Tissue Banking in Community Cancer Centers

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As the technologies for phenotypic and genotypic analyses become simplified and standardized, community cancer centers have increased interest in preserving tumor tissue for testing. Submission of tumor tissue is increasingly a component of clinical trials in order to correlate phenotypic and genomic features with therapeutic response and survival in a defined clinical setting. Although formalin-fixed, paraffin-embedded tissues are sufficient for many types of analysis, snap-frozen tumor tissue and even cryopreserved tumor cell suspensions may be needed for certain tests. The good news: technology related to long-term tissue storage is evolving rapidly. The bad news: early diagnosis of microscopic cancer (prostate, breast, melanoma) and pathology assessment can make long-term tissue storage unfeasible in certain clinical situations.

To date, commercial efforts to establish tissue banks for individual patients have enjoyed limited success, in part because of lack of reimbursement for tumor preservation services. Hoag Cancer Center has maintained a cell biology laboratory with tissue banking capabilities for 20 years. Maintaining a tissue bank in a community hospital requires an ongoing financial commitment to support personnel, facilities, equipment, operating procedures, and data systems. Close working relationships with operating room personnel and pathologists are crucial. In addition, long-term tissue banking involves long-term contractual and legal responsibilities. Prior to establishing a tissue bank, community cancer centers should first evaluate whether operating a tissue bank is consistent with the center's local patient care mission. Second, cancer center and hospital management must look at the tissue bank with a cost-to-benefit perspective.

The Importance of Biospecimen Resources

The heterogeneity of cancer is one of the major challenges in cancer treatment. Just as people are similar, but also unique, cancers are in some ways similar, but also unique in each individual. Until recently, cancer treatment was directed by empiric observations of populations of cancer patients, but this practice is clearly not ideal. As medical researcher J.D. Kanofsky, MD, MPH, once said, "In a town where half the men are 6 feet tall and half are 4 feet tall, it makes no sense to make a suit for the average man." There is no "average" cancer patient. Just as we would tailor a man's suit to fit; ideally we would "tailor" cancer therapy, individualizing it for each patient's tumor and biologic system.

In 1977, Nobel laureate Peter Medawar predicted that: "…the cure for cancer is never going to be found. It is far more likely that each tumor in each patient is going to present a unique research problem for which laboratory workers and clinicians between them will have to work out a unique solution." Now more than two decades later, because of advances in genomics, proteomics, immunology, biotechnology, and computer technology, we appear to be on the verge of "personalized medicine" becoming a standard commercial approach, rather than "a unique research problem." The key to this paradigm shift is the correlation between clinical outcome and individual genomics and proteomics, or individual genotype and phenotype.

Why Tissue is an Issue

Historically, resected tumor tissue was controlled by pathologists who were expected to make a histological diagnosis, identifying the tissue of origin, and determining the extent of tumor and whether surgical margins were clear. Blocks of formalin-fixed, paraffin-embedded tissue were often saved for varying periods of time in case additional examination was needed. Pathologists and clinicians have

PhD cell biologist viewing tumor cells growing in a plastic flask.

identified microscopic features that have prognostic implications, including:

- Cell differentiation
- Tumor grade
- Perineural, lymphatic, or vascular invasion
- Degree of vascularity.

The availability of monoclonal antibodies has facilitated phenotypic characterization of tumors, and identification of specific tumor markers, some of which are specific targets of today's "targeted" therapies. Expression of certain genes and molecules influence the biological behavior of tumor cells, and contribute to sensitivity or resistance to cancer therapies. Chromosome analysis is now part of the standard evaluation of patients with liquid tumors, but this practice requires having suspensions of single cells rather than the matrix of tumor tissue, and a number of cells in mitosis, which requires short-term cell culture. A limited number of tests that analyze multiple genes have been validated sufficiently to justify commercialization, and one is now widely used to facilitate clinical decision-making in breast cancer (Onco*type* DX).

We are on the verge of being able to perform a complete genomic analysis of each individual's cancer. At the moment such genomic analyses take place in research laboratories with an emphasis on clinical correlations between therapies and histologic, phenotypic, and genotypic findings. As these technologies are perfected and standardized, and validated with clinical correlations, for-profit companies have begun to offer such testing. As usual, when tests first become available, reimbursement is challenging. Eventually, such testing will be used to guide individualized therapy for all patients rather than relying on the results of clinical trials comprised of biologically diverse human beings.

