Published in final edited form as:

World J Surg. 2009 April ; 33(4): 723–731. doi:10.1007/s00268-008-9877-1.

Developing a Tissue Resource to Characterize the Genome of Pancreatic Cancer

Georgios Voidonikolas, MD^{1,3}, Marie-Claude Gingras, PhD^{1,2}, Sally Hodges, A.A.S^{1,3}, Amy L. McGuire, PhD⁴, Changyi Chen, MD, PhD^{1,3}, Richard A. Gibbs, PhD², F. Charles Brunicaudi, MD, FACS^{1,3}, and William E. Fisher, MD, FACS^{1,3,*}

¹ Michael E. DeBaakey Department of Surgery, Baylor College of Medicine, Houston, Texas, USA

² Human Genome Sequencing Center, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA

³ The Elkins Pancreas Center, Baylor College of Medicine, Houston, Texas, USA

⁴ The Center for Medical Ethics and Health Policy, Baylor College of Medicine, Houston, Texas, USA

Abstract

With recent advances in DNA sequencing technology, medicine is entering an era in which a personalized genomic approach to diagnosis and treatment of disease is now feasible. However, discovering the role of altered DNA sequences in various disease states will be a challenging task. The genomic approach offers great promise for diseases like pancreatic cancer in which the effect of current diagnostic and treatment modalities is disappointing. To facilitate the characterization of the genome of pancreatic cancer, high quality and well annotated tissue repositories are needed. This article summarizes basic principles guiding the creation of such a repository including sample processing and preservation techniques, sample size and composition, and collection of clinical data elements.

Keywords

Pancreatic cancer; tissue bank; genomic analysis

Introduction

It is estimated that more than 37,000 people in the United States and over 200,000 patients worldwide are diagnosed with pancreatic cancer each year, making it the 11th most common cancer in the US [1]. However, in terms of fatality rates, pancreatic cancer ranks number one with a 5-year survival rate of <4% and is the number four cancer killer overall in the US among both men and women [2]. Only the creation of a high quality pancreatic cancer tissue bank can provide the resources necessary for large-scale systematic study of this disease.

*Corresponding author: William E. Fisher, M.D., F.A.C.S., Director, Elkins Pancreas Center, Michael E. DeBaakey Department of Surgery, Baylor College of Medicine, 1709 Dryden, Suite 1500, Houston, TX 77030, Tel: 713-798-8070, Fax: 713-798-4530, wfisher@bcm.edu.

This work was presented at the Molecular Surgeon Symposium on Personalized Genomic Medicine and Surgery at the Baylor College of Medicine, Houston, Texas, USA, on April 12, 2008.

Recent advances in DNA sequencing technology have made it possible to contemplate a personalized genomic approach to pancreatic cancer. The existing reference genome, NCBI build 36, consisting of a haploid copy of the euchromatic DNA of the human genome (2.8 billion bases) was completed after 13 years of work and a cost of 3 billion dollars by an international consortium [3]. Using a new generation sequencing technology, an individual's diploid genome (6 billion base pairs) was sequenced in 2 months for less than 2 million dollars, a 78-fold reduction in time and a 10,000-fold reduction in cost [4]. In recent years, advanced sequencing techniques such as 454 pyrosequencing, Solexa and SOLiD, and technologies like Nimblegen microarrays have vastly surpassed di-deoxy chain termination on capillary sequencers (Sanger technique) in productivity. These new platforms will soon be focused on the sequencing of disease states such as cancer [5].

Several large-scale pilot projects are already underway such as the Tumor Sequencing Project (TSP), launched by the NHGRI, to study lung adenocarcinoma [6,7]. They have completed the sequencing of 623 candidate genes in the tumors of 188 cancer patients [8]. Last year the National Human Genome Research Institute (NHGRI) and the National Cancer Institute (NCI) jointly launched The Cancer Genome Atlas (TCGA) whose aim is to sequence approximately 1200 candidate genes in 500 patients each stricken with glioblastoma multiforme (GBM), squamous cell carcinoma of the lung and ovarian carcinoma. In addition, this study is also collecting expression, copy number, SNP, and methylation data from the same samples that are being sequenced. In 2008, 3 GBM tumor genomes will have been completely sequenced.

