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Banking Brain Tumor Specimens Using a University Core Facility

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Within the past three decades, the significance of banking human cancer tissue for the advancement of cancer research has grown exponentially. The purpose of this article is to detail our experience in collecting brain tumor specimens in collaboration with the University of Miami/Sylvester Tissue Bank Core Facility (UM-TBCF), to ensure the availability of high-quality samples of central nervous system tumor tissue for research. Successful tissue collection begins with obtaining informed consent from patients following institutional IRB and federal HIPAA guidelines, and it needs a well-trained professional staff and continued maintenance of high ethical standards and record keeping. Since starting in 2011, we have successfully banked 225 brain tumor specimens for research. Thus far, the most common tumor histology identified among those specimens has been glioblastoma (22.1%), followed by meningioma (18.1%). The majority of patients were White, non-Hispanics accounting for 45.1% of the patient population; Hispanic/Latinos accounted for 23%, and Black/African Americans accounted for 14%, which represent the particular population of the State of Florida according to the 2010 census data. The most common tumors found in each subgroup were as follows: Black/African American, glioblastoma and meningioma; Hispanic, metastasis and glioblastoma; White, glioblastoma and meningioma. The UM-TBCF is a valuable repository, offering high-quality tumor samples from a unique patient population.

Introduction

The banking of human cancer tissue has grown tremendously over the past 3 decades. Banking operations have existed for over half a century, as the United States Navy established the first bank of human specimens for transplantation in 1949.[1] However, it was not until 1989 that many researchers became aware of the Cooperative Human Tissue Network, an early effort of the National Cancer Institute (NCI) to make human tumor tissue available to cancer researchers nationwide.[2] As cancer research modernized over the following decades and studies increasingly focused on the genetic mechanisms of cancer, the importance of high-quality tumor tissue for research continued to grow.

Today, the advent of personalized medicine and tailored treatment protocols has turned knowledge of the individual mechanisms of cancer formation and growth into potential treatment strategies. Research into human disease has historically relied heavily on animal research, making applicable results and quality specimens challenging to acquire. But as knowledge of human pathologies and disease mechanisms has grown, discoveries have increasingly relied on properly preserved human tissue samples as a source of information for investigations of gene expression, proteomics, and intracellular pathways. Advances in technology are requiring large numbers of samples that are needed for high-throughput assays used in drug screening.[3]

The demand for biospecimens has increased four-fold over the past 20 years and is predicted to continue to increase over the next 10 years.[4] Nevertheless, the collection of samples may be a fragmented, disjointed process, even within one institution. The lack of standardization leads to variations in collection, processing, and storage, which undoubtedly affect the quality and reliability of any research done using such samples.[5] The establishment of a tissue bank addresses these concerns, aiming to provide an adequate supply of high-quality samples to researchers.

During the past 4 decades, many tissue banks have been established with the aim of supplying high quality, sterile tissues to hospitals, other medical institutions, and research centers, for their use in medical treatment and research.[6] Despite the growing number of tissue banks now operating worldwide, only a limited number can procure and process all types of tissues with the highest possible quality.[6] Best practice guidelines have been formulated by the National Institutes of Health (NIH) and by ISBER (International Society for Biological and Environmental Repositories) in order to standardize the processes associated with tissue banking, although they provide considerable opportunity for individual centers to adapt these guidelines to the running of their own tissue bank.[7,8]

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The purpose of this article is to detail our experience in collecting brain tumor specimens in collaboration with the University of Miami/Sylvester Tissue Bank Core Facility (UM-TBCF), to ensure the availability of high-quality samples of central nervous system tumor tissue for research. Although our standard operating procedures for biospecimen procurement and processing developed at the University of Miami may not be directly applicable to other institutions, we offer a generalized framework to facilitate the development of institution-specific protocols.

