Establishing the Japan-Store House of Animal Radiobiology Experiments (J-SHARE), a large-scale necropsy and histopathology archive providing international access to important radiobiology data

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Abstract

Purpose: Projects evaluating the effects of radiation, within the National Institutes of Quantum and Radiological Science and Technology (QST), National Institute of Radiological Sciences (NIRS), have focused on risk analyses for life shortening and cancer prevalence using laboratory animals. Genetic and epigenetic alterations in radiation-induced tumors have been also analyzed, with the aim of better understanding mechanisms of radiation carcinogenesis. As well as the economic and practical limitations of repeating such large-scale experiments, ethical considerations make it vital that we store and share the pathological data and samples of the animal experiments for future use. We are now constructing such an archive called the Japan-Storehouse of Animal Radiobiology Experiments (J-SHARE).

Methods: J-SHARE records include information such as detailed experimental protocols, necropsy records and photographs of organs at necropsy. For each animal organs and tumor tissues are dissected, and parts are stored as frozen samples at -80 ˚C. Samples fixed with formalin are also embedded in paraffin blocks for histopathological analyses. Digital copies of stained tissues are being systematically saved using a virtual slide system linked to original records by barcodes. Embedded and frozen tissues are available for molecular analysis.

Conclusion: Similar archive systems for radiation biology have been also under construction in the USA and Europe, the Northwestern University Radiation Archive (NURA), and STORE at the BfS, respectively. The J-SHARE will be linked with the sister-archives and made available for collaborative research to institutions and universities all over the world.

Introduction

As has been discussed in several recent scientific reports (Wang 2010, Abbott 2012) and also highlighted in the media, there is a growing recognition of the value of making historical data from large-scale animal experiments freely available to the scientific community. Unlike animal experiments conducted to test the effects of a particular chemical or pharmaceutical compound which might be performed under the auspices of a single regulatory authority or company sponsor, and have occurred in a single laboratory within a reasonably short period of time, experiments into the effects of radiation are quite different. The large number of variables includes but is not limited to physical parameters such as radiation type, quality, dose and dose-rate, as well as experimental parameters such as exposure route, age at exposure, animal model, housing conditions, diet, sex and of course the biological endpoint(s) measured. Experiments on the effects of radiation in animals have been conducted around the world for decades, and sponsored by a variety of institutions and research bodies with differing priorities and protocols. The heterogeneity of these studies complicates direct comparisons between experiments, while at the same time providing a rich source of data from which to uncover interaction and assess the generalisability of effects across different models (Haley 2015, Tran 2017).

Efforts to date to provide access to some of the earliest large-scale experiments have demonstrated the utility of such data (reviewed in Zander et al. 2019), and have also uncovered some of the challenges that such an endeavor poses. In one example, data from more than 36,000 mice stored in the JANUS archive were re-analyzed to determine low dose- and dose-rate extrapolation factors (Tran 2017) showing the power of working with

data collected from a single effort over a long period of time; while conversely, combining JANUS data with compatible data in the European Radiobiological Archives revealed that the dose-response model did not fit the expanded cohort (Haley 2015). This effort highlighted the challenge of taking a large population of available animal data and carefully extracting experiments that met strict inclusion criteria. Work on data from internal alpha-emitter experiments in both mice and dogs stored in the International Radiobiological Archive has shown the utility of comparing data that are not conducted under the same conditions (Sazykina and Kryshev, 2016). The use of stored data in these studies rather than new experiments on stored specimens is a sign of the additional challenges posed by molecular investigations in archival samples from radiobiological archives conducted many decades earlier (Tapio and Atkinson 2006; Tapio et al. 2010). The pioneers of current archives have shared their experiences, providing invaluable advice to those seeking to introduce their own data into the public sphere.

The pioneers of current archives have shared their experiences, providing invaluable advice to those seeking to introduce their own data into the public sphere. Here at the National Institute of Radiological Sciences (NIRS), a National Institutes of Quantum and Radiological Science and Technology (QST) in Japan, as well as the common hurdles faced in digitising and sharing large volumes of historical data, we have encountered additional challenges that arise due to issues of language. Although existing archives include data from a range of locales, we believe ours known as the Japan-Store House of Animal Radiobiology Experiments (J-SHARE), is the first attempt to share original data which was recorded in a language other than English, and one where the data are not only historical but also contemporary and ongoing. Here we outline the structure and principles underlying our recently launched experimental archive, and share our experiences which may prove useful to others.