Since large cohorts of high-quality tumor and matched normal samples are difficult to obtain from a single academic center, cooperative groups must collaborate to construct the tissue banks required for cutting edge genomics studies. Many tissue resources for other cancers have already been created and serve as models. Examples include the Japanese, United Kingdom, Swedish, Austrian and other European biobanks as well as international networks like the International Bladder Cancer Bank (IBCB), the European Human Frozen Tumor Tissue Bank (TuBaFrost) and European Organization for Research and Treatment of Cancer. (EORTC) [9–14]. There are also examples within the United States including the Cooperative Prostate Cancer Tissue Resource (CPCTR) and the Indiana University Cancer Center-Lilly Research Labs human tissue bank [15,16]. Other tissues being stored in an organized fashion include mesothelioma, brain, breast and gynecologic tumors [17–21]. The National Cooperative Human Tissue Network (CHTN) was founded in 1987 to stimulate and organize the collection and distribution of human tissues in the US [22]. Today, TCGA has established the Biospecimen Core Resource (BCR) which can function as a model [23], and issues relevant to biobanking are also addressed by forums like the International Society for Biological and Environmental Repositories (ISBER) and web-based portals like BioBank Central [24–26]. We have consulted all of these resources and reviewed the literature to formulate an action plan for the construction of a tissue repository to be used to characterize the genome of pancreatic cancer.

Standardized operating procedures and a prospective approach

Any residual tissue that is not used for diagnosis is a valuable source for both basic and translational research. However, the creation of a large biorepository is a complex endeavor requiring the collaboration of surgeons, pathologists, and basic scientists at multiple centers, using clearly delineated procedures for the handling of valuable specimens, careful quality control, and accurate data tracking. Although centralization of previously collected tissue can bring centers together and foster inter-institutional collaboration, there are disadvantages to organizing such a resource on an ongoing or retrospective basis. For instance, collecting specimens that have already been processed and are stored in different institutions leads to a

lack of standardization and wide heterogeneity. So the first step in organizing a tissue bank for the analysis of the genome of pancreatic cancer is to organize a functional group and establish leadership.

All surgeons, nurses, pathologists, technicians, and genomic researchers involved in the handling and managing of the biospecimens should be informed of the procedure and trained so adherence to the protocol is maximized. Frequent meetings will ensure team work and adherence to the protocol. Furthermore, their risk of exposure to chemicals and potentially infected tissue should be minimized by adherence to bio-safety policies [27–29].

The process of tissue and data collection should be standardized as much as possible [30]. Specific criteria of quality and quantity should be set and met by all collaborative institutes. DNA, RNA and protein require different preparation and fixation methods. The types of preservation solutions and priorities if specimen quantity is limited need to be established. Shipping procedures that prevent thawing and standardized labeling and tracking must be utilized. Finally, a uniform set of clinical parameters should be defined and collected into a universal database for each specimen. Many of these criteria have already been established by the TCGA and guidelines given by the National Cancer Institute (Best Practices for Biospecimen Resources) [27–31].

Informed consent issues

The generation of genomic information on a large scale requires specific attention to assure patients' rights and confidentiality [32]. The potential for personal risk in genome sequencing must be explained to participants during the informed consent process. Broad data sharing is required for most federally-funded studies as outlined in the NHGRI Data sharing policies, and the risks and benefits of broad data release as well as an explanation of the possibility of the extent of data sharing must be provided in language that the patient understands. When data sharing is not required to achieve the goals of the project, it would be advisable to offer opt-out provision for public and/or restricted data broadcast [32,33]. The ability of patients to control whether the sequencing results should be released to a restricted group of investigators or to be made available to the general public must also be explained.

Whether research results involving extensive genome sequencing should be given to study participants is also a cause for concern. If the research reveals results of clinical relevance, arguments have been made that the results should be reported to participants [34–40]. The language in the consent forms should be very carefully worded to prevent potential legal liability. A template of appropriate consent language regarding sharing of genetic information is given below.

Risks associated with genetic analysis

There are additional risks associated with the genetic analysis. Genetic analysis may reveal that you are at risk for other genetic diseases. This information could make it hard to get life or medical insurance. This risk is very small. Your identity will not be known when your sample is analyzed. However, if you consent to the release of your DNA sequence information into the public domain for other scientists to use, there is a small risk that others will be able to trace this information back to you. There is potential risk in this genetic analysis for uncovering and conveying unwanted information regarding parentage or specific risk of disease. No information regarding parentage will be specifically analyzed in this study. No information about risk of disease will be revealed to you, unless you specifically requested that it be. A genetic counselor will be available to help explain the results of this study for you, if you want that information.

Who will have access to my genetic information?

In order to speed research, other researchers would like to have access to your genetic information so that they can compare it to the genetic information of others from other research studies and use it to answer future research questions. This information is most valuable when it is linked to information about your medical history (clinical information).

