored in LuCa. Although the isolated DNA is highly fragmented, it was shown to be suitable for PCR amplification of short fragments (<500 bp).

3.2.2. Lung Cancer

In addition to the material from the pathological archive, 81 DNA samples isolated from blood samples of lung cancer cases of the German case–control study (Indoor Radon) on lung cancer in former Wismut uranium miners (1990–1995) were provided by the Institute of Epidemiology of the HMGU (Prof. Dr. Wichmann) to be stored in the BfS Wismut Biobank for future research. Age, exposure and smoking distribution are demonstrated in Figures 2, 3 and 4B, respectively. DNA samples have already been typed for whole-genome SNPs by OncoArray 500-K Chip analysis.

3.3. Available Samples and Data

In summary, the GUMB is currently composed of biomaterial and data from the subgroups WiProBa, LuCa and Lung Cancer (Figure 7). The available materials and data are summarized in Tables 1 and 2, and the available OMICs data are displayed in Figure 8.

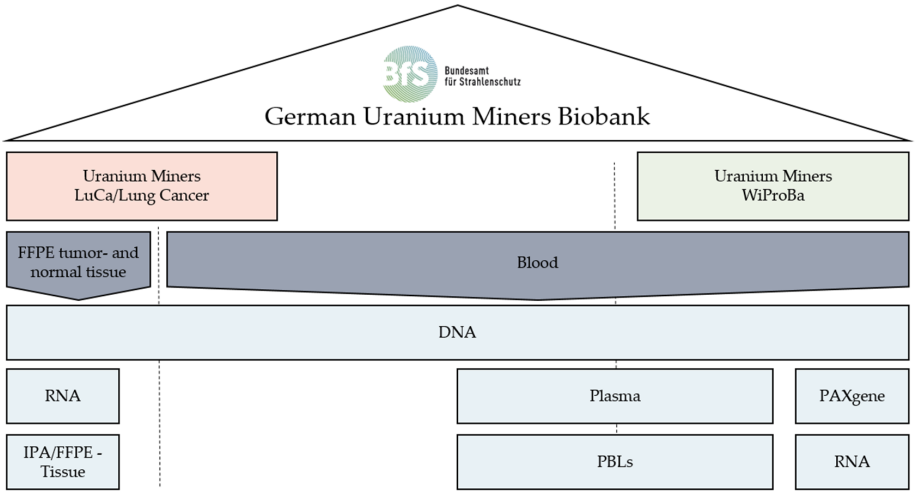

Figure 7. Overview of the available biomaterial stored in the German Uranium Miners' Biobank (GUMB) located at the BfS. FFPE tissue is still available in the pathological archive stored by the IPA.

Table 1. Available biological material of individuals in WiProBa and LuCa.

Cohort	Number of Individuals	Origin of Material	DNA	RNA	Cells	Plasma	PAXgene
WiProBa	419	Blood	419	418	399	369	418
LuCa	617	FFPE tissue					
		Tumor	617	612			
		Non-Tumor	612	607			

Table 2. Experimental data for individuals in WiProBa.

Experimental Data	Number of Samples	Literature	Technique
DNA methylation:			
ATM methylation	441	Pesch et al. 2015 [14]	Bisulfite/PCR
LINE1 methylation	441	Pesch et al. 2015 [14]	Bisulfite/PCR
SNP typing	413	Rosenberger et al. 2018 [13]	Infinium OncoArray-500K chip
RNA expression:			
ATM expression	438	Pesch et al. 2015 [14]	qRT PCR
GAPDH expression	438	Pesch et al. 2015 [14]	qRT PCR
Whole genome RNA expression	196	Bonin 2015 [22]	Affymetrix (Human Genome U219 GeneChip)
RNAseq analyses	22	Bonin 2015 [22]	NGS
miRNA expression	61	Johnen et al. 2014 [23]	Custom-based Microarray
Chromosomal aberrations	120	Unpublished	mFISH