Materials and Methods

Infrastructure and personnel

The UM-TBCF is comprised of 3.5 full-time employees (FTE): 1 manager, 2 pathology assistants, and 1/2 programmer (shared with another core facility). Funding for the UM-TBCF is provided by the Sylvester Comprehensive Cancer Center. Although a small fee is charged to investigators using this service, it is not sufficient to cover expenses and external funding is strongly recommended (such as federal administrative grants).

Ethics and informed consent

Under an Institutional Review Board (IRB)-approved protocol, patients with brain tumors are approached by a pathology assistant, an attending neurosurgeon, or a clinical research coordinator at their initial visit to the University of Miami Miller School of Medicine, and asked to give consent to the collection of blood and residual tissue samples in the case of a surgical resection. The collection site is dependent on the location of the surgery and may occur at either the University of Miami Hospital or at Jackson Memorial Hospital. The consent form is available in English, Spanish, and Creole to accommodate the population demographics so that patients may read and understand the form in their native language. Interpreters are available in over 150 languages to assist via telephone on a 24-hour, 7-day-per-week basis.

Patients who agree to participate must sign an informed consent form. A copy of the consent form is given to the patient and another copy is attached to the patient's chart. It is preferable that all patients sign their informed consent pre-operatively, but in cases where this is not possible, post-operative consent is acquired. If post-operative consent cannot be obtained within 14 days of the surgical resection, the collected samples are discarded. The original, signed informed consent is kept at the Core Facility where it is processed, coded, and filed. The administrative unit of the Core Facility is locked at all times with a card-reader door lock, which allows access only to authorized personnel.

Patients are informed that their consent authorizes the collection of tissue, blood, and/or other bodily fluids, which are part of standard clinical practice, and not necessary for clinical diagnosis. Moreover, patients are further instructed that the collection of these tissue(s) will not interfere with the surgical process and that any tissue collected is secondary to the management and treatment of their disease. It is also made clear that participation is not obligatory, that they may opt out at any time, that there is no cost to the patient, and that they will not receive any compensation for their participation. The consent form does not discuss what patient samples will be used for specifically, but it does indicate that samples may be used for IRB-approved, future/current research projects. The informed consent also discusses storage of samples, as well as potential risks, likely benefits, and management of confidentiality.

Specimen collection and management

A standard workflow has been implemented for the collection of surgical tissue in order to optimize specimen integrity, as shown in Figure 1. Scheduling of tissue collection

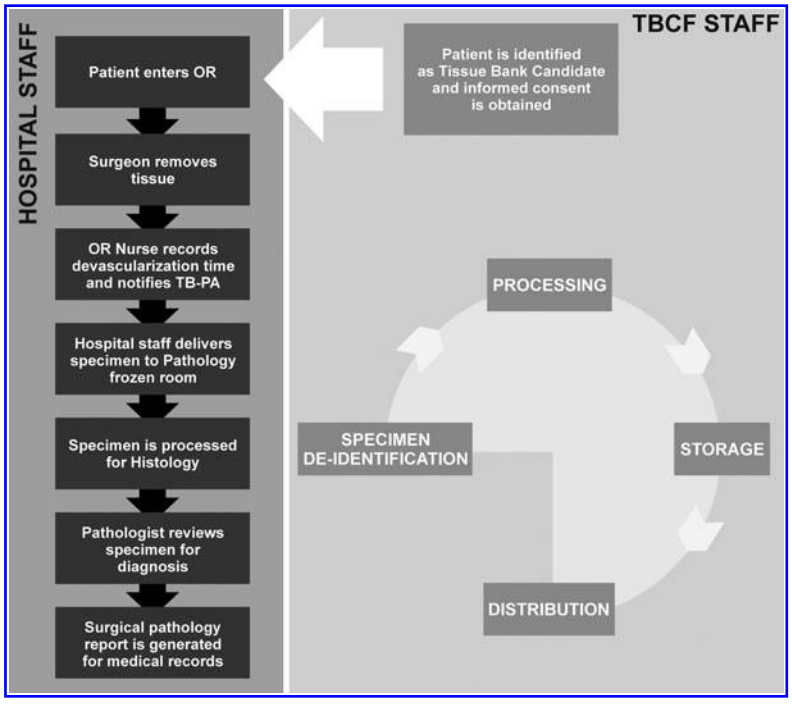

FIG. 1. Biospecimen collection workflow. TBCF, Tissue Bank Core Facility; TB-PA, Tissue Bank Pathology Assistant.