Release of information into publicly accessible scientific databases

If you agree, parts of your genetic information and in some instances, some clinical information, will be released into one or more scientific databases that are publicly accessible. This will help advance medicine and medical research by allowing other researchers to use this information to help solve questions of what causes cancer of the pancreas and other diseases. There are many publicly accessible scientific databases where your genetic and clinical information may go. Neither your name nor any other personally identifying information about you will ever be released. Nobody will be able to know just from looking at a database that the information belongs to you. However, because your genetic information is unique to you, there is a small chance that someone using a publicly accessible database could trace the information back to you. The risk of this happening is very small, but may grow in the future. As technology advances, the information in these databases will become more valuable to scientists, but there may also be new ways of tracing the information back to you, increasing the risk over time that your privacy would be breached.

Release of information into restricted scientific databases

If you agree, parts of your genetic information and in some instances, some clinical information, will be released into one or more scientific databases that are restricted and can only be accessed by approved researchers. This will help advance medicine and medical research by allowing other researchers to use this information to help solve questions of what causes cancer of the pancreas and other diseases. There are many scientific databases with restricted access; some are maintained by Baylor College of Medicine, some are maintained by the National Institutes of Health, and some are maintained by private companies. Neither your name nor any other personally identifying information about you will ever be released. With restricted databases, researchers who access your genetic and clinical information have a professional obligation to protect your privacy and maintain your confidentiality. Therefore, there is a very small risk that your privacy would be breached.

The decision of whether to allow genetic and clinical information about you that will be gathered in this research to be released into all scientific databases (both publicly accessible and restricted), restricted databases only, or no databases is completely up to you. There will be no penalty to you if you decide not to allow release of your information, and your decision will in no way affect your participation in this research.

Please initial below whether you consent to have your genetic information released into scientific databases (please initial only one option):

_____ I consent to release of my genetic and clinical information into scientific databases, both publicly accessible and restricted.

_____ I consent to release of my genetic and clinical information into restricted scientific databases only.

_____ I do not consent to release of my genetic or clinical information into any scientific databases, other than those maintained and used for the purposes of this study.

To protect patient privacy, all tissue sample storage vials must be de-identified and permanently labeled with a sequential number, tissue type, and preservation method. The number/code is linked in a database that includes all information about the tissue and the patient from which it was obtained as well as tracking of sample quality analysis. For this purpose, the Brady labeling system or the Radio Frequency Identification (RFID) system can be used. Regardless of the system, coded labeling will follow the sample through the whole procedure until the end of sequencing and analysis of the results.

We are currently using the Brady-Soft 8 software, BP-1344 printer and Freezer-Bondz labels, which prints customizable labels which can utilize numbers and text as well as barcodes and the Code Reader 3 to read the barcodes. For storage, we are using 2.0 ml sterile freestanding screwcap tubes with clear o-ring caps and 81-place polycarbonate freezer boxes with clear lids. All products can be purchased through Phenix Research Products (Candler, North Carolina).

Another issue that must be addressed in the consent process is the transport of tissues to a central biorepository for storage and research and who will have access to the tissue. Among the participating institutions of the tissue consortium, a material transfer agreement (MTA) should be signed to formalize the transfer of materials. These documents are needed to clarify intellectual property rights, specify the terms under which samples have been procured, and the conditions under which the biobank will use the samples. It should be clear to all parties that if pancreatic tumor is included in the TCGA list of tumor to be sequenced and a tissue repository participates in this effort, the sequencing results will immediately become public property, since they will be broadcast in a public database.

Study design and patient characteristics

Of the 37,000 patients diagnosed with pancreatic cancer each year, only 15% or about 5500 patients are eligible for resection. Cooperative tissue consortiums, organized primarily by surgeons, could facilitate the rapid collection of appropriate specimen from this relatively small patient population. In order to create a pool representing the whole population, the demographics of the disease should be taken into consideration. For pancreatic cancer, males and females should be equally represented, and the prevalence of different races and ethnicities should match the pancreatic cancer patient population (Table 1 and Fig. 1).

Obtaining tissue from patients participating in a clinical trial offers the advantage of uniform patient selection, standardized therapy and well-documented clinical correlations. However, the details of the clinical trial protocol must be carefully considered. Most clinical trials for pancreatic cancer enroll patients with locally advanced unresectable disease or metastatic disease, and FNA biopsies represent the only available tissue. However, preoperative fine-needle aspiration (FNA) biopsy specimens are frequently inadequate in size. Additionally, specimens in which no neoadjuvant chemotherapy or radiation was given prior to surgery are preferred since this treatment theoretically might change the biologic profile of the tumor cells. Specimens from patients undergoing pancreatic resection are generally larger than pre-treatment needle biopsy specimens and are thus preferred because they usually provide sufficient tissue for research purposes.

