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HOW TO EFFICIENTLY OBTAIN HUMAN TISSUES TO SUPPORT SPECIFIC BIOMEDICAL RESEARCH PROJECTS

Walter C. Bell, Katherine C. Sexton, and William E. Grizzle

Department of Pathology, University of Alabama at Birmingham, Birmingham, AL

Abstract

The purpose of this article is to facilitate access of biomedical researchers to human tissues by describing the types of tissue resources available to researchers, common problems with tissue requests which may limit tissue availability to specific investigators, and steps that can be taken to simplify requests to avoid these problems and enhance access to tissue. Types of human tissue resources available to investigators are described and reviewed, and the experience of the UAB Tissue Collection and Banking Facility (TCBF) is described.

Our experience indicates that typical problems with requests for tissue fall into the following categories: 1) size and number of specimens, 2) type (rarity and availability), 3) time constraints, 4) demand vs. supply, 5) limitations and goals of the resource, and 6) time and resources that can be devoted to a specific request. Investigators should review their requests for human tissues to support their research if they are not receiving adequate quantities of tissue. This is best accomplished by discussing their requests with the tissue resource and correcting specific limitations which block access to the tissues they need.

Introduction

Many investigators have difficulty in obtaining high quality, well characterized, human tissues to support their research. The experience of the Tissue Collection and Banking Facility (TCBF) at UAB in providing over 200,000 tissue specimens to researchers indicates many of the problems result from inappropriate requests, incorrect expectations, as well as limitations of tissue repositories(1–3). Our tissue resource cannot provide a whole “normal” beating heart, or 10 cm of normal ascending aorta from surgical procedures, as have been requested. We can, however, provide extensive numbers of well characterized human tissues to support a wide range of biomedical research projects if the requests for specific tissues are reasonable. Our goal is to explain the role of the major tissue resources, why the more demanding is an investigator’s request, the fewer tissues a repository can supply, and how researchers can work with their tissue resource to improve tissue availability.

Tissue Collection and Banking Facility at UAB

The TCBF at UAB is composed of multiple components including the Southern Division of the Cooperative Human Tissue Network (CHTN), the Tissue Procurement Service of the UAB Comprehensive Cancer Center, the Tissue Repository of the Pancreatic SPORE at UAB, the Collection and Preparation Facilities of the Pulmonary Hypertension Breakthrough Initiative, and the Tissue Resource of the Skin Disease Research Core at UAB. Prior facilities have

Address for Correspondence and Reprint Requests: William E. Grizzle, M.D., Ph.D., Professor of Pathology, University of Alabama at Birmingham, Zeigler Research Building Room 408, 703 South 19th Street, Birmingham, AL 35294-0007, Phone: (205) 934-4214, Fax: (205) 975-7128, E-mail: E-mail: grizzle@uab.edu.

included the Tissue Resource Centers of the Ovarian and Breast SPORES at UAB and the Banking Demonstration Project of the Early Detection Research Network (EDRN). Over the past 20 years the TCBF at UAB has received over 4,000 requests for human tissues and provided over 200,000 tissue specimens to researchers. Many initial requests are found to be difficult or impossible to meet for a variety of reasons.

Tissue Resources

Basically, tissue resources work under one of two models: prospective collection or tissue banking. The investigator must understand the model under which a given tissue resource operates. For example, the Cooperative Human Tissue Network (CHTN) collects tissues prospectively based on the requirements of a specific investigator and does not have a large bank of previously collected tissues. Using this “model of prospective collection”, an investigator may establish specific collection requirements such as requesting that fresh lung carcinoma be minced and supplied in RPMI 1640 and such tissues are collected and processed to meet the investigators needs (1–3). One major disadvantage of this model is that clinical information is usually limited; however, if permitted, clinical information may be obtained from medical records. Because samples have just been collected, it may be years before clinical outcomes are available. Large numbers of specimens are not immediately available because samples are collected prospectively (1).

In contrast, each Specialized Program of Research Excellence (SPORE) has a tissue bank focused on an organ system. SPOREs exist for all major cancers with over 50 spore center grants currently funded¹. In this “tissue banking model,” samples are collected and processed according to a standard protocol. For example, all solid tissues might be divided into 3 aliquots and frozen using the same or different preparations. The banking model has the advantage that samples may be associated with both detailed clinical information and with clinical outcome. Also, relatively large numbers of specimens may be available initially; however, tissue processing may not meet investigator needs.

