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Review

Advanced Omics and Radiobiological Tissue Archives: The Future in the Past

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Abstract: Archival formalin-fixed, paraffin-embedded (FFPE) tissues and their related diagnostic records are an invaluable source of biological information. The archival samples can be used for retrospective investigation of molecular fingerprints and biomarkers of diseases and susceptibility. Radiobiological archives were set up not only following clinical performance such as cancer diagnosis and therapy but also after accidental and occupational radiation exposure events where autopsies or cancer biopsies were sampled. These biobanks provide unique and often irreplaceable materials for the understanding of molecular mechanisms underlying radiation-related biological effects. In recent years, the application of rapidly evolving "omics" platforms, including transcriptomics, genomics, proteomics, metabolomics and sequencing, to FFPE tissues has gained increasing interest as an alternative to fresh/frozen tissue. However, omics profiling of FFPE samples remains a challenge mainly due to the condition and duration of tissue fixation and storage, and the extraction methods of biomolecules. Although biobanking has a long history in radiation research, the application of omics to profile FFPE samples available in radiobiological archives is still young. Application of the advanced omics technologies on archival materials provides a new opportunity to understand and quantify the biological effects of radiation exposure. These newly generated omics data can be well integrated into results obtained from earlier experimental and epidemiological analyses to shape a powerful strategy for modelling and evaluating radiation effects on health outcomes. This review aims to give an overview of the unique properties of radiation biobanks and their potential impact on radiation biology studies. Studies recently performed on FFPE samples from radiobiology archives using advanced omics are summarized. Furthermore, the compatibility of archived FFPE tissues for omics analysis and the major challenges that lie ahead are discussed.

Keywords: FFPE; ionising radiation; radiobiological archive; biobank; cancer; omics; RNA sequencing

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1. Introduction

Formalin-fixed, paraffin-embedded (FFPE) tissue archives and their well-annotated clinical records represent an invaluable source for prospective and retrospective studies. FFPE tissues are routinely provided from biopsies or autopsies and stored in significant quantities over many years. These biomaterials offer an extensive resource of normal and diseased tissue for screening identification and validation of biomarkers, investigation of disease mechanisms and the development of new therapies [1–4].

Biobanking has a long tradition in radiation research [5–7]. The radiobiological archives contain not only the clinical samples obtained during cancer diagnostic or therapy but are also exclusively collected after different radiation scenarios including occupational or accidental exposure [5,6]. The radiation biology biobanks also contain the samples collected from well-established animal studies [6]. In addition to blood and cells, a large number of FFPE tissues were also stored in these archives. These samples, which frequently

are the only source of biomaterial available, can be used to understand the molecular mechanisms involved in the biological effects of radiation exposure,

Advances in high-throughput molecular analysis in recent years have revolutionised our knowledge of biological processes. Global analyses of proteins, RNA, genes, metabolites and lipids are the goal of highly advanced omics technologies such as proteomics, transcriptomics, genomics, metabolomics and lipidomics. Recently, the number of studies using omics approaches to profile the FFPE tissues has markedly increased. However, the application of omics technologies remains challenging mainly due to the harsh fixation and embedding process that severely impacts the integrity and quality of biomolecules [3]. Considerable efforts have been made to develop the optimized and standardized protocols for extraction and separation of protein, DNA and RNA from FFPE samples by testing different factors such as buffer components, detergents, pH, temperature, pressure and others [8–11]. These methods aim to optimise reproducible, cost-effective and efficient protocols that are compatible with omics techniques. Analytical protocols for the profiling of FFPE samples have been also established, optimized and used in radiation biology studies [12–14]. In this review, we describe the characteristics of radiobiological archives with available FFPE samples and the recent advances in omics analysis on FFPE tissues in radiation research. The main problems and challenges to be overcome for the optimal application of omics on radiobiological FFPE samples are further discussed.

2. Radiation Biology Archives

Biobanking has a very long history in radiation research. Over the past 100 years, the large scale of animal and human biomaterials from different radiation exposure scenarios were collected, proceeded, and stored. These archival samples often originated from non-repeatable experiments or unique events. Retrospective studies of such biobanks allow understanding of the biological effects of radiation exposure and improve interpretation of the epidemiological data for radiation risk assessment. Accordingly, efforts were made to rescue both endangered data and biomaterials [15].

The FFPE samples and, in part, their characterized tagged data are a substantial part of radiobiological archives. The majority of FFPE blocks in radiobiological archives are from animal experiments, but human tissues are also available. The information of several radiobiological biobanks and associated large-scale data collections are available in the STORE database (https://www.storedb.org) (accessed on 18 November 2021). In the following section, we summarize the information about the biobanks with available FFPE samples and related databases. Both the archives and the databases are listed below, with a concise overview in Table 1 for animal material and in Table 2 for human material.