Tissue processing and storage

After excision of tissue from the patient, it should be placed in chilled Tis-U-Sol® (Baxter Healthcare, Deerfield, IL) immediately to inhibit degradation, and transferred as rapidly as possible to the pathology department [41]. The time in Tis-U-Sol® until immersion into preservative or snap freezing should ideally be kept under 15 minutes, to minimize the alteration of cellular state due to ischemia and because of the sensitivity to degradation,

especially of RNA. A realistic time from excision to preservative immersion could be up to 15 minutes for RNA or 30 minutes if only DNA is to be studied, but any delay should always be tracked and recorded.

The first priority of the pathologist is diagnosis and only if there is residual tissue, should it be used for research purposes. The average weight of the research samples should be about 200 mg, with a minimum weight of 200 µg needed if sample size is limited and only DNA isolation is possible. An effort should be made to harvest the samples away from necrotic, hemorrhagic, or fibrotic capsular tissue since these factors risk the purity and abundance of tumor cells. When a large tumor specimen is available, serial sectioning should be done, with the sections or slices not more than 5–7 mm thick. If the slices are wider than 1 cm, it should be cut into 5mm horizontal strips. The ends of each strip should be trimmed off and placed into 10% neutral buffered formalin in separate cryovials to be embedded into paraffin for permanent sections. The remaining middle portion of each sample is then put into a pre-labeled cryovial with RNA later, proteinase (see below), or snap frozen in liquid nitrogen. (Fig. 2) Labeling must distinguish which top and bottom histology sections correspond to which frozen sample. This is a critical quality control measure because the pathologist cannot accurately distinguish tumor from surrounding fibrosis or pancreatitis on gross examination at the time of specimen procurement. Specimens should be handled with gloves, and preferably sterile instruments and dishes changed between tumor and matched normal tissue, to prevent contamination with foreign DNA, RNA etc. The processing of tissue samples should not be left to the pathologists, but should at least be overseen and preferably done by a member of the research team to assure adequate sample collection.

Preservation techniques for DNA, RNA, protein, and tissue architecture need to be considered. For example, it is believed by some that protein structure is better preserved by formalin fixation and paraffin embedding (FFPE) rather than fresh freezing in OCT [42]. On the other hand, formalin fixation is known to cause cross linking of DNA and RNA and results in very short fragments that may not be appropriate for sequencing and mutation discovery. However, these samples could be useful for genotyping, or validation of known mutations. RNA and DNA can be isolated from FFPE specimen using commercially available kits but fresh freezing is still considered best for RNA and DNA isolation [43–45].

Recently developed preservation solutions like *RNAlater* (Ambion, Austin, TX), have facilitated long term storage of tissue and eliminated the need for immediate RNA, allowing flexibility without compromising RNA quality [46–48]. Irrespective of the exact preservation solution, the time that elapses between the procurement of the specimen until fixation or immersion into preservative and freezing is the most important factor. The temperature at which the sample is kept, transported, and processed also needs to be considered and standardized. These parameters need to be standardized since both proteins and nucleotides are subject to enzyme degradation and chemical modification. This is particularly true for a pancreatic cancer tissue resource since pancreatic specimens have an abundance of pancreatic enzymes which may be active during warm ischemia [49–51].

The specimen for DNA study should be fresh frozen using an isopentane bath readily available in most hospital frozen section facilities. The specimen is then transferred to a –80°C freezer. The specimen for RNA study should be incubated overnight in *RNAlater* solution at 4°C so that the solution penetrates the tissue, the fluid drained, and then frozen at –80°C. The specimen for protein study should be immersed in a proteinase inhibitor solution (Roche) and then frozen in an isopentane bath and stored at –80°C. Screw cap cryovials should be used for all specimens because they are leak proof and stable to freezing. In cases where the tissue quantity is ample, extensive study can be achieved using

a sample piece for immunohistochemistry after freezing in OCT and tissue microarrays can be constructed from FFPE tissue.

Each tumor sample should also have a matched peripheral blood sample which will be used to isolate germline DNA. Normal matched DNA, RNA, and protein can also be obtained from surrounding normal pancreatic tissue (usually at the margin of resection) that is not infiltrated with tumor. However, areas of pancreas around the tumor may contain genetic changes associated with the development of the tumor; therefore, blood samples are preferred for determining the germline DNA sequence. Blood component samples can be stored as whole blood, although frozen separated peripheral blood lymphocytes (PBLs) or frozen separated PBLs in Dimethyl Sulfoxide (DMSO) are preferred.

We recommend use of PAXgene blood collection tubes, which are commercially available and simplify storage of blood samples without the need to separate blood constituents. These tubes contain a proprietary blend of reagents that can stabilize the cellular constituents of blood for up to 14 days at room temperature, 28 days at 4°C, or indefinitely at -80°C allowing some time until the DNA is extracted. However, caution should be exercised as these blood collection tubes do have expiration dates.

