



## Significance of biological resource collection and tumor tissue bank creation

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### Abstract

Progress in the molecular oncology of gastrointestinal carcinomas depends on high quality cancer tissues for research. Recent acceleration on new technological platforms as well as the "omics" revolution increases the demands on tissues and peripheral blood for research at the DNA, mRNA and protein levels. Tissue bank creation emerges as a priority. Tumor tissue banks are facilities that are organized to collect, store and distribute samples of tumor and normal tissue for further use in basic and translational cancer research. The samples are generally obtained immediately after excision, prior to fixation, to ensure optimal preservation of proteins and nucleic acids. It is possible for surgeons or pathologists to collect fresh tissue prospectively during their routine dissection procedures. Most tissue banks are "project-driven" tumor banks, which are specialized collections of tumor samples on which their research is based. Systematic collection of all available tumor tissue is much rarer. High quality tissue banks need the collaboration of clinicians and basic scientists, but also

the informed consent of patients and ethical approval. Through the standard operation procedure, snap frozen fresh tissue collection, storage and quality control for cryopreserved tissues are the pivotal factors on tissue bank construction and maintaining. The purpose of the tissue bank creation is enhancing the quality and speed on both the basic and translational research on gastrointestinal cancer. The quality assurance and quality control are handled based on reviewing HE staining slides or touch imprint cytology by pathologists.

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**Key words:** Tissue bank; Bioresource; Cryopreservation; Quality control

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### INTRODUCTION

Tumor tissue banks are facilities that are organized to collect, store and distribute samples of tumor and normal tissue for further use in basic and translational cancer research. Surgeons and pathologists play an important role in collecting fresh tissues and body fluids during their routine procedures. Along with the occurring of "omics" platforms, requirement of high quality cancer tissues or peripheral blood for research of genome, transcriptome, proteome and metabolome is increasing rapidly. Therefore, the creation of high quality banks of biological resources at large medical centers or institutes is a fundamental and valuable work.

## SIGNIFICANCE OF TISSUE BANK CONSTRUCTION

Collection of high-quality biosamples, such as tumor tissue and peripheral blood, is a material basis for discovering new biomarkers as well as drug targets. Good biosamples are the rate limiting factors on translational medicine. Many tumor tissue banks have been constructed in the United States, Canada, United Kingdom, France and so on<sup>[1,2]</sup>. For example, at the MD Anderson Cancer Center of America, a tissue bank for head and neck tumors has been constructed since fifteen years ago. At the end of 2008, there were over 25000 tissue samples accumulated in this tissue bank<sup>[3]</sup>. Since 2000, a tissue bank for pancreatic cancer has also been initiated at the MD Anderson Cancer Center. This new tissue bank shared patient information resources among twenty-five satellite tissue banks in the Houston area<sup>[4]</sup>. In China, the cancer tissue bank construction was begun at the end of twentieth century. Currently, different kinds of cancer tissue banks have been established at large teaching hospitals or cancer centers<sup>[5,6]</sup>.

## PROCEDURE OF TISSUE BANK SETUP

The workflow of tissue bank creation involves both sides, i.e. doctors and patients. It is a team play among doctors, nurses, technicians, office clerks and computer scientists. According to the international standard operation procedure (SOP), the necessary steps of a tissue bank setup include: (1) Approval by the local hospital Ethical Committee; (2) Obtaining the informed consent from patients before blood and/or tissue sample collection; (3) Distributing the procedure to the operation rooms as well as to the pathology departments for sample collection; (4) Storage of samples at very low temperature as quickly as possible after collection in the operation room; (5) Collection of the cancer tissue after recording its macroscopic features: this is done by a pathologist, who supervises the whole cutting procedure; (6) If the size of the tissue sample is equal or less than 1 centimeter, then it must be used, as a priority, to establish the clinical diagnosis; tumor and normal tissue (at least 3 centimeter far away from the tumor lesion) are allowed to be taken to create a tissue bank in case of a larger tumor sample. In addition, one piece of tissue should be fixed in 10 percent formalin for H&E staining (Figure 1A and B); (7) A high-quality tissue means that the integrity of protein and nucleic acids is well preserved. Tissues must be put in liquid nitrogen within 30 min after excision; (8) Tissue samples are transferred from the operation room to the laboratory and put in -80°C freezers for long-term storage (Figure 1C); (9) A detailed clinical information of patients must be collected along with the tissue or blood sample; (10) The electronic medical record of patients is established at many centers. In order to preserve the privacy of patients, the data have to be supervised by an authorized administrator; (11) A serial number has to be used for tissue or blood sample storage. In many large tissue banks, a bar code is introduced for sample

management; (12) The sample types are always different depending on the specific research needs. Some tissue banks are focusing on head and neck cancer, while others may focus on breast or ovary cancer<sup>[7]</sup>. In general, at the beginning of tissue bank creation, cancer tissue samples represent most of the material being collected. Subsequently, peripheral blood, urine and other body fluids are also collected<sup>[8]</sup>; and (13) It is preferable not to harvest tissue from patients who are infected with pathogens like HIV or hepatitis B or C viruses.