2.1. FFPE Blocks from Animal Experiments

A major archive of biological animal material is hosted by Northwestern University, Chicago, USA. The so-called NURA archive houses the JANUS Mouse Archive including data and tissues from 50,000 mice exposed to gamma and neutron irradiation during 11 different studies, as well as the Argonne Dog Archive including data and tissues from 5000 beagle dogs exposed to gamma irradiation and internal emitters at Argonne National Laboratory. It also includes the Lovelace Dog Archive with data and tissues from beagle dogs exposed to internal emitters and inhaled radionuclides at the Lovelace Inhalation Toxicology Research Institute (ITRI). A Radiation Biology Wiki (http://janus.northwestern.edu/nira/index.php/Main_Page) (accessed on 18 November 2021) holds summary reports and documentation of the research related to these three archives [7,16,17].

The Southern Urals Biophysical Institute (SUBI) in Ozyorsk, Russian Federation, conducted a large number of animal studies, mostly with Wistar rats. For six of these experiments study descriptions are available through the STORE database (http://dx.doi.org/doi:10.20348/STOREDB/1056) (accessed on 18 November 2021); biological samples and the related data can be accessed on request. The studies dealt with the effects of incorporated

tritium or tritium oxide, incorporated plutonium and external gamma radiation, partly with a mixture of these.

Along with the German Thorotrast Study [18], a large study was conducted by the German Cancer Research Center (DKFZ) on rats to investigate the roles of the radioactive and chemical components in Thorotrast gel-induced tumours of the lung, liver or spleen. About 6000 annotated FFPE blocks are held by the German Federal Office for Radiation Protection (BfS), Neuherberg, Germany (http://dx.doi.org/doi:10.20348/STOREDB/1017) (accessed on 18 November 2021). Both the study-related data and the blocks are available on request.

In Japan, the Japan-Store house of animal radiobiology experiments (J-SHARE) has been established, aimed at providing access to data and samples from experiments with mice and rats conducted at the Institute of Radiological Sciences of the National Institutes for Quantum and Radiological Science and Technology (QST), Chiba, Japan [19]. Exposures included X-rays, gamma-rays, neutrons as well as a range of heavy ions, including carbon ions as used in radiotherapy. Currently, efforts are made to make the J-Share website accessible to the scientific community.

The Institute for Environmental Sciences (IES) in Aomori, Japan, has a unique Low Dose Radiation Effects Research Facility (LERF) which was established to study the biological effects of long-term low-dose-rate irradiation on large populations of mice [20]. IES maintains and stores, among other materials, all FFPE samples. Data and samples can be accessed on request [21].

After the Fukushima catastrophe, an archive system was established at Tohoku University, Japan, composed of frozen tissues, FFPE blocks, blood samples and extracted DNA and RNA from cattle and wild Japanese macaques in and around the evacuation zone [22–24]. These materials and their associated data are available on request [23].

The French Nuclear Safety Authority (ASN), Montrouge, France, conducted a number of large-scale experiments with rats for different exposure scenarios including plutonium or neptunium inhalation, intravenous plutonium citrate injection and wound contamination by actinides [25–27]. Descriptions of these studies are available through the STORE database (https://www.storedb.org/store_v3/study.jsp?studyId=1005) (http://dx.doi.org/doi:10.20348/STOREDB/1005, http://dx.doi.org/doi:10.20348/STOREDB/1007 and http://dx.doi.org/doi:10.20348/STOREDB/1022) (accessed on 18 November 2021).

Table 1. The FFPE animal tissue listed in STORE DB and/or available in the radiobiological archives.

Place	Name of Archive	Species	Tissue	Exposure *	Source of Information	DOI	Reference
BfS [a]	n.a.	Rats	Various	Internal (Thorotrast)	storedb.org	http://dx.doi.org/doi:10.20348/STOREDB/1017	[18]
SUBI [b]	n.a.	Wistar rats, CBA mice	Various	Tritium; tritium and external gamma; Pu	storedb.org	http://dx.doi.org/doi:10.20348/STOREDB/1041 http://dx.doi.org/doi:10.20348/STOREDB/1056	
ASN [c]	n.a.	Rats	Lung	Inhalation Pu; Np	storedb.org ERA; study ID 23.1	http://dx.doi.org/doi:10.20348/STOREDB/1005	[25]
ASN	n.a.	Rats	Bone	Pu citrate i.v.	storedb.org	http://dx.doi.org/doi:10.20348/STOREDB/1007	[26]
ASN	n.a.	Rats	Various	Wound contamination with actinides	storedb.org	http://dx.doi.org/doi:10.20348/STOREDB/1022	[27]
QST-NIRS [d]	J-Share	Mice; rats	Various	Various	storedb.org	http://dx.doi.org/doi:10.20348/STOREDB/1138	[19]
IES [e]	n.a.	Mice; rats	Various	External gamma	storedb.org	http://dx.doi.org/doi:10.20348/STOREDB/1139	[20]

Table 1. *Cont.*

Place	Name of Archive	Species	Tissue	Exposure *	Source of Information	DOI	Reference
Tohoku University [f]	n.a.	Cattle wild Japanese macaques	Various	Gamma (external and internal)	storedb.org	http://dx.doi.org/doi:10.20348/STOREDB/1141	[22–24]
Northwestern University [g]	NURA	Mice; Beagle Dogs	Various	External neutron; and gamma	storedb.org http://janus.northwestern.edu/wololab/index.php?go=archives	http://dx.doi.org/doi:10.20348/STOREDB/1094	[7,16,17]

[a] Federal Office for Radiation Protection, Germany; [b] Southern Urals Biophysical Institute, Russian Federation; [c] The French Nuclear Safety Authority, Fontenay-aux-Roses, France; [d] National Institutes for Quantum and Radiological Science and Technology (QST)-National Institute of Radiological Sciences (NIRS), Chiba, Japan; [e] Institute of Environmental Sciences, Aomori, Japan; [f] Department of Pathology, Institute of Development, Aging and Cancer, Tohoku University, Miyagi, Japan; [g] Northwestern University, Chicago, USA; n.a. non-applicable; * All animal experiments include at least one unexposed control group. (All DOIs have been accessed on 18 November 2021).