Patient serum and pancreatic juice are also very valuable and should be collected for future studies that will concern possible clinical correlations with soluble biomarkers. These studies may lead to much needed diagnostic tests for pancreatic cancer. Genetic changes, such as *KRAS* mutations, are already beginning to be studied in pancreatic juice [52]. Pancreatic juice can be obtained in the operating room at the time of resection by aspiration from the pancreatic duct at the transection margin using a small syringe and plastic angiocath. The juice is placed in a cryovial, frozen in an isopentane bath, and stored at -80°C.

Corresponding clinical database

Complete clinical information about each patient should be recorded and remain protected but accessible and subject to validation. We have established a Health Insurance Portability and Accountability Act (HIPAA) -compliant prospective database using Velos Eresearch software, (Fremont, California).

The Velos eResearch system was chosen because it is a comprehensive system for electronic capture and management of clinical and tissue bank data which allows reporting, and interfaces efficiently with other existing systems that are in operation at various pancreas centers in the US. The Velos system is built in n-tiers with XML, Enterprise Java Beans and Oracle datastore, and provides a full, flexible and enterprise scalable solution to data management. There is no limit to the number of institutions or patients for which the system can be used to track, manage, and collect data. Our data is stored on off-site secure servers hosted, maintained and backed up by Velos, but there is the flexibility of being stored on facility servers, if it is required.

The system is web-based, and there are no site or location restrictions for Velos users allowing simultaneous use of the system by multiple clinicians across all participating centers. Only licensed users can gain access to the system over the web through a password protected mechanism to register patients, and enter patient and specimen related data. Data can be imported or exported to MS Excel, SAS or other applications as desired. Access to data management and reporting is similarly controlled, and restricted to appropriately licensed investigators. Use within these boundaries can be limited and monitored by the system administrator and protocol manager. All use of the system is tracked by each user's unique identification.

In order for the biospecimens to have high quality clinical annotations, the use of controlled vocabularies is mandatory. Common data elements (CDE) have already been developed by groups of scientists including oncologists, pathologists, researchers and experts in bioinformatics for other cancers. CDEs for breast and prostate cancer and mesothelioma have been developed by the National Cancer Institute (NCI) [30]. Although such an initiative has not been performed for pancreatic cancer, there is a need to establish a core set of data elements for annotating specimens for the pancreatic cancer that would be used in a genome project. These CDEs will facilitate accurate data collection and sharing among institutions and allow correlation with clinical data in future studies. Many of the pancreas centers across the US collect a large volume of clinical data for each patient, but there is considerable heterogeneity and differences in data terms across existing centers. Based on our review of the literature and CDEs for other sequencing projects, we have selected a minimum data set for all specimens (Table 2).

Tissue storage and logistics

Storage in liquid nitrogen vapor-phase freezers or mechanical freezers at less than -80°C can preserve fresh tissue for years [53]. RNA first starts losing its integrity after 5 years in the mechanical freezers [43]. In case longer periods of storage are desired, even lower storage temperatures (-170°C) and cryoprotectants such as dimethyl sulfoxide (DMSO) can be used. The samples should be stored in multiple freezers rather than in one location to minimize the loss of samples should mechanical failure arise. The power supply for the freezer system should be backed up with automatic emergency power. The freezers should have monitoring equipment and software that automatically telephones support personnel and the investigators if there is any problem with the system. Alternative freezers should be nearby should the need arise to move samples from a defective freezer. Liquid nitrogen freezers provide lower and more stable temperatures and do not depend on the electrical power supply network but mechanical freezers are more economical, practical, easier to install and maintain [43]. Paraffin blocks and slides can be preserved in vacuum sealed bags with a commercial oxygen absorber at room temperature.

If the samples need to be shipped to another site, appropriate insulation and temperature preservation should be assured by using chipped dry ice. The process details should be well documented. It is important to align with the regulations of National and International Air Transport Association (FAA, IATA) and Occupational Safety and Health Administration (OSHA) [53–55].

Quality assurance and quality control (QA/QC)

In every step of this process, standards of quality should be validated. Before sequencing begins, data from the collection process such as any periods of delay before freezing or thawing of the sample during storage or transport should be reviewed. An experienced gastrointestinal pathologist should confirm the diagnosis and record the percentage of viable cancer cells. It is important to minimize the extent of necrosis and the level of contaminating stromal tissues in samples intended for genomic analysis. In the case of pancreatic cancer the fibrotic reaction around the tumor can be a limiting factor. In such cases, technology such as laser micro-dissection has been used to extract tumor cells out of surrounding stromal non-malignant cells. However, with the development of the next-gen sequencing platforms, which have increased sensitivity, it is expected that will not be necessary.