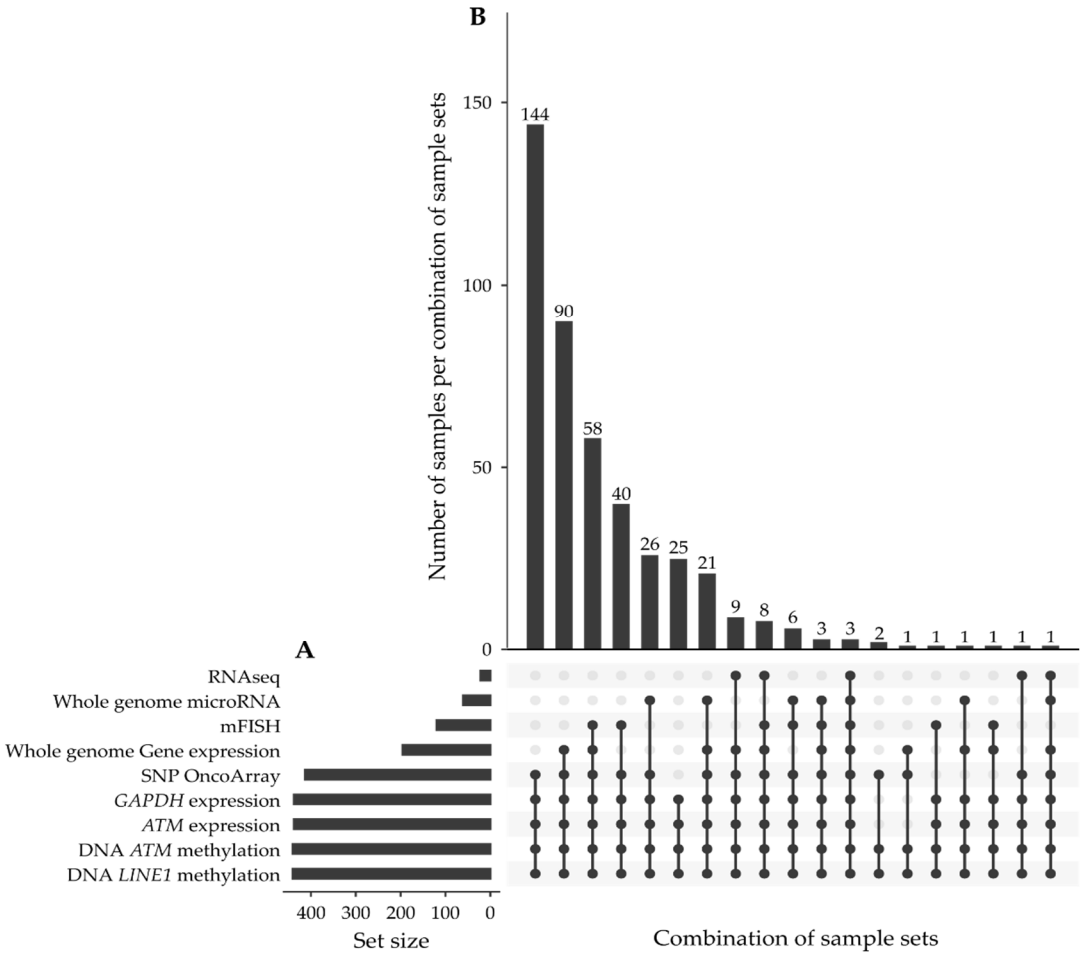

Figure 8. Present experimental data and their relationship on the individual level visualized in an UpSet Plot. (**A**) The graph represents the total number of individuals for which OMIC and functional data are available. (**B**) Number of individuals for which overlapping data are available for the various biological levels and combinations.

For a large part of the individuals, multiple analyses have already been performed on different biological levels, generating data from the genome to the transcriptome to functional cellular data.

Data have been generated by DNA SNP microarray typing [13] for non-cancer controls from WiProBa and lung cancer cases from WiProBa and Indoor Radon.

Data on whole-genome microarray RNA expression and RNAseq analyses [22] are available for high- and low-exposed individuals from WiProBa classified according to their red bone marrow dose. Furthermore, data on whole-genome miRNA microarray analyses are available for a subgroup of high- and low-exposed individuals classified according to their radon lung exposure [23] from WiProBa, as well as expression and methylation data for candidate genes, e.g., *ATM* for all individuals recruited in WiProBa [14].

In addition, chromosomal aberration analysis (mFISH) has been performed on 120 individuals of WiProBa.

Experimental data are included in the STORE database [17] or stored at the BfS server with access after publication and upon request. In summary, data on various biological levels are already available for a large number of individuals in WiProBa; for LuCa, data are currently being generated.

With respect to biosamples, WiProBa and LuCa DNA is stored in two different aliquots. One of the aliquots from WiProBa has never been thawed and will also be suitable for more demanding techniques, such as methylation analysis. For Lung Cancer, no additional DNA

is available, since most of it has been used up for SNP typing. Currently available samples include 1039 PAXgene samples for further RNA and miRNA isolation from 418 individuals and 3654 plasma samples from 369 individuals of WiProBA. For 22 or 10 individuals, PAXgene or plasma samples, respectively, have been obtained at two different time points.