occurs the day before surgery after careful review of the surgical schedule. On the morning of surgery, Tissue Bank Pathology Assistants (TB-PAs) notify the operating room staff of the intent to collect tissue, as well as verify the patient information to ensure the collection of the correct sample.

Blood samples are collected before the incision is made and stored with all tissue specimens. Blood is drawn in conjunction with other routinely scheduled diagnostic tests whenever possible so that an additional venipuncture is not needed.[5] The time of the collection is noted on the Tissue Bank Candidate Form, a running log of the tissue collection information specific to each patient. One drop of blood is applied to a Whatman Fast Technology for Analysis (FTA) card, which lyses the blood cells and preserves the released DNA for potential later use. This serves as a secondary record of the sample, in the event that the initial sample needs to be verified at a later time. Blood samples are held at 4°C until further processing, typically for less than an hour, while the FTA card remains at room temperature.

The vials of blood are spun down for 10 min at $1,500 \times g$ to obtain the plasma and buffy coat. The buffy coat is mostly platelets and white blood cells, whereas the plasma contains all dissolved proteins, electrolytes, clotting factors, and hormones. The plasma and buffy coat are then carefully aliquoted into sterile cryovials, and stored frozen until the end of the day. The plasma and the buffy coat pellet aliquots are stored at −80°C for long-term storage. All patient samples are labeled with a barcode number, assuring patient confidentiality. The same barcode number is used to label all samples from the same patient, and serves as the record number within the database system.

Upon surgical resection, the time of devascularization is recorded by the OR nurse, and the surgeon immediately places the specimen in a sterile cup. The time between tissue devascularization and freezing or fixation of retrieved tissue is about 30 min and should not exceed 60 min. If the elapsed time is greater than 60 min, the specimen can be banked, but this must be clearly documented as the quality of the sample might be impaired. Tissue needed for diagnosis by a pathologist has priority, and only if there is any remaining tissue does the operating room nurse notify the TB-PA that the tumor sample is ready for collection. Samples are transported immediately to the frozen section area, where they are weighed and prepared for sectioning. The times of TB-PA notification and beginning of tissue processing in the pathology suite are noted.

All samples to be banked have a frozen section taken for later examination by a clinical pathologist who will assess the percent tumor nuclei and percent necrosis of the banked specimen. Samples are processed by (a) freezing in Optimal Cutting Temperature compound (OCT), (b) snap freezing (if requested), or (c) fixing in formalin (for after-hour cases). The process should follow this order unless otherwise specified by the investigator. Multiple specimens are banked whenever possible.

In order to avoid cross-contamination of specimens, a clean benchtop is used with clean forceps, gloves, and scalpel blades. Each sample is frozen in OCT and a 4.5 μm section is cut to generate a frozen tissue section, which is examined by the TB-PA under a microscope. Each slide is labeled with the patient's tissue bank barcode number. The tissue samples frozen in OCT are collected individually in small nylon biopsy bags, and stored in a small vial labeled with the patient's tissue bank barcode number. The vial is frozen at −80°C until the end of the day, and the time is recorded. All samples must be processed within 60 min of surgical excision in order to preserve their integrity. To ensure high quality is maintained, the UM-TBCF randomly tests a small subset of samples annually to assess DNA and RNA quality.

Throughout the processing of the tissue sample, the TB-PA records the required information on a Tissue Tracking Notification Form. The amount of time between surgical resection and freezing, weight of the sample, patient tissue bank barcode number, and relevant notes about the specific case are recorded. This form accompanies the tissue sample to the main Tissue Bank Laboratory.

