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REVIEW

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Tumor banking for health research in Brazil and Latin America: time to leave the cradle

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Abstract

Cancer is rapidly growing to be one of the major health burdens in Brazil and Latin America. Access to tumor samples is one of the many barriers that need to be removed in order to promote clinical and translational research aimed at developing and improving cancer prevention and treatment in this region. Although there is a growing interest in establishing tumor collections in many hospitals and institutions, success is limited by the lack of knowledge of the complexities of this activity. This article reviews the regulatory, pathology, and molecular aspects that are relevant to the establishment of tumor banks in Brazil and Latin America. It also provides an overview of key players in the region.

Keywords: Tumor banks, Regulatory aspects, Pathology and molecular aspects, Brazil, Latin America

Background

For a long time, research in Brazil and Latin America with human biological material had been restricted to universities and a few teaching hospitals. Difficulties in getting sufficient and long term research funding might have accounted for a common characteristic among these types of human biological collections: they were exclusively project driven collections, usually accessed only by those investigators involved in the research itself. Applying a recently proposed schema for sample collections, they would be classified as mono-user or oligo-user biobanks [1].

From the second half of the past century, some events concurred to change this scenario. An increase in development indicators led most countries in South America to experience a socioeconomic transition. Despite the persistent socioeconomic inequality, this transition led these countries to face an aging population and a marked change in the ranking of death causes [2]. In Brazil and many other Latin American countries, cancer is now a major cause of death, second only to cardiovascular diseases. The technical advances and promises brought by the endeavor to sequence the human genome in the late nineties was the second event that led many institutions to decide to create the so called Tumor Banks, so that common cancers in Latin American countries could be studied. However, as opposed to the notion of "research directed-use" associated with project driven collections – which would require that patients knew exactly the research objectives to which the collection of its biological material is associated – this new biobanks proposed that the collection of biological material were authorized for future projects, whose specific objectives were not yet known ("future undefined use"), and that would be conducted by third parties ("poly-user biobanks"). This new concept of "tissue donation" to a central core facility that distributes samples to different projects/researchers brings new ethical problems which are mainly related to issues such as the right of privacy, the ownership and commercial use of human biological material and right of access to information.

Main text

Regulatory aspects

In Brazil, it was felt that the existent legislation did not take into account the differences, both in ethical and operational terms, of these types of biobanks. As a result, a new regulatory framework came into force in 2011 [3]. The Brazilian Guidelines for Biobanks and Biorepositories dedicated to Health Research (Brazilian Ministry of Health Ordinance 2201/11) and the National Health Council Resolution CNS 441/11 (governing the

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ethical analysis of research projects using human biological material) seek to recognize and better regulate both mono/oligo-user and poly-user collections of human biological material. Biobanks (defined in both normative as an institutional facility dedicated to the systematic collection of human biological material to support multiple, future studies) need to be registered at the Brazilian National Research Ethics Commission (CONEP). Since 2014 the ethical analysis of research projects that use human biological material is put on hold if CONEP and/or local ethics review boards find out that the samples come from unregistered biobanks. To be recognized as institutional biobanks, they must adopt a set of procedures to standardize the collection, storage, processing and distribution of human biological material, ensuring its quality and fitness-for-purpose and also the individual rights of donors.

It is expected that in the coming years specific national regulations on biobanking will come into force in Argentina, Chile and other countries in South America. Until then, biobanking and the use of human samples in research will be governed in these countries by specific chapters from prior resolutions, decrees or laws enacted for other purposes (such as those regulating transplant banks or establishing general guidelines for research involving human beings).

Tissue collection and storage requirements

For the biological material collected for biobanks to be effectively used in translational research, special care must be taken during the stages of collection and storage. In the case of tissue samples, it must be kept in mind the potential end-use when defining the best preservation method. Formalin-fixed and paraffin embedded (FFPE) tissues are traditionally used in diagnostic pathology, considered the gold-standard for the morphological preservation of original tissue and can be used in techniques such as immunohistochemistry, *in situ* hybridization and even gene sequencing. However, depending on the molecule of interest and molecular technique to be used, the cryopreservation method is most suitable and has been adopted by many biobanks. There are however crucial factors that need to be controlled.

Cold ischemia is a major factor that can influence the utility of banked samples. It is defined as the elapsed time between the interruption of blood supply and cryopreservation of the tissue sample, and if not controlled may result in significant global gene expression changes [4–6]. Currently there is no universally accepted maximum cold ischemia time [7, 8], and although the guidelines of the A C Camargo Biobank suggest a maximum of 30 min, collections that exceed this time are allowed in order to detect and correct deviations from the maximum time window. On average, 80% of the samples at the A C Camargo are collected within 30 min and the average collection time for the role inventory of tissue samples is 24 min.