J-SHARE

The QST-NIRS has several independent research departments which have conducted large-scale rodent experiments over the past decades, including some which are still actively generating new data. Many of these experiments have been lifespan studies, where tumor incidence is recorded over several years after radiation exposure. The availability of different types of radiation exposure apparatus has permitted the investigation of X- and gamma-rays, neutrons as well as a range of heavy ions from the Heavy Ion Medical Accelerator at Chiba (HIMAC), including carbon ions as are used in ongoing radiotherapy treatment at our institute (Table 1). The majority of the animals in these studies have been housed under specified pathogen free (SPF) conditions within the past 10 years. Since one of these groups, now known as the Department of Radiation Effects, has focused on investigating the effects of age-at-exposure on cancer risk, a large number of lifespan studies have been performed using a range of exposure ages in parallel. Recently, a purpose-built low dose-rate exposure facility has allowed the addition of chronic exposures across different age ranges to the experimental repertoire. In the wake of the Tokyo Electric Power Co. Ltd., Fukushima Dai-ichi Nuclear Power Plant accident of 2011, recognition of the value of such data to the estimation of cancer risk in exposed populations led to government funding of a project to establish a public archive of the experimental results, and the J-SHARE project was established.

Experimental Designs and Initial Data Format

The majority of experiments were performed in B6C3F1 mice originating from founder stocks available in Japan; however, the J-SHARE system has been designed to eventually include experiments which have used other mouse strains, rats, and transgenic animals (Table 2). The experiments typically include 50 male and 50 female mice per

radiation treatment group, and a single 'experiment' may contain a single radiation source/dose across several age groups, or even multiple doses across multiple ages. The mice are all housed, maintained and monitored under the same conditions until an necropsy is performed at or immediately prior to natural death according to consistent criteria as agreed upon by independent observers, which provides confidence in the estimates of tumor latency and lifespan. The mice are necropsied by a member of a trained team of researchers who apply a fixed analysis protocol to each mouse, and record the gross observations and necropsy findings on a standardised necropsy record. A veterinarian participated in necropsy, a cause of death decision. Five mice are bred in one cage, and the individual identification of the mouse is carried out at the necropsy. In the study of the breast tumor, a palpation record is taken during the experiment period. The records for routine procedures and operation of animal facility are written in Japanese and can be traced. The records are, however, not uploaded and linked to this archive for the moment. If there is an inquiry depending on need, the records could be provided.

These paper-based records are sequentially issued with a fixed and unique necropsy number which provides a primary key to any animal across all past and future experiments. The necropsy records have been printed in a mixture of English and Japanese text, with some common terms such as 'Body Weight' and 'Date' written in English while other terms are printed in Japanese logographic characters. Notes are recorded by systematic entry (filling in dates, circling relevant information e.g. ♀/♂) as well as by handwritten note-taking and diagrams drawn with reference to printed anatomical maps of the mouse, written in a mixture of Japanese logographic characters, Japanese phonetic abbreviations (equivalent to an English short-hand) and occasional English letters/words.

Although the conversion of freeform notes to a systematic nomenclature is always a challenge in digitisation projects, many existing projects rely on coding only major information for each record with the details available directly to readers via a scanned copy of the original record. In the case of Japanese, there is no simple method by which readers who are not fluent in Japanese can read the text since use of a dictionary or machine translation is still hindered by how one can identify or enter non-roman characters. The use of optical character recognition (OCR) to convert the handwriting into digital text proved too unreliable in our hands with existing technology. This means that the scanned copy of the necropsy record is inadequate as a means to share the information for each mouse. The solution we have adopted is a multi-pronged approach which seeks to maximise the usefulness of the data and confidence in the results of a search, while minimising the time required to convert each record. Given that there are 10,220 mice necropsy records in the current scope of J-SHARE, a labour-intensive conversion method would prove a significant obstacle to sharing the data.

Digitisation, Conversion and Translation

One significant advantage in the task of sharing the necropsy data is the availability of histopathology data for each mouse. For all mice, a standard set of tissues/organs as well as any additional lesions/tumors which are discovered at necropsy are fixed in formalin before long-term storage as paraffin-embedded blocks. Normaly, histological sections are made from the following organs; pituitary, femur, sternum, cerebrum, harderian gland, thyroid, adrenal, eye, testis, liver, lung, kidney, heart, spleen, pancreas, small intestine, large intestine, stomach, submandibular gland, mesenteric lymph node and tumors, if present. The organs are dissected at a representative position. The remaining tissue pieces of the organ are stored to wax blocks. By the experiment to

analyse a specific tumor, for example, pulmonary tumor, the whole organ is divided into some peace, and histological sections covering the whole organ are made.