At the end of each day, the frozen blood and tissue samples and accompanying pathology slides must be transported from the surgical area to the Tissue Bank Core Facility Laboratory. Samples are submerged in liquid nitrogen and carried via a transfer vessel to the Tissue Bank Laboratory. The paperwork corresponding to each sample is logged, along with the day's surgical schedule. The tissue bank barcode number of each sample is verified to match that on the Tissue Tracking Notification Form. The tissue slides from each sample are kept and later reviewed by a clinical pathologist. Slide review includes confirmation of the diagnosis and assessment of quality by quantification of necrotic tissue. The information is then recorded in the database, along with the data obtained from the original review of the clinical specimen immediately following surgery.

Adjudication of banked specimens

All requests submitted to the UM-TBCF must be reviewed and approved by the Tissue Adjudication Committee (TAC) before tissues can be used for research. The TAC consists of clinicians and scientists selected to serve as reviewers for all TBCF tissue requests at the University of Miami and the Sylvester Comprehensive Cancer Center. Membership is comprised of experts in multiple areas including, but not limited to, basic science, biostatistics, hematology-oncology, pathology, imaging, radiation oncology, and surgery. Wherever possible, a member of the appropriate Site Disease Group is included. The TAC aims to provide academic review of the validity and design of the research proposed for use of banked tissue. The committee works to ensure that all research using fresh, frozen human tissue is meeting the highest standards and is an appropriate use of these invaluable resources.

Long-term storage of specimens

Collected specimens enter long-term storage at the Tissue Bank Core Facility Laboratory. Incoming samples are added to the freezers at the end of each day and stored at −80°C. Each group of samples from the same patient is labeled with the same unique barcode number, which matches the sample with the specific patient to whom it corresponds. Samples are then available for use by researchers from this site. All freezers are monitored by an alarm system and plugged into emergency power outlets. Freezers are hard-wired and linked directly to the security department. When the alarm is triggered, a security officer is dispatched and he/she must visually inspect the freezer. If the freezer requires immediate assistance, TBCF personnel are contacted. In addition, the freezer temperature is recorded daily in a freezer log.

Collection of clinical patient information

Multiple safeguards have been incorporated into the institutional tumor bank infrastructure to maintain patient confidentiality and ensure compliance with Health Insurance Portability and Accountability Act (HIPAA) guidelines. Our institution maintains a secure, intranet-based database that records details of all specimens. The UM-TBCF uses the caTissue software for recording and tracking all events related to specimen use in research. The caTissue software, developed with financial support from the NCI and the NIH, is an open-source platform that allows tissue bank administrators to record tissue sample requests, organize tissue sample shipments, and track samples once they leave the tissue bank.

In this way, samples are closely followed and maintained in the proper form. Up to 50 data points are collected for each sample, including demographic data, such as name, age, and ethnicity; surgical information, such as diagnosis and specific sample collection details; consent information, including date(s) and languages used; and specimen data, including pathological status and number of samples available.

Facilities management and technology

The Tissue Bank Core Facility is comprised of two rooms of approximately 450 square feet. Equipment includes: (1) VWR −80°C upright freezer; (1) Kenmore 4°C refrigerator; (1) Brady Ip Printer-300dpi and barcode scanner; (1) NUAIRE Tissue culture hood; (4) computers; (4) standard light microscopes. Software for specimen tracking is CaTissue Suite version 1.1p5.

Immunohistochemistry

Standard immunohistochemical procedures were performed by the Department of Pathology at the University of Miami. Immunohistochemistry for the cellular markers (see Results section) was performed using formalin-fixed, paraffin embedded tissue using a standard labeled streptavidin biotin (LSAB) method.

