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Current concepts in biobanking: development and implementation of a tissue repository

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TABLE OF CONTENTS

1. ABSTRACT

Biobank repositories are actively contributing to modern medical research. The availability of tissues to researchers has been hampered by the lack of adequately characterized high quality tissues, increasing concerns because of privacy issues and the inability to integrate the follow-up or clinical outcome with the data generated. A properly organized biobanking facility is a critical resource for molecular-based biomedical research. In this article, we provide an overview of the essential steps involved in procuring tissues and storing them. The need to maintain patient privacy and the logistics of sharing tissues with collaborators is discussed. With the advent of the era of personalized medicine, a repository of tissues holds immense promise to provide an improved understanding of the disease process, monitor response to therapy and identify novel drug targets.

2. INTRODUCTION

As we begin to better understand the molecular basis of disease, the doors to both predictive and personalized medicine are starting to open. The inception of "personalized medicine" based upon the molecular and genetic characteristics of a patient's tumor sample has dramatically changed the way we analyze and perceive a patient's diagnosis and treatment. The repository of tissues into a dedicated facility, such as a biobank, combined with data on the associated clinical outcomes is a key enabler to improve genetic analysis and screening. This holds great promise for the future of medicine, providing a way to significantly improve the effectiveness and efficiency of clinical trials through an improved understanding of the disease process. This pathway to understanding begins with the careful procurement and preservation of

diseased and/or cancerous tissues, with the biobanking facility as the centerpiece for making such a complex process successful.

The concept of preserving and storing pathologic tissues of all types for future use is not new (1). Throughout history, others have stored and preserved tissue for later observational studies or for research experiments. At present, the pathologist will normally render a final diagnosis based upon the tissue staining of the paraffin-embedded block. This provides adequate diagnostic information in most cases in regards to the accurate pathologic diagnosis, and is considered the gold standard for diagnosis. However, in many cases, there is a wealth of tissue samples that remain unutilized or even discarded, if there is not an active biobanking protocol in place. Thus, the critical components of a successful biobanking endeavor are based upon the procurement and preservation of quality human tissue samples followed by the integration of clinical correlation of the data obtained from subsequent molecular analysis. The quality of the tissues is important, as undue delay can greatly impact the isolation of high-quality nucleic acid. The clinical correlation is essential in order to incorporate the basic data obtained from the tissues into relevant information that may impact either the prognosis or treatment decisions for a patient. Having one of these components without the other, as often is the case currently, results in a much more difficult task in developing correlative studies that link the discoveries from the tissue with the clinical outcome of the patient.

The importance of biobanking is best exemplified in the field of cancer research. The mapping of the human genome has opened up the field of oncogenomics, a field of research that involves meticulous analysis of tumor tissues at the genetic level. Before we are able to fully realize the potential of oncogenomics and proteomics, we must first examine the difficulties in developing, establishing and integrating a fully functional biobank at a hospital, research laboratory or other such facility (2,3). This review focuses on the essential issues in the biobanking of human tumors, providing a resource from which to successfully develop and implement a successful biobanking program for application of the following:

- Discover the genetic basis of cancer
- Identify potential cancer genomic and proteomic biomarkers
- Identify predictors of disease outcome
- Provide trends in tumor-specific outcome and survival
- Identify those patients that will (or will not) respond to a particular cancer therapy
- Provide drug targets through the identification of critical genes involved in cancer
- Translate fundamental discoveries to improved cancer therapeutics

We have long recognized the importance of these broad and overlying concepts of cancer discovery, concomitant with the challenges that are involved with the direct application of such technology in our daily clinical practices. We will focus on several important issues of biobanking and how it relates to molecular characterization of the diseased tissue, primarily examining the role of each in cancer research.

3. BIOBANKING BEGINS WITH THE PATIENT

The discussion of whether a patient voluntarily wishes to contribute their tissues to a biobanking effort begins upon the first visit to the hospital. Previously, many facilities provided a “blanket consent” that the patient would sign as part of the initial registration and admission to the hospital. This “consent” often allowed the hospital to utilize tissues for whatever use they may have, research or otherwise. In general, there was little, if any, discussion with the patient in regards to what specifically was going to done with the tissue or further details that outlined and discussed the confidentiality and privacy of the information obtained.