Tissues that are unavailable at one resource may be obtained from a different resource (1–3). Because of the controversial nature of some specimens (e.g., fetal or HIV tissues), some tissue resources elect not to collect such tissues; however, other tissue resources do (e.g., the AIDS and Cancer Specimen Bank). Investigators should be aware of the major resources available and the model under which each resource works (Table 1)².

Investigators should check with their local IRB and HIPAA privacy board as to what is required to accept and use human tissues received from an outside source. Such tissues may be supplied as de-identified so that HIPAA requirements do not apply³.

Requests for Tissue

Tissue resources typically rely on remnants of tissue which are not needed for diagnosis and which are available from clinical activities. Procedures that may furnish excess tissues can be surgical, diagnostic, clinical research, transplantation and/or autopsy. When developing a request it is important to understand that some tissues and/or medical information may not be readily available. Thus, requests for tissues or information that are not available may either cause the rejection of the request and/or a delay in processing the request.

¹http://spores.nci.nih.gov/current/index_current.html

²<http://resresources.nci.nih.gov/categorydisplay.cfm?catid=631#653>

³<http://www.hhs.gov/ohrp/humansubjects/guidance/cdebiol.pdf>

Problems with requests fall under specific categories: 1) size and number of specimens, 2) types (rarity and availability), 3) time constraints, 4) demand vs. supply, 5) limitations and goals of the resource, and 6) time and resources that can be devoted to a specific request. Even when informed of specific limitations which prevent the investigator's requests from being met, the investigator's refusal to compromise on their requirements may result in disappointment and delay in the investigator's ability to perform his/her research(1–5).

Size of Specimens

Before tissues can be utilized in research, all diagnostic requirements of the tissue must be fulfilled. For breast cancer, diagnosis may require about 1 g of cancer to ensure that the histopathologic description is adequate and biomarkers such as estrogen receptor, progesterone receptor, and Her2-neu status may be evaluated. Thus, a 1 cm breast tumor would just fulfill diagnostic requirements. Also, pre-invasive neoplastic lesions such as ductal carcinoma *in situ* (DCIS) are submitted completely to exclude invasion. If a resource receives 1 g of remnant breast tissue, an investigator who requests 1 g of breast cancer is unlikely to receive a specimen from this case, in that rather than providing 1 g of breast cancer to a single investigator, the tissue source is likely to choose to supply 10 investigators with 0.1 g specimens; thus meeting the needs of a greater number of researchers. Requesting a gram or more of high demand tissues is destined to lead to receiving few specimens. Investigators should adapt their experimental methods to small specimens if breast, prostate, and pancreatic cancers or other small, rare or high demand tissues are requested. Similarly, brain, pancreas, and small cell undifferentiated (oat cell) lung cancers may be treated following needle biopsies, so no fresh or frozen tissues may be available except from autopsies unless the patient is consented to obtain "extra" biopsies for research. Such consent and extra biopsies are being obtained by some SPOREs. In contrast, obtaining grams of tumor for one investigator is possible from some types of more common, bulky tumors for which resections are performed (e.g., ovarian carcinomas, renal cell carcinoma).

Number and type of tissues requested

Some tumors are relatively rare (e.g., paragangliomas) so that requests for multiple samples of such tumors or for many tumors in high demand (breast, prostate, pancreatic cancers) may be impracticable unless a special collection of such tumors is available. Another limitation to the availability of some tumors is treatment prior to surgery (e.g., sarcoma, breast cancer). Neoadjuvant radiation or chemotherapy together with successful early screening for neoplastic diseases can result in small overall tumor size at the time of resection. These approaches have begun to severely limit access to many tumors and to large tumors.

Similarly, some types of tissues are unavailable from diagnostic procedures, especially "truly normal tissues" or certain diseased tissues not treated by surgery. Normal tissues are not removed unless the surgery removes normal adjacent tissues. "Uninvolved matching" tissues may be available (e.g., colonic epithelium from a patient with colon cancer); however, this is not truly normal tissue because molecular changes may have occurred and be present in the normal appearing tissue(5). In contrast, from operations for colon cancer, normal smooth muscle can be collected because colon cancer does not affect muscles unless it invades or the cancer is hereditary.