Table 2. The FFPE human tissue listed in the STORE DB and/or available in the radiobiological archive.

Place	Name of Archive	Tissue	Exposure	Source of Information	DOI	Reference
BfS, Germany	Wismut Uranium Miners Biobank	Lung	Radon, Radon progeny, longlived Radionuclides, external gamma	storedb.org	http://dx.doi.org/doi:10.20348/STOREDB/1034	[28,29]
UA, RUS	Chernobyl Tissue Bank	Thyroid	Chernobyl catastrophy	storedb.org www.chernobyltissuebank.com	http://dx.doi.org/doi:10.20348/STOREDB/1092	[30]
RERF, Japan [a]	Adult Health Study (AHS)	Various	Atomic bomb survivors	storedb.org https://www.rerf.or.jp/en/programs/research_activities_e/outline_e/progahs-en/	http://dx.doi.org/doi:10.20348/STOREDB/1137	[31]
Washinton State University, USA	NHRTR/USTUR	Various	Internal; actinides	storedb.org https://ustur.wsu.edu/	http://dx.doi.org/doi:10.20348/STOREDB/1140	[32]
Nagasaki University, Japan	The Nagasaki Atomic Bomb Survivors' Tumor Tissue Bank of Atomic Bomb Disease Institute	Various cancer and surrounding tissue	Atomic bomb survivors	storedb.org https://www.genken.nagasaki-u.ac.jp/pathology/en/tt-bank/index_e.html	http://dx.doi.org/doi:10.20348/STOREDB/1142	[33]
SUBI [b]	RHTR	Various	Actinides; external gamma	storedb.org http://www.rhtr.subi.su/?requests/new	http://dx.doi.org/doi:10.20348/STOREDB/1149	[34]
Nagasaki University, Japan	The Nagasaki Atomic Bomb Survivors' Tumor Tissue Bank of Atomic Bomb Disease Institute	Various	Thorotrast	https://www.genken.nagasaki-u.ac.jp/pathology/en/tt-bank/index_e.html		[7,23,35,36]

[a] Radiation Effects Research Foundation, Hiroshima, Japan; [b] Southern Urals Biophysics Research Institute, Ozyerk, Russian Federation. (All DOIs have been accessed on 18 November 2021).

2.2. FFPE Material from Humans

From 1946 to 1990, extensive uranium mining was conducted by the former SDAG Wismut in Saxony and Thuringia which are southern parts of the former German Democratic Republic (GDR). The health services of the SDAG Wismut included an Institute of Pathology [37,38]. In the 1990s, the BfS began constructing the German uranium

miners cohort to investigate the potential health risks associated with occupational radiation exposures and dust [28,39,40]. The archived biological samples including FFPE tissues are now hosted by the Institute of Prevention and Occupational Medicine (IPA) in Bochum, Germany. Overall, it harbours 28,975 autopsy cases and health data [41] that have been collected from 1957 to 1994. Among these cases, 17,466 were identified as uranium workers [29]. The long-term study of cohort and its follow-up includes information on radon, quartz and arsenic exposure derived from work history, as well as information on tumour subtypes and non-cancer diseases [28,39]. For more than 600 workers, DNA and RNA were extracted from the FFPE tissue and stored at the German Uranium Miners Biobank (http://dx.doi.org/doi:10.20348/STOREDB/1034), (accessed on 18 November 2021) hosted at BfS.

The Chernobyl Tissue bank inherits human thyroid material and was established after the 1986 Chernobyl accident. Both data and material can be accessed on request [30]. The US National Human Radiobiology Tissue Repository (NHRTR), which is associated with the United States Transuranium and Uranium Registries (USTUR) (https://ustur.wsu.edu) (accessed on 18 November 2021), contains a variety of biological material. Primarily it comprises tissues obtained at autopsy from USTUR, including among other material tissue blocks. The NHRTR also houses a collection of tissue material obtained from the terminated Radium Worker study at Argonne National Laboratory.

For the Japanese Adult Health Study, a sub-cohort of 15,000 individuals of the life-span study (LSS) of atomic bomb survivors, biological samples have been collected including FFPE (https://www.rerf.or.jp/en/about/organization-en/chart-e/bio_e/) (accessed on 18 November 2021). Beginning in April 2008, a cohort study has been initiated at Nagasaki University to analyse solid cancers and haemopoietic malignancies among atomic bomb survivors. Biomaterial includes tumours and surrounding normal tissues [33]. These tissues are removed at surgery and archived together with personal, historical dose and demographic data.