Each block is routinely sectioned and stained with haematoxylin and eosin (HE) and the slides stored as a reference. Through the use of a high-throughput automated slide digitisation platform, we have been able to link the digitised necropsy information, the scanned necroopsy record and the histology images together using a barcoding strategy to use the unique necropsy record number across all relevant data. Since the high-resolution histology images allow a user of J-SHARE to inspect each tissue of interest and validate the status of each tissue/mouse according to their own criteria, this removes the need for users to rely solely on a translation of gross necropsy findings. Rather, users can identify a shortlist of animals with suspected lung pathology (whether that be based on an observation of discolouration, unusual size or shape, a mass of unknown significance or a frank tumor and apply their own analysis approach (for example, enumerating the number of animals with lung adenocarcinoma from their own inspection of the tissues). Classifying each necropsy record according to relevant tissues/organs with suspected pathology greatly increases the speed with which useful information can be shared. In addition, organ weights can provide a universal screening method to identify suspected cases of pathology, such as in the case of thymic or splenic lymphoma. Where available, digital photographs associated with a particular mouse will also be accessible from the digital record.

Over time, the digitisation efforts will allow the precise findings for each mouse to be coded (first from the freeform notes into systematic Japanese, which will automatically be translated into the systematic English terminology), but in the meantime, data can be made available much more rapidly. Eventually, J-SHARE digital records will contain both the initial necropsy data as well as our own pathology diagnosis and analysis.

To further aid information sharing, the pathology analysis will be coded directly using the international MPATH nomenclature (Schofield, 2013). In addition, experiments may already contain additional data, such as immunohistochemistry staining or molecular analyses which can be linked into the records.

Unlike purely historical archives, the mice in J-SHARE can also have frozen tissues, RNA extracts, protein lysates, chromosome preparations and other samples available depending on the experiment and pathology identified at necropsy. State of preservation and the use number of times of the freeze sample will be input in the “note” box of freeze sample in future. These samples can be shared on a collaborative basis with interested parties. Data searching will allow users to select experimental parameters (such as X-ray doses above 2 Gy given at 1 week of age) and then refine the results by the outcome (e.g. to mice with lung adenocarcinoma) and finally by sample availability (those with frozen tumor and adjacent normal tissues). The results of such searches and all associated data can be exported for further analysis outside of J-SHARE, and the search results list will provide a direct link to each individual mouse. The data which are now being made available through J-SHARE have been used in a number of studies to date, reflecting the transition from retrospective entry of completed experiments into the archives towards real-time integration of our animal experiments and the recording of data directly into the J-SHARE platform. (Shang 2017, Yamada 2017, Showler 2017, Takabatake 2016, Tsuruoka 2016, Imaoka 2016, Sunaoshi 2015, Blyth 2015, Morioka 2018).

A web address of J-SHARE is http://133.63.23.41/J-SHARE/login.aspx . User submits the user profile (name, e-mail address, affiliation) and the login information (user ID, password) in the registration page, then the ID is activated after examination. When user access demonstration data, the user input "guest" into User ID and "guest" into

Password in login page. In the page of Data Search, the user input "S11273" into a box of Sample number and click "search", then demonstration data of the mouse is appeared.

The J-SHARE is a data sharing platform. In the use of data (including the transfer of a link, presentation of the results of the study, publish of a paper, and analysis of the samples), the conclusion of the collaborative research agreement with QST-NIRS is required.

Further Scope of J-SHARE

In addition to radiation experiments, J-SHARE will eventually provide access to experiments with interactions with chemical carcinogens, dietary interventions and other more complex experimental designs. The web-based platform of J-SHARE has been designed with flexibility and expansion in mind, allowing future experiments to be integrated with existing historical and contemporary data. Efforts are underway to enable the data in J-SHARE to be linked with similar data in ex isting sister-archives in the US and Europe (NURA; the northwestern University Radiation Tissue Archives, STORE; the EC Euratom Program) (Figure 1). From 2018, new necropsy and pathology data from the department will be entered directly into J-SHARE, with the archive serving as a primary information record. New mice will be directly issued with a digital identifier and all paper-based records will be imprinted with barcodes assisting data entry. New conventions for image analysis will be adopted that preserve the consistency of analysis with existing data while simplifying the digitisation process into the future.