When "normal" tissue is requested, it is important to specify what is acceptable as "normal". Is uninvolved tissue acceptable, or does the investigator need only tissues from non-cancer cases? Is diseased tissue acceptable? What types of diseases or abnormal conditions are acceptable? An age range should be specified in requests for "normal" prostate because many prostates in men ≥ 50 years of age may have involvement by benign prostatic hyperplasia (BPH).

Some investigators request normal tissues from trauma cases. Tissues from surgical trauma are very difficult to obtain because these surgeries are rarely scheduled, and often are performed after working hours. Similarly, repositories may not obtain tissues from forensic autopsies because these may be performed without consent.

Effect of time constraints

Unreasonable time constraints reduce the availability of tissues. Some specimens can be obtained within 15 minutes of surgical removal; however this requires the tissue organization to devote special resources to accomplish very rapid processing. Also, only a few aliquots can be processed rapidly because of the time required for dissecting, processing, and labeling of large specimens. For example, obtaining 10 samples from one large, complex specimen may take over an hour to collect and process, and processing may be slowed if multiple specimens from this or other cases must be processed simultaneously. Rapid collection may not permit critical diagnostic review and could compromise a specimen's diagnostic usefulness, and diagnosis always must take precedence over research. It is important for investigators to have a basic understanding of the types of changes which tissue may undergo and how these changes may impact their results.

The tissue collection timeline can be broken down into several phases including *in vivo*, *ex vivo*, and storage phases. *In vivo* warm ischemia changes occur during the surgical procedure following induction of anesthesia and compromise of the vascular supply. This phase may be prolonged in some procedures such as recently developed robotic procedures for prostatectomy. A similar ischemic process may be seen *in vivo* as a result of tumor growth exceeding vascular supply, particularly in high grade tumors resulting in degradation of biomolecules. Studies of these changes and their time course have not been performed. Although human tissues to support research should be collected and processed rapidly following the removal of a specimen from the patient, the scientific importance of rapid collection following lengthy warm ischemia remains controversial. Several studies, based upon observations of intact ribosomal bands of RNA and lack of smudging of mRNA on gel analysis, have indicated that the rRNA does not change rapidly after tissues are removed from the body (4,6–8). This may be due to a more rapid degradation of RNA during warm *in vivo* ischemia after organs have their vascular supply compromised; however, relying on intact ribosomal bands considers only degradation, not alteration in expression levels of various genes which may occur.

Huang *et al.* (6) reported modest changes in gene expression in colonic epithelium during *ex vivo* warm ischemia in tissue held at room temperature (5 minutes versus 60 minutes post-operative). Type I genes (63.8% of all genes) had an average increase in expression of 27% over 60 minutes; 67% of Type II genes (17.8% of all genes), had an average increase in expression of 12%, and 33% of Type II genes had an average decrease in expression of 12%. For Type III genes (13.4% of all genes), half increased on an average of 50% and half decreased on an average of 50%.

Dash *et al.* (7) reported that less than 0.6% of the genes they evaluated in prostate cancer demonstrated increased expression at one hour at room temperature. Spruessel *et al.* (8) using Affymetrix chips, RT-PCR, and SELDI-TOF-MS reported no changes in the 28S:18S ribosomal bands of RNA at 30 minutes; however, 15% of genes and proteins changed by at least 2.0 fold within 5 minutes after removal and 20% by 2.0 fold within 30 minutes of removal.

The above studies indicate that after tissues are surgically removed from humans, ribosomal bands usually do not degrade rapidly after tissues have experienced *in vivo* warm ischemia, that most genes are likely to increase in expression rather than to degrade, and that the expressions of some genes return to baseline within 1 hour. In addition, newer methods of real

time quantitative PCR have been developed which use short amplicons and hence can utilize partially degraded mRNA (9). Also, some proteins such as phosphoproteins have been reported to degrade rapidly, but such degradation is likely to vary with specific tissues (10,11). The effects of anesthesia and *in vivo* ischemia on all these genes are unknown and may be greater than the changes observed after removal of the tissue from the patient. Thus, requiring rapid removal and processing of tissues may not always be warranted, especially because this may reduce tissue availability. Each investigator must match the requirement and time restraint needed for their specific research with effects of these on tissue availability.