The Russian Radiobiological Human Tissue Repository (RHTR), known as the Mayak Worker Tissue Repository, samples and stores for a long period of time the human tissues after chronic, low-dose radiation exposure. The RHTR enrolled two cohorts between 1951 to the present time including exposed workers at the Mayak facilities as well as the local residents who were never occupationally exposed to ionizing radiation as controls. The repository consists of surgical tissues and autopsy samples, together with blood samples and DNA from parental-offspring trios [34]. The detailed information of the samples, including FFPE tissues, are available on RHTR website (www.rhtr.subi.su) (accessed on 18 November 2021).

Data and material from the Japanese Thorotrast study developed by Tohoku University were transferred to the Atomic Bomb Disease Institute of Nagasaki University, Japan. Efforts are currently being made to make the website of the Thorotrast study accessible to the scientific community.

2.3. Databases

The diversity and quantity of the samples available in radiobiological archives and the volume of the datasets associated with them are prompting the radiation community to design different platforms to manage big data collections. In addition to the above mentioned JANUS database held by the Northwestern University, there are the European Radiobiological Archives (ERA) (https://era.bfs.de) (accessed on 18 November 2021) [42,43] and the STOREDB database (https://www.storedb.org) (accessed on 18 November 2021) [7]. The ERA databank aims to preserve the information provided by the long-term animal studies between the 1960s and 1990s on the effects of radiation exposure and radioactivity to make them available to the scientific community for further evaluation. STOREDB hosted by the German Federal Office for Radiation Protection (BfS) (https://www.bfs.de) (accessed on 18 November 2021) is a central access portal to information from radiobiology experiments distributed across scientific institutions worldwide [7]. The database was developed with

funding from the Euratom Research and Training Programme and is intended to provide a repository of primary data to support publications, protect data at risk of being lost to the community, and maintain legacy data and links to archives, as well as links to biological resource collections for radiobiology projects to facilitate systematic data sharing and archiving [7]. The platform provides an opportunity for addressing newly arising questions by re-analysing existing information or application of new analytical approaches including omics on archival samples. Furthermore, the database provides Standard Operating Procedures (SOPs) on the storage and use of the biological samples. Access to the STOREDB is free and is provided by users' ORCID IDs through an intuitive web interface. Any type of data or information can be uploaded, e.g., text or data files, PDFs, specs, JPGs etc., i.e., no special format is needed. Copyright remains with the laboratory, and the uploader can decide whether the data are locked, are accessible only on request, or are donated to the public domain. The form of the license can be applied to all data by the originating laboratory.

Not included in this review are repositories held at clinics and hospitals for their own purposes (e.g., [44,45]), as it is not certain whether FFPE blocks from these institutions will be made available to interested scientists from outside. Moreover, the authors are well aware that the number of archives listed here might be incomplete.

Tables 1 and 2 provide an overview of the FFPE tissues that are made available on-demand to interested scientists, although the procedures for accessing this valuable material vary. While the process of how to obtain access to data and material is well structured in some cases, particularly for the Chernobyl Tissue Bank, in most cases personal contact to the Principal Investigators of previous studies or to those who oversee hosting the archive is recommended. However, it must always be described precisely what scientific question lies behind the request for access and how it is to be answered.

3. Main Challenges with Omics Analysis of FFPE

For decades FFPE tissues offered the standard materials for histopathological analysis due to the well-preserved morphological architecture, ease of sample preparation, and stability during long-term storage. The rapid development of emerging molecular technologies provides new opportunities for retrospective studies. For a long time, applying such advanced approaches as omics analysis to archival samples were considered an almost impossible task, mainly due to the loss of macromolecular integrity and quality during the rough fixation, delayed embedding and conditions of long-term storage. The sequence of events strongly affected the quality and quantity of protein and nucleic acids yielded from FFPE tissue and hampered the accurate qualitative and quantitative analysis [3,46].

The changes occurring during cross-linking of proteins during the fixation of FFPE tissue were investigated in several studies [47–49]. The most significant consequence of formaldehyde fixation is the generation of a methylol modification at free lysine residues that severely interferes with protein extraction and separation [47,50]. The modified lysine residues remain inaccessible to protease during digestion, a phenomenon that results in a preference for identifying tryptic peptides with the C-terminal arginine over lysine [47,50]. Furthermore, the use of conventional protein labelling techniques for quantitative proteomic analysis of FFPE tissues is even less effective [47,50]. Since the majority of the chemical labels used in quantitative proteomics target lysine residues, the formalin modification of lysin leads to inefficient labelling of FFPE material [51–53]. Several studies were conducted to evaluate different protocols for the extraction of proteins of FFPE tissues [8–11].