3.4. Access to GUMB Material and Data

For radiation research purposes, access to anonymized material and data is possible after signing a transfer agreement including compliance with the GDPR. Access to data is limited to the necessary information and material as outlined in the study protocol. Informal requests need to be sent to Dr. Maria Gomolka (mgomolka@bfs.de). The BfS review board decides upon material and data research requests from the GUMB.

4. Discussion

The German Uranium Miners' Biobank will contribute to biological research projects on multisystem levels and represents a sustainable research infrastructure provided by the BfS. It allows researchers to investigate the effects of occupational radiation, dust exposure and lifestyle on genetic, biochemical and possibly also metabolic factors associated with adverse health effects. The biological materials associated with the available exposure assessments, lifestyle and health data are a powerful tool for addressing important research questions in radiation science, such as

- the risk of low doses for organs other than lungs,
- individual genetic susceptibility to radon-induced lung cancer,
- long-term radiation-induced biological effects and their associated adverse health effects other than lung cancer,
- the combined effects of co-carcinogenic lung exposure such as radiation and arsenic dust,
- potential radon-specific fingerprints in lung cancer tissue depending on tumor subtype.

These research tasks in radiation science have been identified as high priorities in several research agendas developed by experts worldwide, such as the High Level Expert Group on European low-dose risk research and the Multidisciplinary European Low Dose Initiative (MELODI) [24,25].

4.1. High-Dose and Low-Dose-Associated Health Effects

The broad range of organ doses received by the uranium miners allows us to investigate the effects of high as well as low doses on organs and tissues, with a special focus on the dose to the red bone marrow. Cumulative lifetime dose estimates to the lung exceed more than 9 Gy for highly (>850 WLM) exposed individuals, while the cumulative lifetime dose to the red bone marrow is below 1 Gy. The high dose to the lung is the result of high-LET exposure due to radon (99.9%), while the dose to the red bone marrow originates mainly from low-LET radiation from external gamma rays. Underground mining studies, unlike residential radon studies, provide higher exposures and a broader dose range, and therefore contribute significantly to understanding radon risks [26]. Radiation doses to red bone marrow are orders of magnitudes higher than those from residential radon studies.

A low-dose organ effect that has to be evaluated on the epidemiological level [27,28], as well as by molecular studies in the obtained DNA material of GUMB, is the leukemia risk induced by chronic cumulative lifetime gamma and alpha radiation exposure in miners. The aim of a current project [29] is to analyze the genetic variants acquired over the lifetime by next-generation sequencing (NGS) of DNA from blood in the miners' samples. Specific driver variants are thought to give a fitness advantage and support the expansion of certain clones which may lead to cancer [30]. Precancer clonal expansion has been observed in hematological diseases and is associated with an increased risk of developing myeloid neoplasms [31,32]. Deciphering whether radiation exposure promotes clonal expansion and represents the mechanistic link to epidemiological indications of increased myeloid

leukemia risk by radiation in Wismut miners [28,33] is a step forward towards identifying the potential mechanisms of how radon might induce leukemia.

4.2. Radon Risk-Modifying Factors

Individual susceptibility to radon-induced lung cancer depends strongly on lifestyle factors such as smoking [34]. For smokers a submultiplicative radon risk is the proposed model in Wismut uranium miners [35]. However, genetic factors affecting the DNA repair machinery also modify the radon- or radon and smoking-induced lung cancer risk [13,36]. The exact mode of action of each single factor and the combination of genetic and lifestyle factors, in combination with other noxae such as dust, are open questions in radon risk assessments and will also have an impact on proposed mitigation strategies in homes [37]. Research in GUMB has already allowed us to identify genetic variants modifying the radon-related lung cancer risk in miners [13]. Currently, the most promising variants will be validated in two ongoing projects in the LuCa samples.

4.3. Signatures in Radon-Induced Lung Tumor Tissue

Mutational signatures in tumors reflect mutational processes that can be caused by endogenous pathways such as the DNA repair machinery, or by exogenous stressors such as tobacco smoke and radiation exposure [38]. Recently, mutational signatures from the analysis of 23,000 different cancer tissues have been published by the ICGC/TCGA Pan-Cancer Analysis of Whole Genomes (PCAWG) Network [39]. The mutational signatures induced by radon and other ionizing radiations are less clear. Residential studies have shown that mutational tumor burden increased with radon exposure in Korean lung cancer cases [40]. The LuCa material in GUMB offers the possibility of extending and validating these findings in tumor and normal tissue, especially in high-exposure lung cancer cases. Here, the exposure ranges up to 3000 WLM. In contrast, residential radon exposure barely reaches 50 WLM. Additional information on combined exposure to other noxae, together with the information on lung cancer subtypes, will possibly help us to stratify the tumors and decipher radon-specific fingerprints. In national and international ongoing projects, such as RadoNorm, whole-genome DNA sequencing projects are currently performed for subgroups of individuals in LuCa and will generate new knowledge on the mutation signatures derived from high occupational radon exposure.