Results

From July 2011 through September 2013, 225 brain tissue specimens were collected from 225 adult patients operated on by a single attending neurosurgeon (Fig. 2). The rate at which samples have been collected has increased over the duration of the tissue bank; 56 samples were added in the first 12 months, and 113 samples were added in the 12 months following. The current collection year is in progress, though the trend suggests that the rate at which samples are added should increase relative to the previous year. Figure 2 shows the amount of tissue accumulated by month for each year, and Figure 3 illustrates the number of samples accumulated during three consecutive years (2011 to 2013).

Figure 4 shows the distribution of specimens by histological identification. Glioblastoma multiforme (GBM), the most common tumor histology, was identified in 50 out of 225 (22.1%) patients. Meningioma was found in 41 patients (18.1%); 36 specimens (87.8%) were Grade I, and 5 (12.2%) were Grade II. Forty specimens (17.7%) were identified as metastatic adenocarcinoma, mainly of breast, lung, and renal origin. Twenty-two specimens (9.7%) were identified with anaplastic astrocytomas and 17 (7.5%) had low-grade gliomas. Notably, 24 specimens (10.6%) were pituitary adenomas, 19 specimens (8.5%) were reactive gliosis or necrosis, and 13 specimens (5.8%) were benign vascular lesions, cysts, or abscesses. Additionally, our data show that each tumor type collected has represented a similar percentage of samples collected year to year.

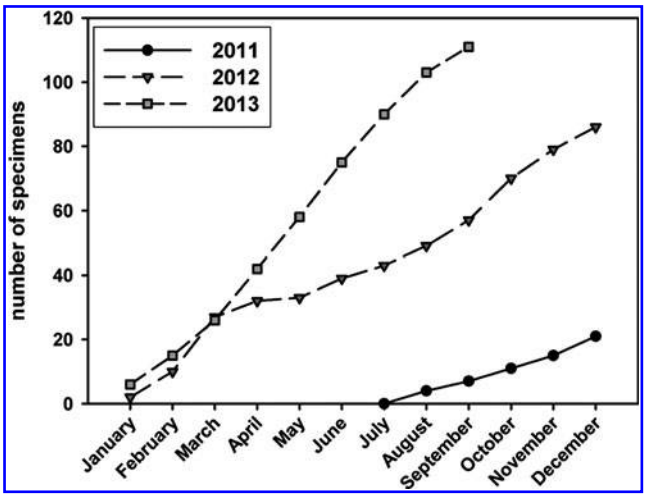

FIG. 2. Cumulative specimen accrual, July 2011–September 2013.

Demographics

Of the 225 patients in this study, 51.6% ($n=116$) were males and 48.4% ($n=109$) were females. Figure 5 displays the ethnicity of each of the patients. The majority of patients (55%) were White, non-Hispanics accounting for 45.1% of the patient population; Hispanic/Latinos accounted for 23%, and Black/African Americans accounted for 14%. The most common tumors found in each subgroup were as follows: Black/African American, glioblastoma and meningioma; Hispanic, metastasis and glioblastoma; White, glioblastoma and meningioma.

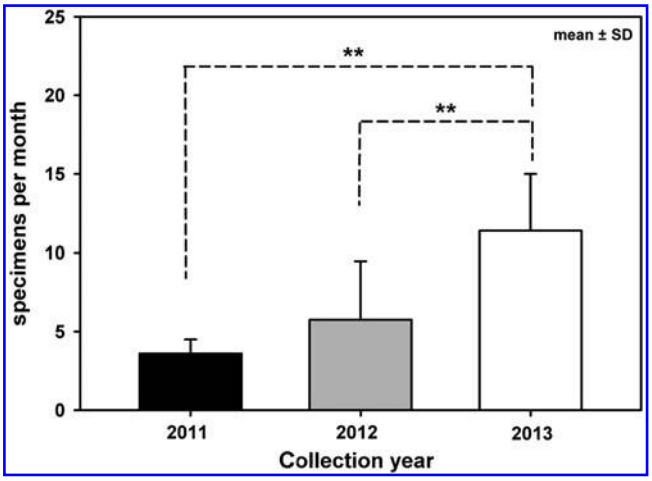

FIG. 3. Monthly tissue acquisition, 2011–2013.