DNA, as the most stable species among biological macromolecules, is subject to the cross-linking of cytosine residues, or spontaneous deamination/depurination of nucleotides and fragmentation during the fixation procedure [54,55]. The formalin fixation not only affects the quantity and purity of the DNA yield, but also causes misinterpretation of the DNA sequence, particularly due to an increase in the level of artifactual mutation of cytosine (C) to thymine (T) and guanine (G) to adenine (A) (C:G > T:A) [56]. Yet another

known problem is the high fragmentation of DNA, probably caused by warm ischemic time in operating rooms, or the type of formalin (buffered versus unbuffered) used, time of fixation that all may result in low quantities DNA yields and therefore low quantities of amplifiable templates for analysis. In addition, formalin fixation produces non-reproducible sequence artefacts due to DNA fragmentation and hydrolytic deamination [57]. Several studies have reported the protocols to overcome these limitations for DNA microarray and DNA sequencing [3,56–58]. These protocols also include bioinformatics or statistical tools to allow for different mutation findings in fresh-frozen or FFPE tissue, considering different allelic fractions, or distinguishing between clonal and subclonal mutations. In the same line of evidence, mRNA extracted from FFPE tissues is mostly fragmented, degraded and modified. Formaldehyde modification of RNA interferes with the base-pairing necessary for hybridization and introduces the cross-linking of RNA to other macromolecules [58,59]. However, recent studies have demonstrated the feasibility of RNA analysis approaches including RNA seq in FFPE samples [60–63]. In contrast to mRNA, miRNA showed more stability and compatibility in FFPE for expression analysis [64–67]. The short length of miRNA is probably responsible for the fact that they are minimally affected by methylol cross-links during the fixation procedure, therefore their expression profile is comparable to those from frozen tissue samples [67,68].

The conditions of FFPE preparation also pose a challenge for other omics approaches such as metabolomics and lipidomics. In the meantime, the first reports describe the technical feasibility of metabolome and lipidome profiling in FFPE tissue, opening up the possibility of utilizing MS-based metabolic/lipid profiling of radiobiological FFPE samples in the future [4,69].

More than general difficulties for analytical approaches using FFPE samples, some concerns need to be addressed specifically for radiobiological archives. The first issue is handling the FFPE tissues with long-lived internal emitters, in this case, the radioactivity released by tissues needs to be carefully assessed before and during analysis.

A further problem is the lack of knowledge about the availability and quantity of FFPE tissues sampled during or after radiotherapy in clinical biobanks. Although a large volume of samples was continually collected from patients who received radiotherapy in hospitals, it is surprisingly difficult to find accurate information about the number of samples and their diagnostic outcome. It is fully understandable that the growing field of omic-analysis on archival materials raises concerns of privacy, confidentiality and data protection, but the extent to which these limitations affect the data and material sharing surprises the authors. Ensuring an optimal mechanism for data protection and facilitating scientific research is particularly important in the case of radiooncology biobanks.

4. Omics Analysis on FFPE Samples in Radiobiological Archives

The omics studies performed on FFPE samples of radiobiological archives have mainly focused on the analysis of a molecular signature of the response of cancer tissue to radiation exposure. There are only a few omics analyses on FFPE samples provided from normal tissues such as the heart, lung and liver after irradiation. In the following sections, we discuss the available omics studies performed on radiobiological archives.

4.1. Proteomics

The application of proteomics approaches in radiation research is well acknowledged [70–72]. The proteome profiling offers a comprehensive platform to investigate the cellular response of cancer and normal tissue to radiation exposure [73,74]. Proteomic analysis of FFPE samples as an alternative to fresh-frozen tissue has gained growing attention in recent years [48,75–79]. However, the difficulties to achieve an optimal protein extraction and separation make the application of quantitative proteomic analysis on FFPE samples challenging. To optimize the quality and quantity of proteomic analysis of FFPE tissues, a wide range of proteomic techniques were used [80]. The protein profile of FFPE cancer and normal tissues was analysed using the well-established classical 2D electrophoresis and

two-dimensional differential gel electrophoresis (2D-DIGE) [47,52,81,82]. To improve the protein resolution pattern result in 2D, several extraction and focusing methods, including liquid isoelectric focusing (LIEF), were applied before electrophoresis [47,77,81]. However, these platforms often confirmed the difficulty in separating and identifying low abundance proteins, especially those subjected to formalin-induced modification [47]. The separation and identification of proteins from FFPE tissues have been greatly improved by gel-free proteomics. The combination of different chromatography approaches with mass spectrometry makes gel-free proteomics a suitable technology for reducing the complexity of the FFPE protein profile. A variety of methods such as nano-reverse phase LC (nano-RPLC) tandem mass spectrometry [83], capillary isoelectric focusing (CIEF) [84], a multidimensional separation platform, including capillary isoelectric focusing (CIEF)/nano-RPLC [85], two-dimensional image-converted analysis of liquid chromatography, mass spectrometry (2DICAL) [86], and surface-enhanced laser desorption ionisation time of flight mass spectroscopy (SELDI-TOF) [87] have been used to increase the resolution and identification of proteins and peptides. The FFPE proteome has been quantified with label-based approaches such as iTRAQ [51] or DIGE [52,82]. However, in many of these studies, labelling was not optimal as classical labelling targets lysine residues in a protein that are blocked by the formalin modifications. Label-free approaches can markedly address the issues of quantitative proteomic on FFPE tissues [12,14]. Imaging mass spectrometry combined with Matrix-Assisted Laser Desorption/Ionization (MALDI MS) was also conducted on FFPE tissue to identify the biomarker of cancer and drug development [80]. Protocols for the extraction and analysis of proteins from the FFPE samples were established, optimised and used in radiobiology studies [12,14,47].