However, the guidelines and policies encompassing the procurement of tissues from patients have dramatically changed over the last decade, resulting in sweeping changes in our approach to biobanking. In the United States, the Health Insurance Portability and Accountability Act of 1996 (HIPAA) has placed a greater demand on clinicians and researchers to insure that patients are fully informed and aware of their rights involved with tissue donation. Additionally, all patients must have a full understanding of the informed consent process with an absolute requirement by the principal investigator and hospital to insure the security and confidentiality of a patient’s public health information (PHI). Thus, it is now required by federal law that hospitals conform to all of the outlined policies and procedures attached to HIPAA and other federal guidelines for the appropriate collection and storage of human tissues. It should be stressed that the process of obtaining informed consent for tissue procurement is discussed prior to any procedures, surgical or otherwise, being performed. It is inappropriate and unethical to obtain informed consent “after the fact”, solely for the reason that the investigator simply forgot to discuss the tissue protocol with the patient at the original consultation.

Although each hospital and investigator will obtain informed consent in a slightly different setting and circumstance, it has been our experience and practice that the surgeon who is performing the operation is often the most appropriate representative to discuss the informed consent document with the patient. The surgeon, who may or may not be the principal investigator, should be intimately familiar with the tissue procurement protocol for their institution, as it is very likely that there are multiple versions, each involved with the obtainment of different tissues. Thus, a patient interested in donating their tissues for research purposes should have a thorough discussion with the principal investigator, co-investigator or appropriately trained healthcare professional in regards to the type of research being done with their tissues. It is inappropriate to have any other personnel obtain the informed consent, unless they are specifically trained for this purpose.

In general, the informed consent form (ICF) will contain slightly different versions of the HIPAA policy and procedures. However, all should contain the purpose of the research, the type of tissue that is requested, benefits to the participants, reasonably foreseeable risks and the fact that the patient has a choice in participating in this donation. The patient is instructed that, at all times, the privacy of the patient is ensured and that participation is entirely voluntary and will not affect the treatment that they receive. Furthermore, the ICF explains to the patient that all donated tissue will strictly adhere to the governmental requirements concerning the use of human tissues in research specified in Title 45, Part 46 of the Code of Federal regulations (45CFR46) "Protection of Human Subjects" that outlines conditions that must be met when using tissues from living persons for research (1).

4. TISSUE PROCUREMENT AT THE TIME OF SURGERY

Once the fully informed patient has signed the consent and is ready for surgery, it has been our practice to visit with the family and the patient in the pre-operative holding area, to again discuss the tissue procurement of tissues, confirming that all patients have no reservations or change of heart in regards to the tissue procurement. This may often be the first time that other family members are present, allowing for others to ask pertinent questions about tissue procurement. During the operative procedure, under no circumstance should the obtainment of tissue either change or compromise the procedure being performed. Tissue procurement begins once the specimen has been completely removed from the patient. Once removed, it is critical to involve the pathologist at this point. The normal sequence of events will first involve the surgeon handing off the entire specimen to the surgical nursing staff, who alerts a "runner", who is technically trained to collect (3-5), transfer and deliver such tissues to the pathology department.

Here, the pathologist should be standing by and awaiting the specimen, performing any gross or microscopic descriptions or tests deemed necessary for normal pathologic analysis, such as the assessment of surgical margins or intraoperative analysis of the tumor/tissue. The pathologist will determine if there is adequate tissue for routine diagnostic work as well as for biobanking. Often there may not be an adequate specimen for both, with the examination of the specimen for final pathological analysis having priority over tissues obtained for biobanking.

The specimen is usually macro-dissected at this point with a scalpel, obtaining samples from particular areas of the tumor/tissue and avoiding central areas of necrosis where cell viability may be very low. The major point being that whatever sample is being procured, it is essential that this is done in a very short time period in order to prevent undue nucleic acid degradation. The runner should be trained in the proper handling of biohazardous materials, as outlined in the Title 29 Code of Federal Regulations, Part 1910.1030 (29CFR1910.1030), additionally receiving annual re-training in safety programs offered by the institutional OSHA (2). All personnel should be encouraged to obtain hepatitis B vaccinations and treat all human specimens with universal precautions.

The need for rapid and efficient processing of the excised tissue cannot be over-emphasized. In order to preserve the original tissue properties and gene expression patterns, the tissue should first be placed in either cold saline or some type of complete media (RPMI-1640, DMEM) and then placed on ice (4^0C) immediately. Optimally, the tissue sample will be transferred to the Pathology department, analyzed, procured and snap-frozen in liquid nitrogen within 10 minutes (0.5 to 1 gram pieces). Other specimens, such as peripheral blood samples, saliva, urine and bone marrow, should also be processed in an expeditious manner. We have outlined a series of steps in tissue procurement and processing:

1. The tissue is transported to the pathology processing facility by the runner.
2. The pathologist is standing by, receives the tissue and processes the specimen per standard protocols in regards to margin analysis and tumor measurements
3. The pathologist and/surgeon [who may scrub out of the case and be present for this] will macro-dissect a portion of the tumor/tissue, avoiding necrotic areas of the specimen. As a priority, a portion of the sample is processed utilizing standard histologic preparative techniques with formalin fixation and paraffin-embedding.
4. A part of the tissue may be frozen in sections no thicker than 5 mm in order to ensure uniform freezing and placed into liquid nitrogen, subsequently wrapped in heavy duty aluminum foil and stored at -180 degrees Celsius.
5. Another portion of the procured specimen may be placed in a cryomold and embedded with a frozen-section embedding compound (e.g. O.C.T.).
6. A portion of the tumor sample may be placed into sterile culture media and transported to the laboratory for in vitro growth and expansion [if the tissue protocol allows for this].
7. Collected blood samples, bone marrow and other samples may be processed accordingly, with blood aliquoted into cryovials and stored at -800C.
8. The mononuclear leukocytes from blood and bone marrow may be separated using Ficoll-Hypaque density gradient method and frozen if the protocol permits.
9. Saliva and urine are aliquoted in cryovials and stored in a -80°C freezer.

5. INTEGRATIVE DATA TECHNOLOGIES: LINKING THE INFORMATION

Information pertaining to any and all patient PHI must be restricted to those only directly involved in the research. All collected data should be centrally localized with the appropriate security measures in place to insure strict confidentiality. Once the specimens have been transported to the tissue procurement laboratory and storage facility, the information is then manually entered in one of several secure databases, often capable of completely de-identifying all PHI associated with each patient. At minimum, the snap-frozen specimens within

each cryovial are weighed and appropriately labeled with all relevant data, which may simply be a number that corresponds to all other data within the centralized database. Such PHI that is not within a computerized system must be kept in a centralized location, locked in a cabinet if necessary.

A major weakness of tissue biobanking is the lack of efficient integration of the research data collected from each sample with the clinical characteristics and outcomes of the same patient. More often than not, the data obtained from laboratory experiments is not able to be directly linked to the patient, thereby little relevant clinical correlation can be performed. The majority of databases are separately kept, one for all the clinical data for each patient, such as a standard EMR (electronic medical record), and another for all of the data collected for research of the tissue sample. The two data sets are often utilized within several different computerized platforms, severely limiting the integration of these data sets. Efforts are being made to try to develop a single database where all of this information is available within a single platform. This is a very labor-intensive process as all data entry will ultimately require the full time commitment of at least one data entry specialist.

There are several large biobanking efforts that have greatly advanced the integration of data collected from research with human tissues and oncogenomics. A few institutions have begun to develop affiliate networks whereby each affiliate hospital will procure and preserve all tumor histologies, subsequently transporting all collected samples to a single, centralized "home" location for further analysis. This centralization of tumor specimens has the capacity to provide for the rapid collection of thousands of tumor samples that can subsequently be examined through various molecular techniques, such as gene microarray profiling, SNP analysis and proteomics. Such efforts are logistically complex and extremely cost prohibitive, however, the integration of such large sets of data will be critical in order to further delineate important molecular pathways, genes and potential targets for therapy in the future.

Worldwide, there are countries that are beginning to understand the importance of developing a nationwide biobanking effort in order to keep pace with technologic advances in biomedical research. Biobanking has come to the forefront due to its critical role in providing high quality preserved tissues for research. The United Kingdom, Japan, Estonia, Canada, Norway, Sweden and the United States all have national biobanking activities in place or proposed for the near future. There are also several private industry endeavors, originating with the founder of such endeavors, Decode Genetics in Reykjavik, Iceland, which has created a bank of genetic samples from 100,000 volunteers.

The European Commission has recently established a research infrastructure, the European Searchable Tumor Cell Line and Data Bank (ESTDAB), a consortium of seven European laboratories working together to contribute melanoma cell lines to a centralized location for further characterization. The ESTDAB is able to provide an accessible interactive database of a large collection of melanoma cell lines. Although these are daughter cell lines, they are for the most part derived from a melanoma patient where relevant clinical information is readily available. Furthermore, this collection of ~186 cell lines has been extensively characterized for HLA genotype and surface expression, oncogene and tumor antigen expression, cytokine secretion, surface molecule expression, adhesion to extracellular matrix components, cytokine gene polymorphisms and other factors of interest to immunologists . Such steps should be applauded as this is a start to the collaborative efforts that will be necessary in order to develop a searchable databank that is accessible to those contributors of the ESTDAB.

