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A Primer on a Hepatocellular Carcinoma Bioresource Bank Using the Cancer Genome Atlas Guidelines: Practical Issues and Pitfalls

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Abstract

Since the advent of the human genome, the era of personalized genomic medicine is indisputably in progress. In an effort to contribute to the evolving knowledge of genomic medicine, we aim directly at building a bioresource bank for hepatocellular carcinoma. This tumor bank is based on the rigorous guidelines set forth by the National Cancer Institute, and offers analytes to help elucidate the mechanisms of progression from cirrhosis to malignancy, risk factors for recurrence, and applicability of current treatment options to a diverse group of people. Surgeons have a

privileged position between the patient (and their cancer) and the benches of basic science. Thus, we offer a primer based on our own experiences for which the surgeon may take to build their own bioresource bank and use to collaborate with others. We highlight some practicalities and pitfalls that could be overlooked, as well as discussion of solutions.

Keywords

Bioresource bank; Biospecimen repository; The Cancer Genome Atlas; Translational research; Hepatocellular Carcinoma

Introduction

The purpose of our program is to procure tissue samples of resected liver cancers and gather critical patient and research information that will be utilized to develop a comprehensive biomedical database. As the fifth most common cancer worldwide, and the third most lethal, hepatocellular carcinoma (HCC) continues to be a global burden [1]. It is an epithelial cancer of hepatocytes [2] and one million cases are diagnosed annually [1]. Identifying genetic patterns unique to cancer types helps to reveal molecular mechanisms enabling novel therapeutic approaches. Efforts at whole exome sequencing are underway for a variety of cancer types, including HCC, where hepatic resection and/or liver transplantation remain the definitive treatment. Despite this, many HCCs will go on to recur. Examining the role of altered DNA sequences in pathological states could offer mechanisms of progression from cirrhosis to malignancy, risk factors for recurrence, and applicability of current treatment options to a diverse group of people [3–5]. The surgeon is the catalyst for such an ambitious endeavor, as he has direct access to the patient and their cancerous tissue and can maneuver in both the clinical and basic science arena [6]. This article serves as a primer for establishment of a bioresource bank, encapsulating sample acquisition and processing, preservation techniques, pathological considerations and collection of clinical data elements [7–10]. Notably, we will address the practicalities and pitfalls we have encountered in our endeavors in an aim for greater consistency and improvement of design.

Bioresource Bank

In the new era of personal genomics, we are now responsible for translating our molecular and cellular discoveries into practical applications, especially in light of accelerating advances in biomedical technology and analysis [11–12]. Two decades after the Human Genome Project began, we currently find ourselves with more powerful and precise DNA sequencing and genotyping methods, less costly and faster than ever before. The National Cancer Institute (NCI) has commissioned a national, large-scale effort to acquire a repository of cancer tissue, entitled the Cancer Human Biobank (caHUB) [13] in concert with the Office of Biorepositories and Biospecimen Research (OBBR) [14]. This leads to high quality sequences to complete the pilot database aptly titled the Cancer Genome Atlas (TCGA). The NCI Best Practices for Biospecimen Resources serves as a guideline for those interested in collaboration, and poses that biospecimens of human cancers have become the foundation for developing technology platforms, serving as the critical resource of basic bench and translational research [15]. This data is driving the course of therapeutic research,

diagnosis of and preventative measures for cancer, and its characterization. As such, reliability, reproducibility and quality of the input are of the utmost priority, and a standardization of acquisition becomes a necessity for success [16]. Newer biobanks, such as that of our institution, now employ a standard operating procedure to comply with these standards. Standardization is important to translational cancer research as quality of output is based on quality of input [17]. Having said this, biorepositories and the collection process should not be considered a fixed and unchanging activity. It must be flexible to synchornize with the advent of new techniques and new scientific questions [18]. As a dynamic and nascent science, we contribute our experiences as a comprehensive cancer center in building a bioresource respository of human hepatocellular carcinoma (HCC) in alignment with NCI/TCGA standards.