A written quality management system (QMS) should be established by each repository and followed in order to describe the QA/QC programs. Audits should be conducted to check the equipment maintenance e.g. the temperature and alarm of the freezers, electricity backup, the adequate training of the staff, correct documentation of patient data, adherence to safety

standards and ethical, legal and patient consent practices. If there is a discrepancy between the original pathologist and the study pathologist, a consensus conference with the participation of both, if possible, and at least one other independent pathologist should be called to resolve the discrepancy. As far as the specimens are concerned, full details describing warm and cold ischemia time, freezing and fixation time, techniques and conditions used for tissue accrual, and any deviation from the standard procedure when managing the specific sample should be tracked and recorded.

Conclusions

The importance of good organization at the point of project initiation cannot be overstated. The quality of the sequencing data depends on a large volume of high quality specimens that accurately represent the spectrum of pancreatic cancer. An organized approach to sample processing and preservation techniques, sample size and composition, and collection of clinical data elements will significantly impact the conclusions that can be made from the sequence data. The volume of sequence information that is generated in a genome sequencing project is quite large. Although the main goal of such a project is to characterize the spectrum of polymorphisms in cancers, having accurate clinical data to explore correlations between these genetic events and cancer phenotypes is invaluable. In addition, thinking ahead and simultaneously collecting large numbers of RNA and protein samples will allow studies of individual gene expression to immediately follow. These subsequent studies will undoubtedly lead to a multitude of new therapeutic targets and diagnostic tests for pancreatic cancers that will become increasingly tailored to each patient's unique tumor genome.

Acknowledgments

The symposium was supported by a grant from the National Institutes of Health (R13 CA132572 to Changyi Chen).

References

1. Jemal A, Tiwari RC, Murray T, et al. Cancer statistics, 2004. *CA Cancer J Clin* 2004;54:8–29. [PubMed: 14974761]
2. Kleeff J, Michalski C, Friess H, et al. Pancreatic cancer: from bench to 5-year survival. *Pancreas* 2006;33:111–118. [PubMed: 16868475]
3. International Consortium completes Human Genome Project. National Human Genome Research Institute web site. Apr 14. 2003 Available at: <http://www.genome.gov>
4. Wheeler DA, Srinivasan M, Egholm M, et al. The complete genome of an individual by massively parallel DNA sequencing. *Nature* 2008;452:872–876. [PubMed: 18421352]
5. Blow N. Genomics: the personal side of genomics. *Nature* 2007;449:627–630. [PubMed: 17914399]
6. Cancer Sequencing Projects (CSPs). National Human Genome Research Institute web site. Available at: <http://www.genome.gov>
7. Collins FS, Barker AD. Mapping the cancer genome. Pinpointing the genes involved in cancer will help chart a new course across the complex landscape of human malignancies. *Sci Am* 2007;296:50–57. [PubMed: 17348159]
8. Weir BA, Woo MS, Getz G, et al. Characterizing the Cancer Genome of Lung Adenocarcinoma. *Nature* 2007;450:893–898. [PubMed: 17982442]
9. Morente MM, de Alava E, Fernandez PL. Tumour banking: the Spanish design. *Pathobiology* 2007;74:245–250. [PubMed: 17709967]
10. Knox K, Kerr DJ. Establishing a national tissue bank for surgically harvested cancer tissue. *Br J Surg* 2004;91:134–136. [PubMed: 14760657]
11. Asslaber M, Abuja PM, Stark K, et al. The Genome Austria Tissue Bank (GATiB). *Pathobiology* 2007;74:251–258. [PubMed: 17709968]