## TEMPERATURE REQUIREMENT FOR DIFFERENT BIOSAMPLES

Temperature is a pivotal factor for high quality biosamples storage. Low temperature is beneficial to maintain the integrity of biological molecules. Some study have shown that, after the tissue has been embedded in paraffin for slide preparation, most RNA is destroyed<sup>[9]</sup>. Theoretically, the lower temperature the better for biosample storage. The best container for biosamples is a tank of liquid nitrogen. Qualman *et al*<sup>[10]</sup> proposed that the tissues should be stored in liquid nitrogen vapour-phase freezers, because specimens kept at approximately -170°C are present a higher integrity than those maintained at higher temperatures. Freezers at -80°C are used for short-term tissue storage (1 wk or less) and storage of blood and serum, whereas -20°C freezers are used for reagent storage. Nowadays, not every hospital or cancer center is equipped with a liquid nitrogen vapour-phase freezer, so samples should be snapped frozen in liquid nitrogen and transferred to a suitable freezer, usually at -80°C. A temperature of -80°C induces a reversible change in the physical state of the tissue and an enzyme inactivation, which means that nucleic acids and proteins are chemically preserved. Glass vials and pop-top plastic vials are not adequate storage containers for vapour-phase liquid nitrogen temperatures, as they may readily break or pop open. Screw-cap cryovials work well for storage of serum or urine (Figure 1B). If there is no proper tank of liquid nitrogen at the hospital, a low temperature freezer is an ideal choice. In most hospitals, since there is some distance between the operation room and the tissue bank laboratory, a general refrigerator should be prepared for short-time storage of biosamples. For instance, biopsy tissues can be stored at least six months in -20°C freezers. If they are stored at -40°C, tissues can be preserved for three years. Tissues dipped in high concentration of glycerin at 0 to -10°C can be stored for two years, and for long term at -80°C. However, this kind of tissue is not appropriate for RNA analysis. During the process of creating a tissue bank, non-stop electrical back-up is desirable, in order to ensure the quality of biospecimen in case of power failures. Cancer tissue samples should be snapped frozen as soon as possible after excision, ideally within 30 min. On the other hand, blood samples can be drawn along with the other biochemical exams, and then centrifuged, to separate the serum or plasma, at 2500 r/min for 15 min within 3 to 5 h from collection. The serum and plasma should be preserved at -40°C



**Figure 1 Flowchart of tissue bank set-up.** A: The excision location for tissue collecting of gastric cancer and grossly normal mucosa (white arrows); B: The screw-cap cryovials and the liquid nitrogen transporter; C: Tissue samples are kept in  $-80^{\circ}\text{C}$  freezers for long-term storage.

or  $-80^{\circ}\text{C}$ . The pellet is also useful for DNA extraction and should be stored at  $-145^{\circ}\text{C}$  for long time<sup>[11]</sup>. The perfect condition for tissue sample preservation is the temperature of liquid nitrogen, i.e.  $-196^{\circ}\text{C}$ . According to our experience, the activity and life-span of biological macromolecules are decreased after 3 years or more of storage at  $-80^{\circ}\text{C}$ <sup>[6]</sup>.

## THE PUBLIC OPINION ON CANCER TISSUE BANKS

According to a public survey, a majority of the respondents had a positive attitude towards genetic research. Many people agree to donate their excised tissue for tumorigenesis or biomarker research. Kettis-Lindblad *et al.*<sup>[12]</sup> interviewed six thousands patients, and a large proportion of respondents (86%) would agree to donate their blood samples and genetic information for research purposes. Another 3% would provide an anonymous sample for research. At the Vanderbilt University of Nashville (Tennessee, United States), a project has started for collecting the routinely discarded blood samples for the creation of a DNA bank. Based on this project, over 300 000 samples are expected to be accrued within 5 years, enabling genotype-phenotype associations across any disease area represented in sufficient proportion in the patient population. As we know, a large scale genetic study needs a large amount of samples. Therefore, the public's perception of biosample collection is very important. At present, many cancer tissue banks and database are under construction worldwide. Whether or not the privacy surrounding the genetic information is properly preserved is a matter of concern<sup>[13]</sup>. A population-based genetic database, or "genebanks" project, has been proposed at eight different international locations between 1998 and 2002. A genebank is aimed at collecting genetic samples, in the form of blood or tissue, that can be linked with medical or genealogical or lifestyle information within a specific population. The proposed genebanks are located in Iceland, the United Kingdom, Estonia, Latvia, Sweden, Singapore, the Kingdom of Tonga, and Quebec, Canada. Issues regarding confidentiality and patients' consent have resulted in opposition to some of the more publicized projects. As a result, none of the proposed databases are currently

operational and at least one project was terminated due to opposition<sup>[14]</sup>. Therefore, in the setup of tissue banks, the ethical, legal, and social implications of the projects should be carefully considered.