To investigate the mechanism involved in different radiotherapy responses from oropharyngeal carcinoma (OPC) patients, Sepiashvili et al. compared the proteome profiles of human papillomavirus (HPV)-positive and HPV-negative OPC FFPE samples. The multidimensional Protein Identification Technology (MudPIT) analysis showed significant alterations in the proteins associated with the cell cycle, apoptosis, and immune response. The level of the oncoprotein cortactin was enhanced in HPV-negative biopsies. The authors compared their results to the published data on frozen HPV+ and HPV− OPC tissues [88] and confirm the high level (70%) of protein co-identification in both studies. The authors suggested that cortactin as a potential biomarker for radiation resistance contributes to reduced survival in HPV-negative patients [89].

Dunne et al., investigated the proteome of FFPE samples collected from patients treated by androgen deprivation and radiotherapy using nanoflow liquid chromatography-MALDI MS/MS or after separation by 1D or 2D electrophoresis to identify the prognostic biomarkers for prostate cancer (PC) [90]. Comparing the proteomics data of their study to only a few available published data on frozen PC tissue confirmed identification of similar proteins including a high abundance cytoplasmic, cytoskeletal and nuclear histones. The analysis of FFPE proteomics data and further immunoblotting suggested that an alteration in the ANXA2 expression served as a predictive marker for the metastatic potential of prostate cancer [90].

To identify a predictive marker of chemotherapy and/or radiotherapy resistance in patients with oral squamous cell carcinoma (OSCC), Matsukawa et al. compared the proteome of FFPE samples obtained from patients who received preoperative chemotherapy and/or radiotherapy followed by surgery, using nano-flow high-performance liquid chromatography [91]. The FFPE samples of this study were provided from the different groups of resistant and sensitive patients treated daily by fractional radiation exposure. The proteome profiling and immunohistochemistry validation suggested galectin-7 as a predictive marker of tumour resistance in OSCC patients [91].

Netto et al. used label-free proteomics to analyse the FFPE samples of nasopharyngeal carcinoma (NPC) patients who were treated by intensity-modulated radiotherapy (IMRT). The analysis identified that Epstein–Barr (EBV) and Herpes simplex (HSV) viruses-related proteins markedly present in early-stage of cancer [92]. The authors suggested that identi-

fied proteome signatures in their study is well-related to head and neck cancer onset and can serve as potential targets for therapy but need further validation [92].

In contrast to archival tumour samples, the normal FFPE tissue in radiobiological biobanks was not often analysed using proteomic approaches. To investigate the effect of total body irradiation on the heart, Azimazdeh et al. used a label-free quantitative approach to compare the FFPE heart tissues of sham- and total body irradiated C57BL/6 mice as a model system. The mice hearts were isolated and fixed in formalin 24 h after the irradiation and proteins were extracted and separated using an optimised protocol [47]. In good agreement with data observed from proteome analysis of fresh-frozen hearts [12], the study showed immediate alterations in cardiac metabolic enzymes and mitochondrial proteins [12].

The same group used recently sequential urea/SDS extraction and filter-aided sample preparation (FASP) digestion to analyse the late effects of chronic radiation exposure in human FFPE heart autopsies from Mayak workers by label-free proteomics. Here authors compared for the first time the proteome profiles of the fresh frozen and FFPE heart tissues after chronic irradiation [14]. The proteome profile of the FFPE samples confirmed the observations obtained from fresh-frozen cardiac tissue [93]. Although the experimental design, sample size, analytical approaches make a direct comparison of the two studies difficult, the main results of both studies were similar, indicating the changes in main functional clusters of proteins involved in the heart metabolism and structure following irradiation [14].

4.2. Genomics and Transcriptomics

Genomic approaches are widely applied in profiling clinical cancer samples and biomarker discovery of diagnosis and prognosis. Comparison of the mutational burden of a tumour to normal tissue provides insight into the tumour development due to environmental exposures by the finding of specific mutational signatures of genotoxins [94], but also allows to decipher the nature and timing of mutational processes and the contribution of even rare germline variants increasing cancer risk [95].

