Indian Journal of Biotechnology and Pharmaceutical Research
Journal homepage : www.ijbpr.in
ISSN: 2347-3266
2023, 11 (2): 1 –03

Short Communication

Next generation tumor tissue banking: Experiences from a tertiary care cancer center in north India

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ABSTRACT

Biorepositories act as the connecting link between clinicians and cancer research groups. With the advent of the omics era the needs of the researchers are changing from using traditional biosamples like FFPE (Formalin-Fixed Paraffin-Embedded) blocks, slides, frozen tissue, serum, urine, saliva and a scitic fluid to viable samples, which can be used for drug profiling, personalized medicine research and building better models for research. And so our tertiary cancer center based biorepository has entered the next phase of biobanking and has developed a more advanced "Next Gen" repository which includes: viable frozen tissue, Dissociated tumor cells with matched normal, peripheral blood mononuclear cells, and patient derived primary cell lines.

KEY WORDS: Clinical biobanking, biobanking, cell banking, cryopreservation, data management, DNA extraction, digital histopathology

Introduction

The main aim of biobanking has been to provide researchers with high quality samples to enable them to perform basic as well as translational research. Conventionally biobanks have been a source of bio-specimens suitable for genomic or transcriptomic analysis but, lack in providing viable samples that can be used in functional assays which are essential for applications like: drug testing, single cell sequencing, organ on Chip technology, personalized medicine etc.[1]. Biobanks in developed countries do capture the need of the hour and provide with viable samples. But, Indian biobanks thus far, have not been able to cater to the growing needs of the researchers for the same. It is of paramount importance that Indian biobanks catch up with new age technologies to provide researchers with viable samples from Indian patients to allow rapid advancements in disease understanding as well as treatment especially, for diseases specific to the Indian subcontinent like cancers of the head and neck.

Received: 29.05.2023, Revised: 07.06.2023, Accepted: 23.06.2023
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At Rajiv Gandhi Cancer Institute and Research Center (RGCIRC), New Delhi we aim to overcome this limitation of viable samples/ cells and develop a more advanced-"Next Gen" repository which included: viable frozen tissue, Dissociated Tumor cells with matched normal, peripheral blood mononuclear cells, and patient derived primary cell lines. It is crucial that such an advanced repository be supplemented with clinical and pathological annotation, demographic details, treatment and complete follow up history of the patient. This holistic approach is essential for designing more appropriate hypothesis and disease models. Another fundamental aspect is the quality of the samples, for which we ensure that the sample is free from any microbial or fungal contamination prior to freezing. Microbial growth of both gram positive and gram-negative bacteria is ruled out by inoculating the supernatant from cell preparations in bacterial media for 24 hours. Apart from this, automated cell counts and viability (percentage of live cells/dead cells) are recorded so as to provide the researcher with good quality samples.

We have so far isolated paired peripheral blood mononuclear cells and dissociated tumor cells from more

than 100 patients. Apart, from a pan cancer repertoire of nearly 50,000 fresh frozen tissues, plasma samples and buffy coat, we have also preserved more than 100 viable tissue samples. We also customize and prospectively isolate samples for various academic groups allowing researchers to have samples which are "fit-for-purpose" for their studies (Table 1).

As is well established that tumor in each patient behaves differently depending upon various factors such as immune response, angiogenesis, genetic as well as energetic makeup, environmental factors, exposure to carcinogens etc.[2] A similar observation was obtained during both culturing primary cells and their dissociation into a single cell suspension.

Two striking observations from an experience of more than 30 samples are:

1. The tissue obtained from head and neck sites other than the tongue has higher degree of calcification [3]. Hence, are both difficult to digest and yield lesser number of cells/100 mg of tissue as compared to tissues received from resections of other cancer types and typically requires more time for digestion.
2. The ovarian tumors of the mucinous type have a lower cellularity and more mucoid matter hence resulted in lower counts of cells/ 100 mg of tissue [4].

With the technological advancements in the past decade, artificial intelligence and machine learning has picked up pace to a great extent and finds one of its many applications in disease diagnosis[5]. As a part of our objective of supporting new age technologies we have played an instrumental role in providing tumor tissue Haematoxylin & Eosin stained slides to build digital histology libraries as well as use deep learning strategies for standardizing diagnostic classification which could also predict an underlying molecular change. Now, we provide digital pathology as a regular service to start ups working in the arena of Medical Artificial Intelligence. Another interesting project by TeraLumen Solutions that we have supported is an artificial intelligence based model development. It enables a probe to detect margin negativity in oncology specimens intraoperatively[6].

Table 1: Method of isolation and quality checks performed for dissociated tumor cells, peripheral blood mononuclear cells and viable tissue.

Sample Type	Method of isolation/storage	Quality check	Number of samples prepared
Dissociated tumor cells	Transportation of tumor tissue from histopathology section to cell culture laboratory ↓ Washing of tissue with media+ antibiotic-antimycotic solution(5X) ↓ Digestion of tissue with collagenase and DNase1 ↓ Obtaining single cell suspension by spinning at 300Xg ↓ Storing single cell suspension in FBS+DMSO* at -80˚c.	1. Supernatant is incubated at 37˚c for 48 hours for bacterial/ fungal contaminants. 2. Microbial growth check by overnight incubation in media (both gram+ and gram- bacteria) 3. Cell counting manually using Neubaer's chamber. 4. Automated cell counting using Cell Counter model R1 (OLYMPUS). **Note**: Only those samples are chosen which are viral markers and covid-19 negative.	120 samples (3 aliquots each)
Peripheral blood mono nuclear cells	Ficoll density gradient based isolation followed by storage at -80˚c in FBS+DMSO*.	5. Cell counting manually using Neubaer's chamber 6. Automated cell counting using Cell Counter model R1 (OLYMPUS). **Note**: Only those samples are chosen which are viral markers and covid-19 negative.	125 samples (2 aliquots each)
Viable tissue	Tumor chunk is washed in PBS+ antibiotic-antimycotic solution(5X) followed by chopping of tissue in $1mm^3$ bits and stored at -80˚c in FBS+DMSO*.	**Note**: Only those samples are chosen which are viral markers and covid-19 negative.	129 samples (3 aliquots each)

*Fetal Bovine Serum+ Di-methyl Sulfoxide

The overarching aim of the biobank at RGCIRC has been to provide researchers with high quality samples along with patient data to allow valuable cancer research. Therefore, we are entering in the next phase of biobanking where in viable samples more suited for functional assays shall be stored and made available for academic research along with supporting in-house research facility. Keeping stride with the cutting edge machine learning technologies we provide a platform for training various digital pathology software's as well as provide digital pathology as a regular service.

Acknowledgement

AnuragMehta[a], JuhiTayal[b], D.C. Doval[c], SomikaTewari[b], Sudhir Rawal[d].

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Funding Statement:

Not applicable

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How to Cite This Article:

Soni D, Tayal J and Sharma A. Next generation tumor tissue banking: Experiences from a tertiary care cancer center in north India. Indian J. Biotech. Pharm. Res. 2023; 11 (2): 1 – 03.