For all of the above reasons, there is increasing recognition that individual human tumor tissue has such inherent value, that it should be stored in ways that maximize future use. Long-term storage of formalin-fixed tissue blocks is the oldest and easiest means of tissue banking, but is not applicable for all types of tests. Long-term storage requires dedicated space, personnel, equipment, standard operating procedures, and data systems for storage and cataloging of samples. Formalin-fixed, paraffin-embedded samples are adequate for many tests, including gene-array analyses.

However, certain types of analyses require fresh frozen tissue. For most purposes long-term storage can be achieved by snap-freezing small pieces of tissue and storing them in a -70° C freezer. However, if the potential to retain cell viability is a consideration, then cell suspensions have to be created under sterile conditions with storage in liquid nitrogen freezers. This practice may be important if proliferating and/or tumor stem cells are the focus of testing.

Staffing Your Tissue Bank

Personnel needs depend on the scope of the tissue banking endeavor. At a minimum, community cancer centers looking to bank tissue will need to consider the following staff: pathologists, cell biologists, and program and data coordinators.

Pathologists are always involved in this process because most hospitals hold them accountable for making a diagnosis on resected tissue, and deciding what is be done with any residual tissue. Unfortunately, this practice is not without its challenges. Specifically, a number of reputable hospitals and universities have experienced conflicts related to the value of such tissues for research purposes. (For more information, see the Legal Corner

Research associate processing a blood cell product under a sterile hood.

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About Hoag Cancer Center

Hoag Cancer Center includes a 65,000-square-foot, three-story facility that opened in 1991, and the oncology program of Hoag Hospital, a 498-bed, non-teaching, not-for-profit, community hospital located in the coastal city of Newport Beach, California, in Orange County in southern California situated between Los Angeles and San Diego Counties. The county has a population of about 3.1 million. The primary service area for the hospital includes about 1 million individuals with about 680,000 located in three cities on the Pacific Coast (Huntington Beach, Newport Beach, and Laguna Beach) and three adjacent cities that are inland (Fountain Valley, Costa Mesa, and Irvine). The cancer program is accredited as a comprehensive community cancer program by the Commission on Cancer of the American College of Surgeons and was recently one of 15 percent of institutions surveyed in 2007 that were recognized as "outstanding." Dr. Dillman has been medical director of the Hoag Cancer Center since 1989.

column "Tissue Banks: Part 1" in the March/April 2008 *Oncology Issues* and "Tissue Banks: Part II" in May/June 2008.)

Pathologists and their technicians always process tissue to some extent, such as trimming away non-tumor tissue, identifying areas for microscopic examination, cutting tissue blocks, and more. For some tissue banking purposes, pathologists and/or their technicians may only be required to prepare formalin-fixed tissue blocks, which can be shipped, or prepare snap-frozen tissue blocks that are briefly stored at -70° before being shipped to a collaborator on dry ice. From a tissue standpoint, this is the easiest way for community cancer centers to participate in a tissue bank, because there is no need for additional personnel or onsite space and storage issues.

If more extensive cell processing is required, then the tissue may be transported to a laboratory that includes cell biologists who can create single-cell suspensions for storage in liquid nitrogen. Such preparation retains the ability to derive viable tumor cells from thawed specimens, which can then be grown in tissue culture medium. Our original tissue banking program included a PhD cell biologist and a laboratory assistant, in addition to a coordinator.

Long-term tissue storage also requires program and data management.

A staff member (program coordinator) has to be responsible for ensuring that appropriate consent forms are signed and that there is coordination between the operating room and pathology regarding the tissue, and submission and/or storage of tissue. Depending on the scope of the endeavor, these responsibilities can be allocated to an existing staff member, or may require the hiring of a FTE employee. In some situations, the program coordinator may also coordinate the transporting of tissue.

If your tissue bank is to have research value, you must develop a system for correlating clinical outcomes of individual patients with tissue-based testing. To accomplish this goal, the tissue bank must have personnel who are responsible for obtaining and entering such information into data systems. Community cancer centers must determine whether these responsibilities can be done within the context of existing cancer registries, or if the tissue bank will require designated data management personnel.

Tissue Bank Facilities and Equipment

Depending on the specific needs of your tissue bank program, the dedicated space needed can range from a few feet to several thousand square feet. The pathology departments at most community cancer centers are already processing and submitting paraffin-embedded tumor samples to other laboratories, such as:

- Sending samples to other pathology laboratories for second opinion evaluations
- Sending samples to reference laboratories for certain tests
- Sending fresh tissue to commercial companies for various types of *in vitro* chemosensitivity assays.