Likewise, there is no universally accepted temperature for the long-term preservation of frozen tissues. Although many biobanks use −80 °C freezers, the International Agency for Research on Cancer (IARC) recommends that this temperature should be below − 130 °C (which is the glass transition temperature of water) [7, 8]. In theory, tissues that are quickly frozen and kept below this temperature are free of any degradation of macromolecules (e.g., hydrolysis or enzymatic degradation) [9–11]. In a recent study, our group showed no significant change in RIN (RNA Integrity Number) values of cryopreserved tissues stored at −140 °C for up to 7 years [12]. On the other hand, researchers at the Indiana University, examining sets of samples that were stored at −80 °C observed that all samples with less than 18 months of storage had high-quality RNA (also analyzed by RIN), compared to only 48% of the samples that were stored for longer periods (>8 years) [8]. Currently, there is no published study that has compared the quality of macromolecules extracted from tissues that were submitted to the same collection and cryopreservation protocols and stored at −140 and −80 °C, so biobanks that intend to store tissues for long periods of time should consider adopt temperatures below −130 °C (either using cryogenic freezers or liquid nitrogen containers) [11, 12].

Quality assurance – histology and molecular aspects

Histological review by a pathologist is needed to assure that samples represent the tumor that is being banked, and that non-neoplastic tissues represent the histological counterpart of such tumor. Factors that may influence the quality of the sample and the decision to use it in a certain research include the percentage of viable tumor cells, necrosis, and whether the tumor is infiltrating non-neoplastic tissue in a manner that impedes enrichment using a macrodissection technique [13].

Another critical issue in defining protocols for biobanking practices is the preservation of macromolecules for clinical and translational studies. Tissue storage temperature, and the length of time that tissues and purified RNA stay frozen, may directly impact macromolecule preservation, particularly RNA. In the same study in which our group analyzed the storage conditions of tissues, the long-term preservation of RNA molecules was also addressed. With respect to RNA aliquots (which are stored at −80 °C), our results shown that the concentration of stored RNA strongly affects its integrity in long-term storage, indicating that biobanks that intend to distribute not only tissues, but also extracted RNA macromolecules for research must also control this variable, storing RNA in high concentration (>200 ng/μl) [12].

Overview of key players in Brazil and Latin America

In Brazil, three Tumor Banks can be regarded as key biobank players. The Biobank of the A C Camargo Cancer Center (A C Camargo Biobank) started as a Tumor Tissue Bank in 1997 with the goal of providing high quality annotated tumor and non-neoplastic samples to the then called Human Transcriptome Project. In 2004, a central facility for the extraction and distribution of macromolecules (mainly DNA and RNA) was created, in order to maximize the use of samples and to have a more strict control of quality control metrics [13]. Another characteristic of this Biobank is the involvement of surgical pathology residents in its sample procurement activities, so that residents are exposed to the different technical as well as ethical aspects of biobanking. With approximately 62,000 samples from 22,000 patients, the A C Camargo Biobank has provided sample aliquots for approximately 50 different research projects per year in the last three years (internal data as of December 2015). Samples provided by this Biobank are available to the A C Camargo Cancer Center research staff as well as the general scientific community through the establishment of research collaborations. Being the first tumor bank to be created and certified in Brazil, this biobank has been serving as a model for the establishment of other biobanks in Brazil as well as in other South American countries.

The first public initiative to create a Tumor Bank was carried out by the Brazilian National Cancer Intitute (INCA) in 2004, and the first patient to provide samples was included in 2005. Since then, the so called Brazilian National Tumor and DNA Bank (BNT-INCA) has collected 40,609 sample aliquots from 31,128 patients (as of July 2016) [14]. BNT-INCA has initiatives to motivate the creation of other public tumor banks in Brazil, through the establishment of a Brazilian Network of Tumor Banks. BNT-INCA is also part of a group of tumor banks linked to the South American Network of National Cancer Institutes (RINC/UNASUR). The so called "Red de Biobancos de Latinoamérica y Caribe" (REBLAC) was created in 2008 with the aim of establishing tumor banks in public cancer care institutions, as well as harmonizing operating procedures, and ethical/legal guidelines within the scope of RINC/UNASUR [15]. Together with BNT-INCA, 18 institutional Tumor Banks from Argentina, Bolivia, Chile, Colombia, Ecuador, México, Peru and Uruguay are members of REBLAC. In 2015, REBLAC changed its policies to allow nonpublic institutions to join the network. We have no record of researches performed using samples collected by REBLAC, possibly because currently there is no common software for sample procurement among the different biobanks. The REBLAC has been promoting bi-annual meetings in which its members discuss the current situation of the network, provide updates of their biobanks and plan future actions. Some of the proposed actions are: [15]

- To perform technical site visits in institutions that have been recently incorporated in the network or who intend to join in the near future.
- To provide training for professionals involved in biobanking activities (pathologists, technicians, nurses, TI analysts) at the BNT-INCA or the National Cancer Institute of Colombia ("Banco Nacional de Tumores Terry Fox").
- To have at least two thematic workshops to implement common technical procedures and requirements for quality management and accreditation/certification of participant biobanks.