12. Tilstone C. Further plans announced for national biobanks. *Lancet Oncol* 2006;7:195–196. [PubMed: 16538775]
13. Riegman PH, Dinjens WN, Oomen MH, et al. TuBaFrost 1: Uniting local frozen tumour banks into a European network: an overview. *Eur J Cancer* 2006;42:2678–2683. [PubMed: 17027254]
14. Schmitt M, Mengele K, Schueren E, et al. European Organisation for Research and Treatment of Cancer (EORTC) Pathobiology Group standard operating procedure for the preparation of human tumour tissue extracts suited for the quantitative analysis of tissue-associated biomarkers. *Eur J Cancer* 2007;43:835–844. [PubMed: 17321128]
15. Melamed J, Datta MW, Becich MJ, et al. The cooperative prostate cancer tissue resource: a specimen and data resource for cancer researchers. *Clin Cancer Res* 2004;10:4614–4621. [PubMed: 15269132]
16. Sandusky GE, Teheny KH, Esterman M, et al. Quality control of human tissues--experience from the Indiana University Cancer Center-Lilly Research Labs human tissue bank. *Cell Tissue Bank* 2007;8:287–295. [PubMed: 17387635]
17. Mohanty SK, Mistry AT, Amin W, et al. The development and deployment of Common Data Elements for tissue banks for translational research in cancer - an emerging standard based approach for the Mesothelioma Virtual Tissue Bank. *BMC Cancer* 2008;8:91. [PubMed: 18397527]
18. Ponzoni M, Kwee I, Mazzucchelli L, et al. A virtual tissue bank for primary central nervous system lymphomas in immunocompetent individuals. *Pathobiology* 2007;74:264–269. [PubMed: 17709970]
19. Figarella-Branger D, Colin C, Chinot O, et al. AP-HM tumour tissue bank: molecular signature of gliomas. *Med Sci (Paris)* 2006;22:54–59. [PubMed: 16705945]
20. Prime W, Sobel ME, Herrington CS. Utilization of human tissue in breast cancer research. *Breast Cancer Res* 2000;2:237–240. [PubMed: 11250713]
21. Chu TY, Hwang KS, Yu MH, et al. A research-based tumor tissue bank of gynecologic oncology: characteristics of nucleic acids extracted from normal and tumor tissues from different sites. *Int J Gynecol Cancer* 2002;12:171–176. [PubMed: 11975676]
22. Clausen KP, Grizzle WE, Livolsi V, et al. Special communication. the cooperative human tissue network. *Cancer* 1989;63:1452–1455. [PubMed: 2920370]
23. Human Cancer Biospecimen Core Resource (BCR). The Tumor Gene Atlas website. Available at: <http://cancergenome.nih.gov>
24. International Society for Biological and Environmental Biorepositories. Available at: www.isber.org
25. BioBank Central. Available at: www.biobankcentral.org
26. Troyer D. Biorepository standards and protocols for collecting, processing, and storing human tissues. *Methods Mol Biol* 2008;441:193–220. [PubMed: 18370320]
27. NCI Best Practices for Biospecimen Resources. Office of Biorepositories and Biospecimen Research [NCI website]. Jun. 2007 Available at: <http://biospecimens.cancer.gov>
28. Grizzle WE, Fredenburgh J. Avoiding biohazards in medical, veterinary and research laboratories. *Biotech Histochem* 2001;76:183–206. [PubMed: 11549131]
29. CDC and NIH Biosafety in Microbiological and Biomedical Laboratories. Available at: <http://bmbi.od.nih.gov>
30. Morente MM, Mager R, Alonso S, et al. TuBaFrost 2: Standardising tissue collection and quality control procedures for a European virtual frozen tissue bank network. *Eur J Cancer* 2006;42:2684–2691. [PubMed: 17027255]
31. The Cancer Genome Atlas Biospecimen Selection Process. The Cancer Tumor Atlas website. Available at <http://cancergenome.nih.gov>
32. McGuire AL, Gibbs RA. Genetics. No longer de-identified. *Science* 2006;312:370–371. [PubMed: 16627725]
33. McGuire AL, Gibbs RA. Currents in contemporary ethics: meeting the growing demands of genetic research. *J Law Med Ethics* 2006;34:809–812. [PubMed: 17199822]