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References:

Abbott A. 2012. Radiation risks: Raiders of the lost archive. Nature 485: 162–163.

Blyth BJ, Kakinuma S, Sunaoshi M, Amasaki Y, Hirano-Sakairi S, Ogawa K, Shirakami A, Shang Y, Tsuruoka C, Nishimura M, Shimada Y. 2015. Genetic analysis of T cell lymphomas in Carbon ion-irradiated mice reveals frequent interstitial chromosome deletions: Implications for second cancer induction in normal tissues during Carbon ion radiotherapy. PLoS One, 10(6): e0130666.

Haley BM, Paunesku T, Grdina DJ, Woloschak GE. 2015. The increase in animal mortality risk following exposure to sparsely ionizing radiation is not linear quadratic with dose. PLoS ONE 10(12): e0140989.

Imaoka T, Nishimura M, Daino K, Morioka T, Nishimura Y, Uemura H, Akimoto K, Furukawa Y, Fukushi M, Wakabayashi K, Mutoh M, Shimada Y. 2016. A rat model to study the effects of diet-induced obesity on radiation-induced mammary carcinogenesis. Radiat Res, 185(5): 505-515.

Morioka T, Miyoshi-Imamura T, Blyth BJ, Kaminishi M, Kokubo T, Nishimura M, Kito S, Tokairin Y, Tani S, Murakami-Murofushi K, Yoshimi N, Shimada Y, Kakinuma S. 2015. Ionizing radiation, inflammation, and their interactions in colon carcinogenesis in Mlh1-deficient mice. Cancer Sci, 106(3): 217-226.

Sazykina TG, Kryshev AI. 2016. Lower thresholds for lifetime health effects in mammals from high-LET radiation – Comparison with chronic low-LET radiation. J Environ Radioactivity, 165: 227-242.

Schofield PN, Sundberg JP, Sundberg BA, McKerlie C, Gkoutos G. 2013. The mouse pathology ontology, MPATH; structure and applications. J Biomed Semantics 4: 18.

Shang Y, Sawa Y, Blyth BJ, Tsuruoka C, Nogawa H, Shimada Y, Kakinuma S. 2017. Radiation exposure enhances hepatocyte proliferation in neonatal mice but not in adult mice. Radiat Res. 188(2): 235-241.

Showler K, Nishimura M, Daino K, Imaoka T, Nishimura Y, Morioka T, Blyth BJ, Kokubo T, Takabatake M, Fukuda M, Moriyama H, Kakinuma S, Fukushi M, Shimada Y. 2017. Analysis of genes involved in the PI3K/Akt pathway in radiation- and MNU-induced rat mammary carcinomas. J Radiat Res, 58(2): 183-194.

Sunaoshi M, Amasaki Y, Hirano-Sakairi S, Blyth BJ, Morioka T, Kaminishi M, Shang Y, Nishimura M, Shimada Y, Tachibana A, Kakinuma S. 2015. The effect of age at exposure on the inactivating mechanisms and relative contributions of key tumor suppressor genes in radiation-induced mouse T-cell lymphomas. Mutat Res, 779: 58-67.

Takabatake M, Blyth BJ, Daino K, Imaoka T, Nishimura M, Fukushi M, Shimada Y. 2016. DNA methylation patterns in rat mammary carcinomas induced by pre- and post-pubertal irradiation. PLoS One, 11(10): e0164194.

Tapio S, Atkinson MJ. 2008. Molecular information obtained from radiobiological tissue archives: achievements of the past and visions of the future. Radiat Environ Biophys, 47: 183-187.

Tapio S, Hornhardt S, Gomolka M, Leszczynski D, Posch A, Thalhammer S, Atkinson MJ. 2010. Use of proteomics in radiobiological research: current state of the art. Radiat Environ Biophys 49: 1-4.

Tran V, Little MP. 2017. Dose and dose rate extrapolation factors for malignant and non-malignant health endpoints after exposure to gamma and neutron radiation. Radiat Environ Biophys. 56(4): 299-328.