The third tumor bank created in Brazil is the so called "Banco de Tumores Ricardo Renzo Brentani", located at the Barretos Cancer Hospital, a tertiary non-profit cancer care institution located in the city of Barretos, approximately 450 km distant from the city of Sao Paulo. The Barretos Cancer Hospital is known as a reference cancer center for nearby cities, as well as remote areas of the country for patients without access to private health insurance. This biobank was created in 2006, and, as of April 2014, had approximately 115,000 samples from diverse origins (including paraffin blocks from the Department of Anatomic Pathology) in its database. In 2013, approximately 8,000 samples had been used in different research projects. Access to samples for external investigators are granted through the establishment of research collaborations, as occurs with the A C Camargo Biobank.

In Argentina, aside from the public biobanks that are associated to REBLAC, the Biobank of CEMIC ("Centro de Educación Médica y Investigaciones Clínicas", a medical and research center located in the city of Buenos Aires) was created in 2008 and also focuses in the collection of tumors and non-neoplastic controls. To date, samples from approximately 7,300 patients have been accrued (including fresh-frozen tissue samples, FFPET and tissue-derived DNA/RNA). Researches already performed have focused in the clinical application of molecular biology for the detection of micrometastasis (mainly from breast cancer, colon cancer and melanoma). Currently, researchers from the A C Camargo Biobank and CEMIC collaborate in the study of pre-analytical factors and their impact in the quality of fresh-frozen tissue samples.

Conclusions

Challenges and future perspectives

With the exception of Brazil, research tumor banks in other Latin American countries operate without specific regulation. This may pose a problem for the execution

of collaborative projects or the establishment of an active network of biobanks dedicated to the research of a specific disease (like the above mentioned REBLAC, which is still in the process of establishing minimum technical standards and common rules for sample access within the network). Widespread knowledge of existent/future national regulations and efforts to identify minimum common requirements will be mandatory to facilitate trans-border and overseas exchange of samples [16].

The Latin America region also needs to break out the paradigm of public versus not-for profit private biobanks. A common forum in which both types of biobanks can participate is highly desirable, as well as a decisive move towards integration in the international biobanking society. Only the A C Camargo Biobank has representatives at the International Society for Biological and Environmental Repositories (ISBER), a global forum for sharing knowledge on biobanking [17]. We believe that the exchange of ideas and experiences with other biobanking professionals, as well as the sharing of difficulties, are a source of useful information to improve daily operations.

An additional challenge is the absence of specific funding for existent and future biobank initiatives. In Brazil, as well in Argentina, human biological samples cannot be sold. Although the adoption of a cost recovery model is recommended, this alternative may prove insufficient to secure long-term viability of institutional biobanks. Although in Brazil the federal government and some states (particularly the state of Sao Paulo) often provide funding for research-related infrastructure (which could benefit biobanks), such grants are limited in duration and the bulk of available funding is directed at research projects focused on the study of a single disease. This is in sharp contrast with the philosophy of an institutional tumor bank, in which samples from several types of cancers are collected and stored for future unrelated projects. In this particular field, in which cancer samples need time to "mature" (i.e., to have sufficient annotated information and patient follow up in order to maximize their value for translational studies), the current model of funding is clearly inadequate. This may explain why a few biobanks in the South America region have succeeded in establishing and maintaining their operations, and accomplished their role of providing samples to increase the quantity and quality of health research done in South America.

As outlined throughout this chapter, there has been a focus in the establishment of tumor banks in Brazil and Latin America. However, there is a need to develop biobanks to support the study of a variety of diseases related not only to the aging process observed in this region (e.g., neurologic and rheumatologic diseases) as well as those that still pose a burden to the general population because of persistent social inequality (e.g., infectious diseases). There is also a clear need for biobanks aimed at providing samples for the study of other lifestyle diseases related to an increase in socioeconomic indicators, such as obesity and diabetes. To date, due to their characteristics, the collections in Brazil focused in the study of infectious or lifestyle diseases (e.g. the so called "Projeto ELSA Brasil") [18], in spite of the number of samples, are classified by Brazilian regulations as project-driven collections ("biorepositories") because access is restricted to a single researcher or a single research group.

If tumor banks in Brazil and Latin America overcome these challenges and really leave the cradle, they may turn out to be invaluable resources for the study of many human diseases, particularly in an era in which the promise of personalized medicine needs to be based, among other things, on a solid knowledge of the genetic background of each population .

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