Standardized protocols are applied consistently in preparing and storing biospecimens to ensure their quality and to avoid introducing variables into research studies. Ours was developed in conjunction with NCI Best Practices 2010 to comply with TCGA standards. Biospecimens are collected from populations with demographic characteristics and diversity appropriate to the scientific goals of research, and in this case, as it pertains to questions regarding patients with HCC and its causes.

Prior to implementation, it is prudent to assess the resources at hand. To be able to obtain a high-volume biospecimen bank requires resources able to accommodate [8, 15]:

- Collection, receiving, tracking, and shipping
- Immediate and interim processing (ie fine and gross dissection benches)
- Histological preparation and pathology evaluation
- Informatics for validation studies and organization

Taking on such a large scale project, to have fully operational with a sizable tissue bank, absolutely requires a multidisciplinary approach. Personnel involved in Biospecimen resource management and use, including researchers, technicians, nurses, surgeons, pathologists, anesthesiologists, and assistances, must share the goals of the bioresource repository.

Standard Operating Procedure (SOP)

1. Informed Consent

Informed consent for tissue collection is obtained preoperatively from each study participant by the surgical team at the time of consent for operative intervention. The consent explicitly includes permission for DNA, RNA, and protein extraction, genomic sequencing, release of de-identified clinical information, post-treatment re-contact, and release of de-identified genomic and clinical information to scientific databases. The risks of participation, including potential loss of confidentiality of genomic information, and discomfort associated with blood collection is included. Additionally, relevant HIPAA protections are included in the patient consent.

2. Specimen De-identification

At the time of enrollment, patients are assigned a unique sequential identification number. This identifier is associated with all tissue samples collected, as well as all data elements acquired while maintaining patient anonymity (Appendix 1).

3. Tissue Collection

Staff Notification

- The tissue collection staff is notified at the time of initial incision, and is present in the operating room at completion of resection or completion of explant hepatectomy for transport of the specimen from the operating room to pathology suite for immediate processing.

Blood Collection and Storage

- For patient comfort, venous or arterial whole blood is collected from participating patients in two 8.5 cc plastic PAXgene blood collection tubes after administration of general anesthesia. The time of collection is recorded. To eliminate potential contamination with non-native leukocytes, patient blood will be collected prior to the administration of any blood products or, in the case of transplants, graft reperfusion.
- Blood tubes are labeled using the standard de-identified nomenclature indicating center of origin, sample source, patient number (Appendix 1) as well as the time and date of collection.
- Filled blood tubes are stored at progressively cooler temperatures per manufacturer instructions, for ultimate long-term storage at -80°C .

4. Liver Tissue Processing and Storage

- Cryovials are pre-labeled to indicate patient, tissue type, and preservation mechanism according to the standardized de-identified nomenclature (Appendix 1).
- The time of completion for resection or transplant hepatectomy is recorded.
- The resected liver specimen or recipient ex-planted liver is immediately transported by tissue collection personnel to pathology for processing.
- Using a sterile trimmer blade, the specimen is serially sectioned longitudinally into 1 cm slices.
- The number of grossly identifiable and viable tumors, these tumors' locations, as well as the dimensions of each tumor, will be recorded. Multiple tumors are numbered sequentially from right lobe to left lobe.
- Using a new sterile disposable #20 or #22 blade scalpel, a $1\text{cm} \times 1\text{cm} \times 4\text{cm}$ section of grossly viable tumor is removed. (Care must be taken that a sufficient mass of tumor is available to the pathologist for pathologic diagnosis of the HCC.) In the case of multiple tumors, sections from each tumor are acquired. Tumor tissue is cut into $0.5\text{cm} \times 0.5\text{cm} \times 0.5\text{cm}$ pieces, and divided into three 2ml cryovials: one

containing 1ml of RNA Later, and two empty cryovials to be flash frozen in liquid nitrogen. Separately and with a new set of tools, a 1cm × 1cm × 4cm section of non-tumor liver tissue at least 2 cm from the tumor margin is removed. Repeat the same process as above for the processing of the adjacent non-cancerous tissue (Figure 1).