FFPE tissues, with all known difficulties, offer attractive candidates for a comprehensive investigation of the cancer genome. High-throughput transcriptomics, including microarrays and next-generation sequencing (NGS), is an emerging technology that enables the study of genomes, epigenomes, and transcriptomes using limited sample material, often used in cancer samples [3,96]. The potential of archival samples for high-throughput transcriptome profiling has been well identified and discussed [97]. Efforts are given to optimize microarray and next-generation sequencing technologies as well as associated bioinformatics tools to develop a compatible platform to low input extracted nucleic acids from FFPE tissues [61,98]. RNA extracted from FFPE samples is often not well compatible with standard RNA-sequencing methods, where poly(A) selection or ribosomal RNA (rRNA) depletion-based methods are the gold standard. Both approaches suffer from a higher degree of RNA contamination and a lower number of alignable reads [96,99]. Several approaches have been developed to address the problem of degraded RNA in the sequencing analysis. Veldman-Jones et al. used the nCounter platform from Nanostring Technologies, which allows digital readout of up to 800 mRNA targets even when the RNA is degraded [100]. An exome capture approach for RNA-seq from FFPE samples was also developed. The platform maximizes RNA-seq libraries by including most reads in exons regardless of the RNA quality [101]. Vahrenkamp et al. developed a so-called FFPEcap-seq method suitable for sequencing capped 5-ends of FFPE RNA. To generate sequencing libraries, the platform combines enzymatic enrichment of 5-capped RNAs with template switching [96]. A recent comparison ranked platforms on their performance on FFPE samples with different storage times [97]. Thereby 3′-sequencing approaches from Lexogen and Qiagen were identified as highly reliable and cost-effective for old FFPE samples.

Despite considerable progress in the application of whole-genome sequencing on FFPE samples, there are only a few studies available that analysed RNA and DNA isolated

from FFPE materials from patients who received radiotherapy. These studies were mainly designed to identify the tumour signature or biomarkers of radioresistance of cancer.

To investigate the mechanism underlying the poor outcome of radiochemotherapy with and without cetuximab in cervical cancer (CC) patients, de la Rochefordiere et al. screened the hotspot mutations by target sequencing on FFPE samples from patients who were treated by chemotherapy combined with standard pelvic radiation therapy. The authors showed that alterations in the PIK3CA pathway negatively affected the complete response to radiochemotherapy. The analysis showed that CC patients with PIK3CA mutation had trends to poorer disease-free survival (DFS) at 2 years [102].

Nuryadi et al. analysed the mutation signatures of radioresistant tumours by exon sequencing of 409 cancer-related genes in FFPE samples of uterine cervical cancer (UCC) patients who survived multiple rounds of radiotherapy [103]. The analysis indicated activating mutations in PIK3CA and KRAS, and putative inactivating mutations in SMAD4. The authors further validated the association between this mutation signature and radioresistance by cell-based experiments [103]. The same team later analysed the genetic profile of FFPE tumours collected from UCC patients with local recurrence after carbon ion radiotherapy using the same analytical platform [104]. The study identified mutations in FGFR3 and FGFR4 in the recurrent tumour compared with the treatment-naive tumour [104].

To investigate the association between tumour genetic profiles and radiotherapy outcome, Yoshimoto et al. performed exon sequencing on cancer-related genes in FFPE samples obtained from UCC patients following radiotherapy [105]. The analysis identified the mutations in the intracellular tyrosine kinase domain of the FGFR gene family. The observations were compared with data generated on FFPE and fresh-frozen samples in previously published data where PIK3CA was often identified as a marker. The authors argued that a low association of prognosis with PIK3CA is related to the tumour type treatment. The authors also found worse 5-year progression-free survival (PFS) for FGFR mutation-positive patients, suggesting a potential role for the FGFR signalling pathway in UCC radioresistance [105].

In comparison to mRNA, miRNAs in FFPE tissues were shown to be more robust to degradation, partially due to their smaller size. miRNAs are known to contribute to the radiation response in several cancers [106–109].

Pajic et al. compared miRNA isolated from FFPE tumour samples of breast cancer patients with and without local relapse using a microarray to identify the radioresistance associated miRNA [107]. The analysis identified 11 miRNAs significantly differentially expressed between the two groups. Among them, overexpressed miR-139-5p was selected for further validation. The authors showed that the miR-139-5p and its targets were strong predictive biomarkers for radiation sensitivity in vitro and correlated with outcomes in radiotherapy-treated patients in breast cancer cohorts [107].

To identify the miRNAs signature for radioresponse of laryngeal squamous cell carcinoma (LSCC), Maia et al. analysed the expression pattern of miRNAs in radioresistant and radiosensitive tumours of LSCC patients treated with primary radiation therapy [110]. This analysis and validation of additional samples indicated that the expression level of miR-296-5p was associated with radioresistance and recurrence properties of early-stage laryngeal cancer [110].

In contrast to studies performed to identify the radioresistance profile of the tumour, there are only a few studies that investigate the mechanism of radiation-induced cancer using FFPE samples available in radiation archives.

Using array comparative genomic hybridization (aCGH), Selmansberger et al. analysed the genomic copy number of radiation-associated papillary thyroid carcinoma in FFPE tissues of the Ukrainian–American cohort [111]. The study identified a significant association between single-copy number alterations (CNAs) and clinical parameters and patient data, including individual gender and radiation dose [111].

Wilke et al. analysed the genomic copy number signature associated with radiation exposure in post-Chernobyl breast cancer [112]. The authors compared FFPE tumours

obtained from exposed female Chernobyl clean-up workers and evacuees and matched non-exposed control patients by aCGH. The analysis revealed a significant association of a set of nine signatures of CNAs with radiation exposure but not with any clinical characteristics of the patients and radiation dose [112].

Unlike cancer, not many studies have analysed RNA or miRNA from FFPE lung or heart to investigate the negative effect of radiation exposure on normal tissue.