If your cancer center only requires short-term freezing before the tissue is shipped to a central laboratory or company, a small area of space within the pathology department is all that is required. However, if cryopreservation of single-cell suspensions is a goal, more space will be needed, including an area that is maintained as a sterile environment. And for long-term tissue storage the dedicated space can be much greater. For example, the cell biology laboratory at The Hoag Cancer Center operates in 4,000 square feet.

The scope of your tissue banking needs will also dictate your equipment requirements. At a minimum, you will need a dedicated refrigerator and a -70° freezer, depending on how quickly the tissue reaches its final storage destination. To process tissue into cell suspensions that might be used for cell cultures in the future, you will need a sterile Class 100 biosafety cabinet with ultraviolet light. If your program is looking do to long-term storage of viable cells, your equipment needs will, of course, be more extensive. You will need liquid nitrogen freezers, and these require a regular supply of liquid nitrogen and monitoring to be sure that an adequate vapor phase is sustained. For a look at a fully-equipped cell biology laboratory see "A Cell Biology Lab Shopping List" on page 26.

Systems, Policies, and Procedures

The scope of these will vary depending on the scope and size of your tissue bank. Keep in mind, however, that since tissue banking is not a standard procedure, it is not addressed in standard hospital or surgical center, pathology department policies. In our experience, it is important to have in place specific policies and procedures that address the processing and storage of fresh tumor tissue for the operating room, the pathology department, the cell biology laboratory, and the clinical trials office.

For example, some tissue-specific tests are no longer

PHOTOGRAPH COURTESY OF THE HOAG CANCER CENTER

The Hoag Cancer Center in Newport Beach, California, includes a 65,000-square-foot, three-story facility that opened in 1991, and the oncology program of Hoag Hospital.

possible once a tumor specimen has been placed in formalin. At one time, our program had all tumor samples sent fresh to pathology before anything was placed in formalin, but this practice placed an extra burden on staff. On the other hand, patients who want their tumor preserved for possible future uses and who learn that their tumor was mishandled in a manner that eliminates future tissue-related options can be upset and cause issues for your program.

Data Systems

For community cancer centers looking to bank tissue, data systems are critical both in terms of retention of clinical information and for the tissue itself, if it is being stored onsite. Key information in your data system includes patient information, identification of the specific tissues, dates obtained, diagnoses, and storage location. Consider using a bar code system to avoid any confusion regarding identification of samples. If clinical correlation information is required, data systems for storing clinical information are needed. Existing cancer registries may be able to accomplish this; however, it will require additional work on the part of registry staff. If registry staff is not used, then specific personnel will be needed to track clinical data and enter it into a data system that matches tissues with clinical material. This data collection ultimately determines the value of specimens in a tissue bank, because all antecedent and subsequent treatments and their effects, sources of tissue, and dates of relapse and death are all important information for such correlations.

Consents and Contracts

It is now generally agreed that patients should acknowledge awareness of what is being done with the tissue removed from them, and be aware of the expectation for long-term monitoring of their clinical outcome. To educate your patients on these issues, your tissue bank will need a formal consent process. Ideally the consent process would take place before removal of tissue, but sometimes consent can only be obtained after the fact. While it often falls to a tissue bank coordinator to obtain patient consent, legal expertise may be needed to develop or approve of contracts with outside parties who will be receiving the tissue, or with patients, if tissue is being stored on their behalf.

When tissue is being obtained for clinical correlations as part of a prospective clinical trial, patients typically provide written consent for specific treatment and submission of tissue at the same time, during an interaction with clinical trial personnel. In a research setting or to validate a tissue assay, clinicians need to know what happens to a patient in terms of therapies, recurrence, and survival. HIPPA requirements make patient consent a necessity in order to link clinical data to the tissue testing. If the tissue is being sent purely for research purposes, then the surgical consent form may be sufficient, if it contains language to the effect that tissue not needed for diagnostic purposes may be disregarded or released for research purposes.

The most complicated situation arises regarding tissue that is going to a commercial company that might conceivably use the tissue to develop a commercial product. Many patients are surprised to learn that they do not own their own tissue. The landmark case of John Moore vs. the Regents of the University of California provided precedent in this area when it was settled in July 1990. Moore was a patient with hairy cell leukemia whose spleen was used to derive a T-lymphocyte cell line that produced a factor that eventually was identified as granulocyte colony stimulating factor (GCSF), which became a blockbuster drug. The court ruled that Moore had no rights to any commercial profits derived from his tissues, since the tissue was modified by the laboratory. However, the court scolded UCLA physicians for not making the patient aware that his tissue might be used for a commercial benefit. Because of concern about possible future legal challenges, most companies request that all patients grant consent to release their tissue and any rights related to the tissue, to the company. In contrast, some research companies merely want the tissue for testing to validate potential products.