- Two 1cm × 1cm × 0.5 cm segments are removed from each long end of the sectioned tumor and two segments from the non-tumor liver tissue are placed in a 10cc container of 10% buffered formalin for subsequent paraffin fixation, slide preparation, and pathologist review.

Tissue preserved in RNALater will be incubated at 4°C for 24–48 hours, after which time the supernatant is decanted, and the tissue stored at –80°C.

- After 5–10 minutes in the liquid nitrogen, flash frozen tissue is transported on dry ice to –80°C freezers for long-term storage.
- Biospecimen resource personnel must record storage conditions and thawing episodes. Minimizing these episodes eliminates variables which can compromise the quality of the specimens.
- Back-up equipment must be available to accommodate unforeseen emergencies that may jeopardize integrity of the samples.

5. Independent Specimen Review

- A board certified pathologist will review each slide in comparison to the clinical pathology report to confirm diagnosis, evaluate inclusion criteria, and provide further histologic characteristics. Using appropriate pathology technique, magnification, and diagnostic criteria, the pathologist will confirm diagnosis. All elements included are listed in Table 1.

6. Clinical Data and Demographics, and Ethics

Common data elements include demographics, risk factors, viral studies, treatment history, including local regional therapy e.g. Radiofrequency Ablation (RFA) and Transarterial Chemoembolization (TACE), laboratory values, and patient's recurrence history. It is essential to keep a meticulous database, which correlates with a thorough inventory of specimens collected, as directed by the research questions being asked. As this may serve as the basis for many research questions in the future, it is prudent to record all the information potentially pertinent, and refine the data collection as appropriate [4].

Clinical Databases are an essential component of a comprehensive and useful bioresource bank. This first and foremost concern of establishing a data set is security and informed consent. Institutional Review Boards are to be assured of the safety measures taken into place to protect the identity and rights of participating individuals. As this science continues to evolve, and the amount of information available increases, the issue of privacy and ethics is paramount and frankly, more convoluted [19–20]. This is reflected in the literature as Ethical, Legal and Social Implications (ELSI) [21]. Obtaining consents is absolutely an involved process that requires the investigator to be forthright about the risks associated with

genomic analysis and its inherent preclusion to true anonymity [22]. The patient must understand this is a newer field of study, with unanswered possibilities, and their rights' as research participants and as patients are two separate entities. This is especially important in the liver transplant population; the point must be made clear their transplantation and care is not predicated on their participation. The responsibility lies with the investigator to also maintain limited and secure access to the scientific database. The informed consent and clinical information must be protected rigorously, and the security measures must supported by each institution's Institutional Review Board.

Practicalities and Pitfalls

Appropriate design is essential to an effective tissue-banking program. As we hone and perfect our processes and methodology, we have overcome a few obstacles that deserve attention.

One concern to which appropriate attention must be paid is volume and accrual. Hepatic resections and liver transplantations are relatively infrequent and erratic events, with varying degrees of etiology, which require dedication and conscientious collection. To assist in accumulating an acceptable number of samples, collaboration may be key to the success of a nascent tissue bank. Enlisting the collection efforts of neighboring institutions and regional liver transplant surgeons has enabled our institution to increase our amassment.

Evidence exists to suggest the true ischemia time, i.e. the time from tissue excision to the time of flash freezing, is inversely correlated to retrievable protein [17]. Definition of true ischemia time is also difficult to standardize, and some would argue it begins with cessation of blood flow. The practicalities of tissue collection and stabilization under 30 minutes is also logistically difficult in real-time. However, an SOP only helps to regiment the practice and is facilitated by a full-time tissue collection staff, experienced and expeditious in processing tissue. Documentation of tissue collection times enables a footprinting mechanism which can be investigated should any problems arise in quality of specimens.