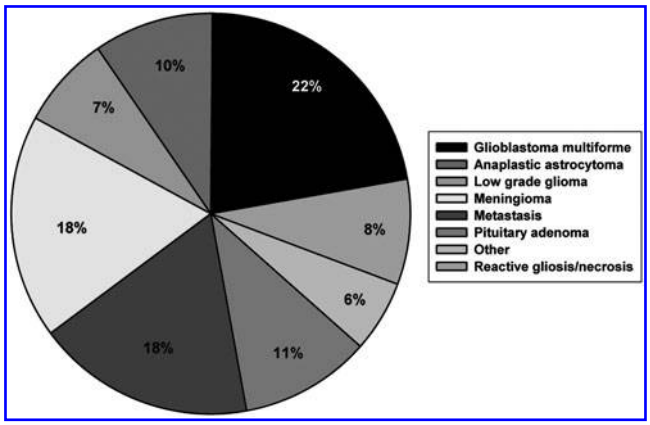

FIG. 4. Tissue pathology distribution. **Statistically significant.

Immunohistochemistry

Immunohistochemistry is an important tool in the diagnosis, classification, and assessment of growth potential of brain tumors by identifying cellular markers of phenotype nuclear markers of proliferation. The following markers were used to test certain specimens:

- **Ki67**: a nuclear protein associated with cellular proliferation and rRNA transcription. It is an excellent marker to determine the growth fraction of a given cell population. It was tested in 68 specimens of various pathologies including GBMs, anaplastic and low-grade astrocytomas, and meningiomas. Fifty-nine specimens (86.8%) were positive and 9 (13.2%) were negative. In our subset, positivity of Ki67 was similar for low- and high-grade gliomas; nearly all (19 out of 20) high-grade gliomas and all (10 out of 10) low-grade gliomas were positive for Ki67.
- **p53**: a tumor suppressor protein encoded from the TP53 gene that is essential in cell cycle regulation. p53 was tested in 32 specimens; 26 (81.2%) were positive and 6 (18.8%) were negative. In our subset, positivity of p53 was similar for low- and high-grade gliomas. The majority (12 out of 16) of high-grade gliomas and nearly all (9 out of 10) low-grade gliomas were positive for p53.
- **EMA**: the Epithelial Membrane Antigen can be detected in non-neoplastic epithelia and it serves as a highly effective marker establishing the epithelial nature of neoplastic cells. It was positive in all 15 specimens tested. Fourteen specimens were Grade I meningiomas and one was Grade II meningioma.
- **GFAP**: Glial Fibrillary Acidic Protein is an intermediate filament protein that is expressed by numerous cell types of the CNS. Among 34 specimens, it was positive in 30 (88.2%) and negative in 4 (11.8%). Out of 30 positive specimens, 8 (23.5%) were anaplastic astrocytomas, 10 (29.4%) GBMs, 9 (26.5%) low-grade gliomas, and 3 (8.8%) others. The 4 GFAP-negative specimens included 1 meningioma, 2 pituitary adenomas, and 1 metastatic melanoma.
- **Progesterone:** receptor was tested in thirty-four (83%) out of 41 meningiomas; twenty-four (70.6%) were stained positive, twenty-two were Grade I, and two were Grade II meningiomas.