If long-term tissue storage is planned as a service for the patient, then a contract is needed that explains the obligations of the laboratory and the patient, in terms of how the service will be paid for, how long the tissue will be stored, what will be done with the tissue when the patient dies, and what the obligations are if the laboratory can longer provide the storage service. Long-term storage can be expensive; so consideration of how costs will be covered over time is important.

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A Cell Biology Laboratory "Shopping List"

The cell biology laboratory at Hoag Cancer Center actually uses specimens to develop patient-specific therapeutic products (e.g., tumor cell vaccines, autologous immune cell therapies). To do so our laboratory is equipped with:

- 14 incubators
- 5 biosafety cabinets
- 2 chemical fume hoods
- 6 standard bench top centrifuges
- 2 microtube centrifuges
- 1 ultracentrifuge
- 1 cytospin centrifuge
- 3 inverted microscopes
- 1 inverted microscope with digital camera
- 3 optical microscopes
- 1 optical microscope with a mercury lamp and time-lapse video recorder
- 1 dissecting microscope
- 1 multiscan plate reader
- 4 regular 4°C refrigerators
- 1 small 4°C refrigerator
- 3 controlled-rate freezers,
- 2 70°C freezers
- 9 liquid nitrogen storage containers
- A remote alarm monitoring system
- 2 cryosafes
- A spectrophotometer
- A fluorescence-activated cell sorter with computer
- A magnetic cell selector
- A gene amplification polymerase chain reaction system
- A gamma counter
- A beta counter
- 2 digital balances
- 2 warm water baths
- A cell harvester
- A sterile tube welder
- An ice maker
- An elutriation machine
- 2 personal cell analysis machines
- A hematology analyzer
- A microbial sterility detection system
- 9 business computers.

PHOTOGRAPH/BIGSTOCKPHOTO

The Hoag Cancer Center Experience

In 1989 Hoag Cancer Center created a tissue storage bank under the auspices of Hoag Hospital, which purchased the assets of a cell biology laboratory that had been created as part of a joint venture between Hoag Hospital and the biotechnology company Biotherapeutics, Inc. (Biotherapeutics, a commercial biotechnology company, was founded in 1986, based on the vision of personalized medicine such as that anticipated by Medawar.)

The purpose of this cell biology laboratory was to cryopreserve individual tumor samples for possible future use, including *in vitro* chemosensitivity assays, establishment of autologous tumor cell lines for potential use as autologous tumor cell vaccines, and to preserve tissue for phenotypic and genotypic analysis. Because of our research interests related to autologous tumor cell vaccines, we needed personnel experienced in sterile cell biology procedures, biosafety cabinets with ultraviolet lighting to provide a sterile environment in which single-cell suspensions of fresh tumor could be created in a sterile environment, -70° C freezers, and liquid nitrogen freezers.

Shortly after creation of the laboratory, we entered into a long-term tumor bank agreement with a biotechnology company. We stored patient tumors onsite, and plans were to link the clinical data from the Hoag cancer registry to testing on the tissue, which the company anticipated would begin early in the 21st century. Unfortunately, a few years later the CEO of the company was diagnosed with metastatic renal cell cancer and died. The subsequent company leadership did not share his vision and elected to discontinue investment in the project. The staff member who served as coordinator and obtained consents was reassigned. We still have several hundred tumor samples which might still be a useful resource for microarray analysis combined with clinical information from our cancer registry data source.

Initially, our cell biology laboratory was a revenue producer in its early years—not so much because of patient payments for tumor cryopreservation, but because of storage of autologous hematopoietic stem cells, and patient-specific cellular therapeutics such as autologous tumor cell vaccines, autologous activated lymphocytes (AAL), lymphokine activated killer (LAK) and tumor infiltrating lymphocyte (TIL) therapy. However, in 1994 after the U.S. FDA published specific rules regarding autologous somatic cell therapy, therapeutic research products could only be given to patients per investigational new drug (IND) exemption, and patients could only be charged for such treatment if/when the product had received regulatory approval. Since then our laboratory has been supported by philanthropy. The current laboratory has seven FTE positions including two PhD cell biologists.

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