Supply vs. Demand

Although breast tumors are frequently removed, their small sizes and the numerous requests for them may make many requests difficult to fulfill. To prioritize samples, most academically based institutions provide tissues using a priority system. Investigators located at the home of the tissue resource may have first priority, academically based second priority, and commercially based users the lowest priority. Subgroups in priority may be based on the scientific impact, peer reviewed extramural or intramural funding, and aid to new investigators. In some cases, a Tissue Utilization Committee decides which tissues are supplied to investigators (1–3,12).

Commercial companies may have difficulty obtaining some samples; however, samples of readily available normal tissues and tissues of tumors that are typically large (e.g., renal cell carcinoma, ovarian carcinomas, and sarcomas) can be supplied. Alternatively, tissue resources which cater to commercial users may be accessed (1,2,12).

Investigator Requirements

A tissue resource may have requirements/agreements which must be documented prior to the receipt of tissues. These are usually rigid, carefully thought out and unlikely to change. The tissue resource may require education of personnel in biohazards (12,13) and signing of an indemnification or material transfer agreement (2,12). Many repositories require that the IRB and Privacy Board of the investigator's institution must agree that the investigator has met their requirements (14). The tissue resource may require that it be recognized in publications. Issues related to commercial use of the specimens also may have to be satisfied. Some resources also may require a Data Use Agreement to satisfy HIPAA requirements if a limited data set is provided. It is important to understand the policies of tissue resources before requesting tissues (12).

Time and resource limitations for a specific request

Most constraints increase the difficulty of obtaining tissue. For example, a sample of breast cancer may be requested from a patient who has Type I (juvenile onset) diabetes. The history of Type I diabetes may be in the medical chart, but most tissue organizations do not have the time/resources to review charts on all patients with breast cancer prior to surgery to fulfill such unusual and demanding requests. Similarly, some repositories may not have permission to review a patient's medical history or to obtain clinical outcome. When patients are a part of an interventional study, consent for use of tissue/medical information in research can be included in the patient consent form; however, it is more difficult and time consuming to obtain informed consent/HIPAA authorization from the majority of patients undergoing surgery or admitted to a medical facility (12,14). Thus, investigator requirements that tissues provided to investigators be from consented patients may limit the availability of tissues unless anonymized specimens can be accepted.

Multiple Constraints

The more restrictive and numerous are constraints, the greater the difficulty in meeting them. When a size constraint is added for a specimen that is already difficult to collect, it frequently makes the specimen impossible to obtain. Thus, a request for 1 gram of breast cancer from a patient less than 30 years of age would be almost impossible to meet because the sample requested is large, breast cancers are in high demand, and breast cancers are uncommon in patients this young.

Limitations of Resource

Extensive services (e.g., isolation of cells or microdissection of specific cell populations) usually cannot be provided by tissue resources; however, if provided, the investigator should expect to pay all costs of such services. If a tissue repository includes or has access to a histology facility, histology services may be available for research. Investigators should check with the tissue resource as to availability and cost of special services.

Conclusions

By understanding the types of resources available and the constraints under which various tissue resources operate, an investigator can select an appropriate tissue resource for his project and work with the resource to tailor tissue requests to maximize yield. Investigators should review protocols to minimize the amount of tissue required and be aware of the difficulty in obtaining large or numerous samples of tissues in high demand or of rare tissues. Difficult or complex tissue requests may limit tissue availability. To ensure that a requester of tissues obtains needed tissues as efficiently as possible, it is important for the requester to understand the constraints under which tissue resources operate.

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Table 1
Tissue Resources Readily Available to Support Research

Resource Name	Collection Model	Brief Description
AIDS and Cancer Specimen Bank	Repository	Bank of collected tissues and fluids with clinical data and followup information from HIV associated malignancies
Clinical Trials Cooperative Groups: Human Tissue Resources	Repository	Bank of tumor specimens from uniformly treated patients including a wide variety of malignancies
Cooperative Breast Cancer Tissue Resource	Repository	Bank of formalin fixed paraffin embedded breast cancer samples with associated clinical information
Cooperative Human Tissue Network	Prospective Collection	Prospective collection of normal, benign, pre-malignant and malignant tissues collected according to researcher specifications
Developmental Therapeutics Program Tumor Repository	Repository	Repository of experimental animal and human tumor cell lines
Gynecologic Oncology Group Tissue Bank	Repository	Repository of benign and malignant ovarian and cervical tissue with clinical information