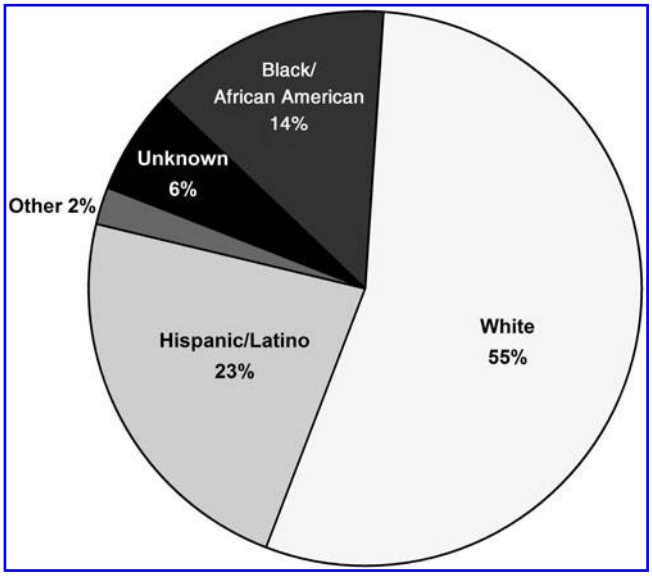

FIG. 5. Race and ethnicity of patients.

Discussion

To our knowledge, this is the first published report of a US-based brain tumor tissue collection that integrates patient and clinical data in a confidential format specific for use in research. Unlike in China and France where there were national incentives to set up brain tumor banks, tissue banking in the United States is still largely a task handled by university centers.[9,10] The largest impediment to the establishment of brain or other tissue repositories (e.g., those at the University of Miami, the University of California at San Francisco, Duke University or University of Texas) are the number of ethical and legal guidelines that must be followed in order to protect research participants and promote ethically responsible research. Key issues include proper informed consent, the paucity of samples that are available for collection, the timing of tissue collection, and the maintenance and protection of private medical information.[11] The fast pace of research makes availability of high-quality samples of the utmost importance, exposing the challenges associated with maintaining a tissue bank.

Successful tissue collection not only requires a well-trained and professional staff, but also collaboration by all team members and the ability to adapt to any unforeseen obstacles in timing, patient location, and/or patient care. Moreover, staff must be vigilant about maintaining the highest standards of ethics and record keeping. It is critical that patients are not unduly exposed to any liability on account of their participation, and researchers must be able to rely on the accuracy of the information in the tissue bank. In this article, we outline our best practices in approaching the challenges of brain tissue banking, as well as the organization and maintenance of a comprehensive database of clinical information.

The Sylvester tissue bank at the University of Miami was initially established to collect samples of other cancers; breast cancer samples were the first to be collected in 2006, followed by expansion of the tissue types in 2009 to include all cancers. The first brain tumor samples were collected in 2011. In all cases, the most recent set of guidelines set forth

by the National Institutes of Health (NIH) and the Best Practices for Biorepositories from ISBER for collection and storage of human tissue were followed where appropriate. Subsequently, we developed our own, institution-specific protocols and refined them as necessary to ensure the proper collection and handling of samples at our institution.[7,8]

Establishing a tissue bank has multiple benefits. It allows easy access to high-quality samples for current and future research projects; accelerates the pace of research; saves time and resources in the collection of samples; and facilitates translational research. A constant supply of high-quality tissue samples allows the various unique cellular signaling pathways that lead to the manifestation of disease to be identified and investigated.[12] The overall pace of research stands to benefit as well, as sharing between institutions allows labs to acquire tissue samples beyond the capability of their own institution. Furthermore, instead of devoting time and effort to the collection of samples for each individual research project, tissue banks allow research laboratories to focus on scientific experiments, rather than administrative and logistical duties.

The failure to control glioblastoma progression is a major challenge for neuro-oncologists and neurosurgeons. Emerging data indicate that genetic and epigenetic changes within cancerous cells play a dominant role in the development of resistant disease and eventually tumor recurrence.[13] Therefore, effective therapy most likely requires the personalized medicine approach using targeted drugs. The goal is to take advantage of the specificity offered by targeted drugs to block proliferation of tumor cells harboring driver mutations. Targeted therapies and molecular profiling comprise the concept of individualized care, which is being put into practice for the treatment of cancer. In this effort, integrated research and clinical trials aim at implementing personalized cancer therapy and improving patient outcomes.