Tsuruoka C, Blyth BJ, Morioka T, Kaminishi M, Shinagawa M, Shimada Y, Kakinuma S. 2016. Sensitive detection of radiation-induced medulloblastomas after acute or protracted gamma ray exposures in Ptch1 heterozygous mice using a radiation-specific molecular signature. Radiat Res, 186(4): 407-414.

Wang Q, Paunesku T, Woloschak G. 2010. Tissue and data archives from irradiation experiments conducted at Argonne National Laboratory over a period of four decades. Radiat Environ Biophys. 49(3): 317-324.

Yamada Y, Iwata K, Blyth BJ, Doi K, Morioka T, Daino K, Nishimura M, Kakinuma S, Shimada Y. 2017. Effect of age at exposure on the incidence of lung and mammary cancer after thoracic X-ray irradiation in Wistar rats. Radiat Res, 187(2): 210-220.

Zander A, Paunesku T, Woloschak G. 2019. Radiation databases and archives – examples and comparisons. Intl J Rad Biol, DOI: 10.1080/09553002.2019.1572249

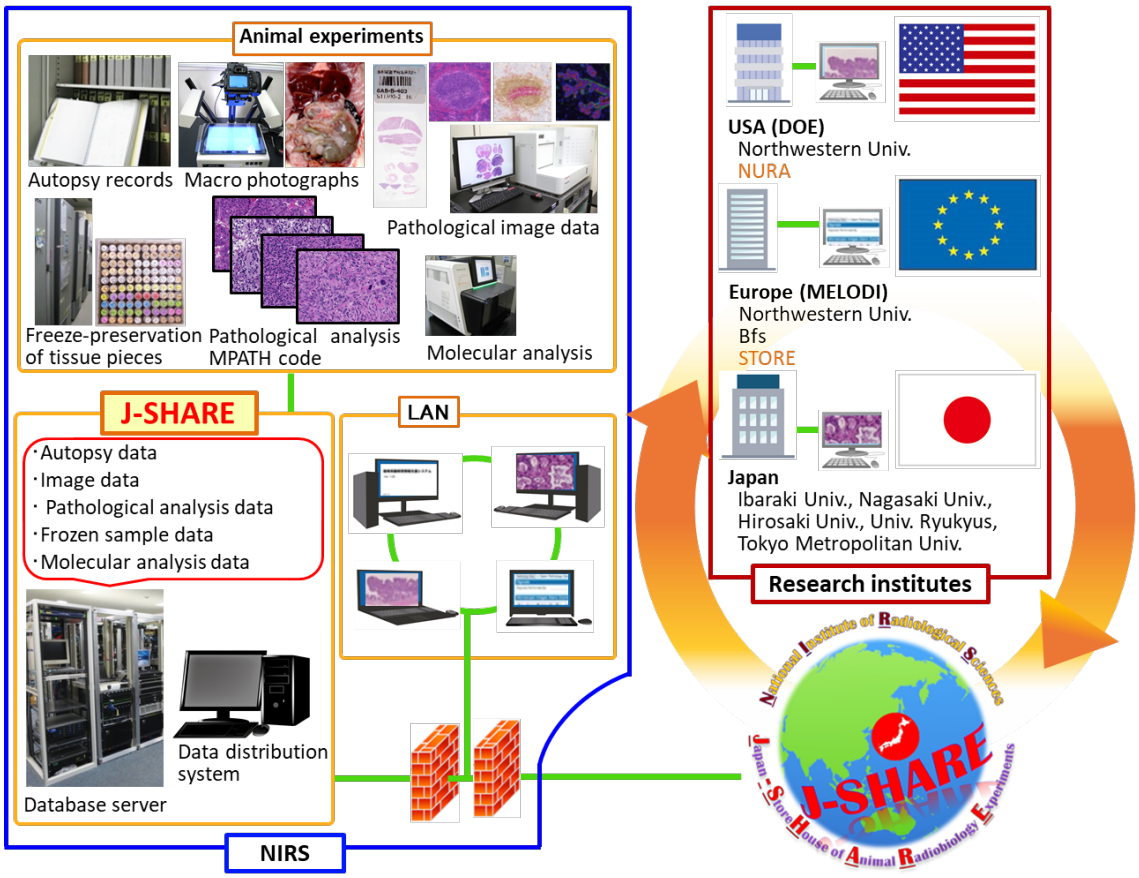

Figure 1. Overview of J-SHARE and collaboration with other archives.