The levels of miR-21 and miR-146a as potential biomarkers of heart disease were measured using FFPE heart tissues in the proteomic study on Mayak autopsies [93]. The analysis showed significant upregulation of both miRNAs in the highest dose group compared to the control and lower dose groups [93].

5. Lessons from Past for Future

With a rapidly growing number of advanced omics approaches being used in translational research, the need for biological samples is continuously increasing. Big data generated by omics technologies need to be validated for biological plausibility and reproducibility, especially if the findings from in vitro or in vivo models are to be translated to humans. The use of a larger sample size provides additional material for further validation to address the problems of variability and heterogeneity of omics data with adequate statistical power. In the absence of sufficient frozen samples, FFPE samples, with their countless numbers, ease of storage and rich clinical information, can indeed provide the optimal materials for such retrospective analyses. Unfortunately, the use of FFPE samples is not hassle-free. The first problem is that the initial purpose for tissue collections is often not prospectively defined based on the requirements and capacity of future analysis platforms such as omics. In addition, the conditions for tissue collection, processing and storage are not yet uniform across the laboratories. Protocols for isolation, extraction and separation of biomolecules from FFPE samples are not consistently standardised, and storage and sharing of archival materials and related information are not yet optimal.

To address these obstacles, the radiation biology biobanking policy and its infrastructure need to be improved. The optimal quality and quantity of FFPE samples must be well foreseen in the main strategy of tissue collection, oriented towards omics analysis.

In an optimal platform, the facility and knowledge are already available to establish uniform protocols for tissue collection and processing, monitor storage condition and status and standardise protocols for biomolecule isolation and extraction. It should be borne in mind that the maintaining of the archival tissues as well as the development of cost- and time-effective protocols require a distinct universal institutional collaboration scientifically and economically.

To evaluate the quality and quantity of omics data derived from FFPE tissues, several studies performed analysis on paired frozen and FFPE samples [113,114]. Comparison of frozen and FFPE tissues is a crucial strategy to evaluate extraction methods and ultimately the validity of FFPE-omics data. Although these studies highlighted the difficulties in FFPE profiling, they were often able to confirm the compatibility, reproducibility, and consistency of FFPE samples for high-throughput omics analyses. Proteomics and transcriptomics analyses have not only demonstrated the applicability of extraction, isolation, and separation of biomolecules from FFPE tissue but have also shown a significant overlap in protein and RNA identification and quantification between FFPE and fresh frozen samples [75,113,114]. Unfortunately, such comparisons for radiological FFPE materials have been very limited. One reason for this is that the additional collection of fresh tissue is so time-consuming and costly that it is not a standard part of routine clinical practice in cancer diagnosis and therapy. This is all the more true for samples taken during a radiation disaster, where priorities are still set differently. In the absence of matched frozen samples, FFPE omics derived results need at least to be compared with available data from published studies using similar analytical platforms. An important issue underscores the importance of principles of findability, accessibility, interoperability, and reusability (FAIR) data sharing in the radiation community. Clinical documentation and subsequently general agreements

on sharing samples and related data also need to be improved. Standardized data acquisition and data storage, as well as machine-readable output data, will facilitate sharing of metadata and available experimental data. General agreements on how to manage archive material and related data are urgently needed for efficient universal material and data sharing.

The available database requires support and further promotion to cover a broader spectrum of archival samples and facilitate sample and data sharing effectively. During the preparation of this review, the authors were concerned to provide adequate information on clinical FFPE tissue samples collected from patients receiving radiotherapy. The lack of information about these types of FFPE specimens and the difficulty in sharing this information is an issue that needs to be addressed. Here an enhanced exchange with the specific existing medical fields is urgently warranted.

Another problem is related to FFPE samples prepared on normal tissues such as the heart, lung, brain or liver. Although the study of non-cancerous diseases such as CVD, pulmonary fibrosis and cognitive impairment has received a priority in radiation research, there is still a significant knowledge gap in these areas. Unfortunately, there are not enough omics studies performed on FFPE samples of normal tissue. Omics analysis on FFPE normal tissue needs to be effectively promoted to uncover the molecular mechanism underlying these diseases.

Despite these challenges, there are promising developments to further boost the exploitation of omics data from radiological FFPE samples. New computational tools for batch corrections will further facilitate the integration of data from different techniques and therefore enable the combination of new data with pre-existing. This seems to be especially important for the limited materials in radiological archives. Great potential to further understand radiation-induced processes may lie in the integrative analysis of FFPE samples with multiple omics platforms followed by a merged analysis of the data, instead of a post-analysis integration [115]. Up to now such analysis unfortunately is missing for radiological FFPE samples. Finally, it is important to note that omics data from FFPE profiles have great potential to be combined with classical oncopathology findings and epidemiologic data to meet the criteria for retrospective studies. Emerging research areas such as radiomics, which employs artificial intelligence or advanced computational data management tools, can improve the status of radiation biobanking and strengthen observations from high-throughput omics on archival samples. All together, radiation biology archives remain as a treasured source of samples and knowledge to be explored for understanding the biological effects of radiation exposure, the knowledge that has been processed in the past serving as a valuable resource to plan for the future.

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