A number of events have converged to create the opportunity to make personalizing cancer care possible. Personalized cancer therapy includes all aspects of individualized patient management driven by the characterization of tumor and its microenvironment; and host characteristics including diagnosis, surgery, chemotherapy, targeted therapy, radiation therapy, and immunological manipulation, either alone or in concert.

The benefits of tissue banking apply especially to cancers and disorders of the central nervous system. The presentation of these diseases can vary widely, and the differences between them can be as important as their similarities. The heterogeneity of samples within a tissue bank allows such differences to be carefully studied. Additionally, malignant nervous system tumors are often associated with poor outcomes, and although prognoses are improving over time, patients stand to benefit immensely from an accelerated pace of research.[13,14]

Though the benefits of creating a tissue bank are evident, the collection of tissue has many associated ethical and legal issues that must be addressed before collection may proceed.[15] Guidelines for patient consent, confidentiality, conflicts of interest, and intended use of samples should be evaluated and approved by an IRB before wide implementation. The potential risks for commercialization of results and/or data, as well as the quality of research for which the samples will be used should also be considered by the IRB.

Once approved, it is critical that all participating patients be treated according to the guidelines. Patients must first sign an informed consent which details the collection process and anticipated use of the sample(s). Anonymity of patient data must be maintained. The use of a de-identifying number and barcode system is designed to keep a virtual wall between researchers and patients, maintaining the delicate balance between researcher needs and patient confidentiality.

The Sylvester tissue bank at the University of Miami operates at two different hospitals, which requires satisfying the rules and expectations of each. As such, it is important that education be an integral part of the mission of the tissue bank. Education and/or continuing education of employees, especially in a field with such high turnover, is essential in order for all staff to be up-to-date on the tissue collection process. The adoption of a joint training program for staff and medical personnel could also be an important activity to be promoted and supported by the institutional administration, ensuring that standards are maintained across both locations and all employees have the most current information.[6] Keeping patients informed of their role in the research generally leads to more satisfied patients.

Our collaborative effort to collect high-quality brain tumor specimens has its limitations. Primarily, all of the samples are obtained from one attending neurosurgeon, which limits the number and types of samples that can be collected, and why the specimen collection has been growing at a slow rate; adding other neurosurgeons to this program could increase the collection rate dramatically. It is also true that each individual sample may not represent the ideal sample for that particular disease, due to variations in presentation, collection, and/or processing.

Banking of a tissue sample that is nondiagnostic may have the unfortunate effect of slowing research, as time is taken to find samples that better fit the needs of the researcher. The UM-TBCF is one repository and while tissues could be shared amongst institutions, a global tissue bank encompassing multiple institutions (e.g., a statewide effort) would have the dual advantage of increasing the numbers of samples and the collection rates. Ultimately, all tissue specimens should be automatically collected (with proper consent) and become part of standard patient care. Tissue samples have a prospective and retrospective value that should be more thoroughly utilized to benefit both the individual, and brain cancer research in general.[16]

Conclusion

We envision the UM-TBCF continuing its growth in the future, and serving as a repository for high-quality tissue samples, accessible to research projects for our institution as well as for other institutions, both nationally and internationally. The formation of partnerships with other Universities and/or companies will facilitate research and promote scientific understanding, and hopefully foster novel therapies and treatments for patients.

We caution other institutions that, although developing a tissue bank is a laudable goal, it is only for those who have the resources for, and institutional dedication to, the process. The Sylvester Comprehensive Cancer Center supports all research core services, which includes tissue banking. This institutional commitment is necessary as the fees charged do

not cover the cost of the infrastructure or the personnel required to have a successful repository. In addition, the lack of uniformity in guidelines represents a challenge with biobanking today. A strong incentive to adopt all—or a subset—of guidelines by all research banking entities would improve biospecimen quality and facilitate the creation of larger networks.[17] We firmly believe that a carefully planned, efficient, and refined tissue bank is essential and would serve as the foundation for current and future biomedical research, and ultimately, better patient care.

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Author Disclosure Statement

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