4.4. Radiation-Induced Long-Term Effects

Until today, long-term radiation effects, including those of radon, have rarely been studied in exposed human population samples, mostly because biological material was either not collected or not collected in a suitable way to allow OMICs research with high-throughput techniques. Additional medical or lifestyle data are often missing, which hampers the consideration of confounding factors and may bias the analysis of radiation effects. The Wismut miners were between 70 and 90 years old when the blood samples were collected. At this timepoint, the occupational exposure was already several decades in the past, as exposure mainly occurred in their early years of employment. For radon, the highest exposure was in the 1950s and that for gamma radiation was in the 1960s [1,4]. Detailed information on medical history and lifestyle is documented in the GUMB, which is essential for evaluating the effects induced by radiation. Thus, the GUMB materials are promising tools for studying long-term radiation effects. Epigenetic effects can be analyzed in a proper study design on genome-wide data. Methylation alterations, and transcriptome changes at the mRNA and miRNA levels will give insights into long-term-induced radiation effects on different molecular processes. Proteomic and metabolomic studies in plasma and also in the stored cell material offer a deeper insight into radiation-perturbed inflammatory or metabolic pathway changes. The integration of functional data such as chromosomal aberrations in the context of immunological or metabolic parameters may support the understanding of the molecular mechanisms and pathologies of long-term radiation effects.

4.5. Outlook

Molecular OMICs technologies for archived biomaterials are improving rapidly and offer new possibilities for analysis [41]. However, while each OMICs technology can only capture a piece of the biological complexity, integrating multiple types of OMICs data on the same set of samples can provide a more holistic view of the underlying biological processes that is suitable for defining key events in pathological processes [42–44]. The GUMB enables the radiation research society to generate knowledge on potential radon-related health effects other than lung cancer that can subsequently be validated in the next step by a specific setup in large-scale epidemiological or molecular epidemiological studies. In fact, some of the identified biological markers indicate cellular or molecular damage of the whole biological system that are a manifestation of developing disorders and could be integrated in future molecular epidemiological study designs addressing the identified putative disorder. These studies have to be at an international and highly multidisciplinary level, including biologists, radiation dosimetrists, medical doctors, bioinformaticians and modelers. The challenge for studies on environmental and occupational radiation exposure is to further improve the process for assessing the risk to human health from single and combined exposure to genotoxic agents (e.g., low and high doses of radiation) by the use of biomarkers predicting the potential risk of the toxic outcomes and pathological changes, and to embed this knowledge in new modeling strategies for risk assessment [45], also using new tools of unsupervised learning [42,44].

5. Conclusions

The high quality of the large part of the biobank and the different biological materials stored in the GUMB allow the identification of biomarkers of exposure, biomarkers of effects or biomarkers of individual radiation susceptibility by using advanced high-throughput OMIC technologies. Biomaterial, together with extensive data (on exposure, medical records, lifestyle and experimental results) and the possibility of crosslinking different biological levels for each individual, provides the opportunity to define key events in the pathological processes of radiation. Highly informative radiation exposure biobanks such as the GUMB are the base for biological research in humans. Together with the newly developed computational (modeling) approaches, such biobanks will contribute essentially to radiation risk assessments and to the development of effective preventive measures against exposures deemed to be hazardous to human health.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Bavarian Ethics Committee (Ethik-Kommission der Bayerischen Landesärztekammer No. 08082, 29 October 2008) and the Federal Commissioner for Data Protection and Freedom of Information (IV-508/020#0021,1 September 2008).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. A written confirmed consent form, which included the use of biomaterial and data for studying the effects of radiation exposure and for analyzing the data on a national and international basis, also including genetic analyses, was obtained from each participating individual. All subjects directly completed anonymized questionnaires with a corresponding unique identity code (ID) per subject. Biomaterial was encoded by a different code. Individuals can withdraw their consent at any time; subsequently, the material will be destroyed. Only for that purpose can the link to personal data and

biomaterial be accessed by a data protection manager at the BfS. Informed consent sheets are stored at a securely locked physical place to which only the data protection manager or his/her deputy has access (no data file exists for these sheets).

Data Availability Statement: Anonymized data will be available on request and after signing a Data Transfer Agreement from the Federal Office for Radiation Protection.

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