One issue we have found in our sequencing is specific to hepatocellular carcinoma. During the pathology review, there is significant heterogeneity of tumor histology, with significant amounts of necrosis and stroma, consistent with the pathology of the disease. Even with significant experience, it is difficult to select only viable tumor with appropriate cellularity, especially in light of the rigorous acceptance criteria for sequencing. Also the top slide used as a representation of the tissue analyzed may not be completely faithful to the genetic sequence of the actual tissue analyzed. Technologies are arising to help expedite pathological assessment of tissue cellularity, enabling faster and more accurate processing. We are currently experimenting with these newer technologies, in the meanwhile submitting only our most histologically reliable samples for sequencing. It is important to note that we bank all of our liver tissue, and while not all of them will meet TCGA criteria, the tissue remains banked. We keep our tumor bank well annotated within our clinical database so that in the future, as technology lurches forth with more precise advances requiring less tissue, our banks can still be culled for viable analytes that were previously passed over.

Another matter of discussion is the inclusion criteria used to assess for acceptable specimens per TCGA standards. While searching for disease biology, it is important to identify and, if possible, separate confounding genetic signatures, which arise from biological stresses [23]. Cancerous tissue is ideal when not treated, specifically with chemotherapeutics. The argument exists that the chemotherapeutics often used in TACE procedures (eg cisplatin, doxorubicin and mitomycin) are DNA altering agents which can change the genomic sequences of the original tumor. The great majority of HCCs for surgical intervention have a history of treatment prior to surgery as standard of care as a bridge to liver transplantation [2]. As such, this limits the number of acceptable specimens per TCGA standards dramatically. Discussions continue on the advantages and disadvantages of excluding such a large number of high-quality samples. While basic science research questions become more difficult to answer with reliability, answers to clinical questions can still offer a great deal of information, especially in regards to therapeutic mechanisms and risks of recurrence.

Informatics is an essential component to a comprehensive cancer center intent on DNA sequencing and validation studies [24]. While it is not integral to the bioresource banking process itself, it is responsible for the quality and organization of the output, which can be sizable and unwieldy if not contained by a strong informatics resource. Validation studies are essential to assure quality and reproducibility of tissue sequences, and serve as the foundation on which to build research questions and results [25]. Our facilities now use a combination of 454 and SoliD technologies to validate our sequences, while continuously searching for more state-of-the-art technologies [9].

Conclusion

Technology has accelerated to a position where we can understand cancer as never before. We have the potential to elucidate the genomic fundamentals of cancer formation, and thus treat it, whether by stratifying risk, treatments and/or surgical options. This point cannot be understated and as such, we must rise to the occasion. It is imperative to use the cancerous tissue to which surgeons have direct access. Creating a bioresource bank, while a daunting task, is a necessity in order to sequence the DNA and identify susceptible genes and possible molecular therapeutic targets. Listed are the obstacles we have encountered as we establish our biobank, which are not insurmountable. Volume, ischemia time, tissue histology, inclusion criteria and informatics are all issues to which one must attend. However, with a dedicated, multidisciplinary team, and the appropriate resources and support, formation of a sustainable, productive system is absolutely feasible and warranted. Most importantly in its creation, an excellent bioresource bank is amenable to the staggering advances and immense progress made each day in personalized genomic medicine, taking us one step closer to better care for our patients.

Appendix 1. Labeling and Deidentification

L= liver, denotes organ; 10 denotes year of 2010; 0248 is the unique identifying number of the patient; TF= tumor frozen, denoting the type of tissue.

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Figure 1.
HCC Tissue Processing

Table 1

Pathology data requirements

<ul style="list-style-type: none"> • Percent Tumor Nuclei (of viable cells present) • Percent Necrosis • Percent Stroma (inflammatory cells, non-tumor liver cells, other cell types of viable cells present) • Degree of Differentiation • Histologic Pattern • Cytologic features • Intracellular inclusions • Vascular invasion • Ductal Invasion • Lymphatic Invasion
<ul style="list-style-type: none"> • The following histologic criteria will be met for all samples admissible by TCGA standards: <ul style="list-style-type: none"> • (80)% Tumor Nuclei • (20)% Necrosis • (20)% Stroma