## QUALITY CONTROL OF SAMPLES

Low temperature provides a long-term preservation condition for organs and tissues. The principle theory of cold storage is to depress the metabolism of tissue and cells. Nearly all biochemical and biophysical processes will be stopped at a sufficiently low temperature, such as that of liquid nitrogen. However, how long will be maintained the activity and/or the integrity of biological macromolecules is still uncertain. A good tissue bank needs a quality control procedure, which is usually managed by the pathologists. The quality analysis includes such parameters as the assessment of tumor diagnosis, the percent of tumor tissue, the percent of stroma, and the percent of necrosis. The pathologists should observe the histological slide first, in order to confirm that there are sufficient cancer cells on the excision tissue samples. A well-qualified sample is one where there are over 75% cancer cells. The tissue should be eliminated from the tissue bank if the cancer cells are less than 65% on the slide. In some tissue banks, touch imprint cytology was used as a quality control assay to estimate the quantity of cancer cells. Touch imprint cytology represents both an inexpensive and a rapid method for maximizing cell recovery when very small pieces of tissue are available. The touch imprint cytology showed higher agreement with histological sections from contiguous tumor<sup>[15]</sup>. All cases enrolled in a tissue bank need to be followed-up. The information about therapeutic effects, clinical evolution and survival is very important. The newly developed molecular techniques are helpful for protein or nucleic acids analysis. High-quality cancer tissue is the basis of molecular analysis<sup>[16,17]</sup>. Reasonable quality nucleic acids and proteins can be obtained from frozen tissue that are suitable for a multitude of molecular analyses, including genomic assays such as comparative genomic hybridization, quantification of gene expression by measurement of transcribed RNA and protein products (proteomics). Compared with the more robust nature of DNA, RNA extraction can be problematic but is at least possible with fresh tissue. Storage of tissues



with intact morphology, proteins, DNA and RNA for use in research and diagnostics is the ultimate objective of a tissue bank. Avoiding RNA degradation is a major challenge in this process. RNA is considered a most fragile molecule. The ubiquitous presence of RNAase requires efforts to avoid RNAase contamination during laboratory work. Micke *et al.*<sup>[18]</sup> analyzed selected tissue types from the local tissue bank representing 47 normal and malignant tissues transported on ice for up to 2 to 3 h before biobanking. RNA prepared from 45 of the 47 samples exhibited distinct ribosomal peaks indicating intact RNA. This data indicate that non-fixed tissue specimens may be transported on ice for hours without any major influence on RNA quality and expression of the selected genes. They believe that RNA degradation detected in some of tissue bank samples more likely is explained by surgical trauma before transport, or by suboptimal storage or mistreatment after biobanking, rather than by a long transport time on ice as a non-fixed surgical specimen. The temperature of -80°C was recognized as a proper condition for the majority of tissue banks. But how long preservation at -80°C affects the activity and integrity of biological macromolecules is still unclear. Many studies have shown that cryopreservation did not affect the DNA quality. Even the DNA extracted from paraffin-embedded tissue can be used for molecular analyses. Demetrick<sup>[19]</sup> used cryopreserved breast cancer tissue for fluorescence *in situ* hybridization and found that both cytologic morphology and hybridization capacity were well preserved in archived frozen tissue, thus easily permitting its use for *in situ* hybridization experiments. RNA is a much more labile molecule than DNA, due to high tissue concentrations of endogenous lytic enzymes, and is substantially degraded by formalin fixation and paraffin embedding<sup>[20]</sup>. RNA is rapidly degraded by RNAase of cells, skin, saliva or environment, if the tissues are not put in liquid nitrogen at once. The quality control of RNA is based on the analysis of the ratio of 28 S and 18 S. RNA electrophoresis is used for assessment the 28 S and 18 S ribosomal RNA bands by ethidium bromide staining. A ratio of 2 represents a good quality RNA. If the ratio is equal to or less than 1, then the RNA is damaged<sup>[5]</sup>. Finally, there is no systematic study about the influence of cryopreservation on protein quality. We need to accumulate the experience of different tissue banks to establish the quality control standard for proteins.

## CONCLUSION

In this article, the significance, collecting procedure, temperature requirements, public opinion and quality control for biological resource collection and tissue bank creation are addressed. It is no doubt that the setup of a tissue bank is a team work involving doctors, nurses, technicians, office clerks and computer scientists. It needs an excellent coordination between multiple disciplines during the whole procedure.

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