34. McGuire AL, Caulfield T, Cho MK. Research ethics and the challenge of whole genome sequencing. *Nat Rev Gen* 2007;9:152–156.
35. Renegar G, Webster CJ, Stuerzebecker S, et al. Returning genetic research results to individuals: points to consider. *Bioethics* 2006;20:24–36. [PubMed: 16680905]
36. Knoppers BM, Joly Y, Simard J, et al. The emergence of an ethical duty to disclose genetic research results: international perspectives. *Eur J Human Gen* 2006;14:1170–1178.
37. MacNeil S, Fernandez C. Informing research participants of research results: analysis of Canadian university based research ethics board policies. *J Med Ethics* 2006;32:49–54. [PubMed: 16373524]
38. Kohane IS, Mandi KD, Taylor PL, et al. Reestablishing the researcher-patient compact. *Science* 2007;316:836–837. [PubMed: 17495156]
39. Sjöblom T, Jones S, Wood LD, Parsons DW, Lin J, et al. The consensus coding sequences of human breast and colorectal cancers. *Science* 2006 Oct 13;314(5797):268–74. [PubMed: 16959974]
40. Getz G, Höfling H, Mesirov JP, et al. Comment on “The consensus coding sequences of human breast and colorectal cancers”. *Science* 2007;317:1500. [PubMed: 17872428]
41. Grizzle WE, Aamodt R, Clausen K, et al. Providing human tissues for research: how to establish a program. *Arch Pathol Lab Med* 1998;122:1065–1076. [PubMed: 9870854]
42. Blow N. Tissue preparation: Tissue issues. *Nature* 2007;448:959–963. [PubMed: 17713539]
43. Ribeiro-Silva A, Zhang H, Jeffrey SS. RNA extraction from ten year old formalin-fixed paraffin-embedded breast cancer samples: a comparison of column purification and magnetic bead-based technologies. *BMC Mol Biol* 2007;8:118. [PubMed: 18154675]
44. Ferrer I, Armstrong J, Capellari S, et al. Effects of formalin fixation, paraffin embedding, and time of storage on DNA preservation in brain tissue: a BrainNet Europe study. *Brain Pathol* 2007;17:297–303. [PubMed: 17465988]
45. van Maldegem F, de Wit M, Morsink F, et al. Effects of processing delay, formalin fixation, and immunohistochemistry on RNA Recovery From Formalin-fixed Paraffin-embedded Tissue Sections. *Diagn Mol Pathol* 2008;17:51–58. [PubMed: 18303406]
46. Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *Am J Pathol* 2002;161:1961–1971. [PubMed: 12466110]
47. Florell SR, Coffin CM, Holden JA, et al. Preservation of RNA for functional genomic studies: a multidisciplinary tumor bank protocol. *Mod Pathol* 2001;14:116–128. [PubMed: 11235903]
48. Mutter GL, Zahrieh D, Liu C, et al. Comparison of frozen and RNALater solid tissue storage methods for use in RNA expression microarrays. *BMC Genomics* 2004;5:88. [PubMed: 15537428]
49. Huang J, Qi R, Quackenbush J, Dauway E, Lazaridis E, Yeatman T, et al. Effects of ischemia on gene expression. *J Surg Res* 2001;99:222–227. [PubMed: 11469890]
50. Blackhall FH, Pintilie M, Wigle DA, et al. Stability and heterogeneity of expression profiles in lung cancer specimens harvested following surgical resection. *Neoplasia* 2004;6:761–767. [PubMed: 15720802]
51. Spruessel A, Steimann G, Jung M, et al. Tissue ischemia time affects gene and protein expression patterns within minutes following surgical tumor excision. *Biotechniques* 2004;36:1030–1037. [PubMed: 15211754]
52. Tada M, Omata M, Kawai S, et al. Detection of ras gene mutations in pancreatic juice and peripheral blood of patients with pancreatic adenocarcinoma. *Cancer Res* 1993;53:2472–2474. [PubMed: 8495407]
53. International Society for Biological and Environment Repositories. Best practices for repositories I collection, storage, and retrieval of human biological materials for research. Available at: <http://www.isber.org>
54. International Air Transport Association (IATA). Infectious Substances Shipping Guidelines. 2006. Available at: <http://www.iata.org>
55. Occupation Safety and Health Administration (OSHA). Regulations on toxic and hazardous substances. Available at: <http://www.osha.gov>

Fig. 1.

Prevalence of pancreatic adenocarcinoma by age group. Although most cases occur in the 60–80 age group, 46 percent of patients fall outside this range and must be represented in the tissue bank. The data is from SEER 2007. http://seer.cancer.gov/csr/1975_2005/, based on November 2007 SEER data submission, posted to the SEER website, 2008

Pancreatic Cancer Sequencing Consortium

Specimen Collection Flowchart

Fig. 2. Specimen Collection. Samples of tumor and normal tissue are collected as soon as possible to minimize warm ischemia time. Sites with tumor and normal tissue are selected by gross examination of the surgical specimen. Each margin of the research sample is fixed in formalin as a critically important quality assurance step to document the histology of the research sample.

Table 1

Demographics of Pancreatic Cancer Patient Population: Prevalence (SEER 2007)

	Race	Ethnicity	Gender
Caucasian	85%		
African-American	12%		
Asian/Pacific Islander	3%		
American Indian/Alaska Native	<1%		
Non-Hispanic		95%	
Hispanic		5%	
Male:Female			1:1

Table 2

Common data elements for tracking specimens for pancreatic cancer tissue bank

Demographics:	- center procuring tissue	
	- date of birth	
	- gender	
	- race	
	- ethnicity	
<hr/>		
Risk factors:	- smoking history (pack-years)	
	- alcohol history	
	- diabetes:	age at onset, exacerbation, insulin or non-insulin dependent
	- chronic pancreatitis:	age at diagnosis, number and dates of incidences, treatments
	- family history of pancreatic cancer	
	- family history of other cancer:	lung, liver, prostate, ovarian, breast, uterine, colon
<hr/>		
Disease:	- date of diagnosis	
	- histologic diagnosis	
	- tumor grade	
	- tumor size	
	- lymph node involvement:	total number, total positive number
	- TNM stage	
<hr/>		
Outcomes:	- surgery	procedure, date of procedure
	- margin status, if resected	
	- chemotherapy:	drugs, dose, date of administration
	- radiation:	dose, fractions, date of administration
	- date of last patient contact:	vital status
	- first recurrence:	date and site