The United States has also begun to integrate and harmonize the efforts necessary to standardize, collect, archive and disseminate collected tissues through the National Cancer Institute's Office of Biorepositories and Biospecimen Research (6,7). Formally developed in October of 2005, this office has begun to develop guidelines and "best practices" over the last year, further supporting several biorepositories around the country (www.biobankcentral.org). Such efforts will take time to develop into its full potential; however, the field of biospecimen science has the greatest future potential for the discovery of potential prognostic biomarkers and novel therapeutic agents.

6. CURRENT OBSTACLES FOR SUCCESSFUL BIOBANKING EFFORTS

6.1. Quality and purity of the specimen

At the center of the biobanking efforts is the steadfast question as to the quality of the specimen procured. It is clear that a macrodissected specimen procured from a tumor mass will contain a markedly heterogenous sampling of tumor cells. It is nearly impossible to obtain a pure cell population of the same genotype/phenotype from a macrodissected specimen. Intrinsic in the tissue procurement process is the collection of cells that are heterogeneous by nature, although attempts have been made to "purify" such samples utilizing techniques such as laser capture microdissection or even manually isolating single cells microscopically by cell sorting. Even so, the particular cells isolated give no assurance that they too will not contain different set of genes and expression patterns.

Others hypothesize that taking a freshly procured tissue sample and placing it into culture will allow for a dominant cell clone to grow over other cells. In principle, this is feasible with some tumor cell types that are quite easy to grow in culture, such as melanoma for instance. However, other cell types are much more difficult to grow *in vitro*, often requiring the addition of specialized growth factors and other additives for cells to successfully grow and expand. One potential pitfall with growing and expanding daughter cell lines *in vitro* is that we have taken such tumor cells out of their natural microenvironment and placed them into a culture flask. This will result in a

completely different set of genes being expressed, such as heat shock proteins and other stress-related genes, in addition to several other genes that will ultimately have no reflection upon the true characteristics of tumor cell behavior within the *in vivo* tumor microenvironment (8-11).

Thus, while some data collected are completely unaffected by storage or handling conditions (e.g., genomic or SNP data), other data (e.g., mRNA or transient metabolite levels) are highly affected (12-14). Such artifactual gene expression as a result of *in vitro* culturing is a major obstacle in working with daughter cell lines established from the parental, freshly procured tumor sample. Such data may lead to incorrect conclusions and precludes accurate analysis and interpretation of the disease process. In some cases, reported data produce results that are impossible to recapitulate *in vivo*.

6.2. Logistics of sharing specimens with collaborators

There is an increasing concern and complexity involved with the sharing of tissue specimens with collaborators at other institutions. First of all, the paperwork and established HIPAA policies that are currently in place for each institution can be overwhelming. It is tedious and time consuming to insure that all of the appropriate paperwork is submitted in order to request for the sharing of procured tissue samples with others outside of the institution. This will often require the step-wise approval of several committees, such as the tissue procurement and biobanking committee, the patent technology and intellectual property division, the IRB and the Privacy Board. Understandably, this arduous process can be extremely frustrating for collaborating researchers who often view such committees as obstructive. It is not unheard of for such decisions to take 6-8 months before a definitive decision is rendered.

This is further complicated by a strong desire for the former committees and boards to keep such important and valuable tissues at the home institution. Thus, this territorial mentality of tissue ownership has greatly impeded the sharing of tissue samples, further limited by the inability to link the clinical data at one institution with the research data [from the tissues] obtained from the second institution. This severely limits the overall relevance of the data obtained, as important concepts and principles may well be overlooked when such comparative data is not readily available.

7. SUMMARY

In summary, the current efforts to establish a successful biobanking repository are noteworthy, providing the critical link between the information obtained from a patient's tumor and clinical outcome. Utilizing this information, we may have the ability to identify novel genes and pathways involved in the malignant transformation and progression of disease. Furthermore, research derived from freshly procured tumors will greatly enhance our current and limited understanding of neoplasia, both at the immunological and molecular levels. A properly organized biobanking facility is able to provide an absolute critical resource for molecular-based biomedical research that conducts such analysis (10), further able to translate new findings into the development of novel drugs or therapeutic agents and determining prognostic markers and diagnostic tools for monitoring/predicting disease outcome. We have just begun such efforts, and the successes of the future will be largely based upon the mutual collaborative efforts of researchers and clinicians from around the world.

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Abbreviations: HIPAA: Health Insurance Portability and Accountability Act of 1996, PHI: Public Health Information, ICF: Informed Consent Form, IRB: Institutional Review Board, ESTADAB: European Searchable Tumor Cell Line and Data Bank, SNP: Single Nucleotide Polymorphism, OSHA: Occupational Safety and Health Administration, EMR: Electronic Medical Record

Key Words: Tissue repository, Biobank, Preservation, Consent, Crypreservation, Review

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