Biobanking: An Important Resource for Precision Medicine in Glioblastoma

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Si Yan Melanie Tan, Edwin Sandanaraj, Carol Tang*, and Beng Ti Christopher Ang*

Abstract

The Cancer Genome Atlas effort has generated significant interest in a new paradigm shift in tumor tissue analysis, patient diagnosis and subsequent treatment decision. Findings have highlighted the limitation of sole reliance on histology, which can be confounded by inter-observer variability. Such studies demonstrate that histologically similar grade IV brain tumors can be divided into four molecular subtypes based on gene expression, with each subtype demonstrating unique genomic aberrations and clinical outcome. These advances indicate that curative therapeutic strategies must now take into account the molecular information in tumor tissue, with the goal of

*Carol Tang and Beng Ti Christopher Ang are Co-corresponding authors for this chapter.

S.Y.M. Tan
Department of Research, National Neuroscience Institute, Singapore 308433, Singapore

School of Biological Sciences, Nanyang Technological University, Singapore 637551, Singapore
e-mail: melanie_sy_tan@nni.com.sg

E. Sandanaraj
Department of Research, National Neuroscience Institute, Singapore 308433, Singapore

School of Biological Sciences, Nanyang Technological University, Singapore 637551, Singapore

Singapore Institute for Clinical Sciences, Agency for Science, Technology and Research (A*STAR), Singapore 117609, Singapore
e-mail: Sandanaraj_Edwin@nni.com.sg

C. Tang (✉)
Department of Research, National Neuroscience Institute, Singapore 308433, Singapore

Humphrey Oei Institute of Cancer Research, National Cancer Centre, Singapore 169610, Singapore

Duke-National University of Singapore Medical School, Singapore 169857, Singapore
e-mail: carol_tang@nni.com.sg

B.T.C. Ang
Singapore Institute for Clinical Sciences, Agency for Science, Technology and Research (A*STAR), Singapore 117609, Singapore

Duke-National University of Singapore Medical School, Singapore 169857, Singapore

Department of Neurosurgery, National Neuroscience Institute, Singapore 308433, Singapore

Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117593, Singapore
e-mail: ang.beng.ti@singhealth.com

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identifying molecularly stratified patients that will most likely to receive treatment benefit from targeted therapy. This in turn spares non-responders from chemotherapeutic side effects and financial costs. In advancing clinical stage drug candidates, the banking of brain tumor tissue necessitates the acquisition of not just tumor tissue with clinical history and robust follow-up, but also high quality molecular information such as somatic mutation, transcriptomic and DNA methylation profiles which have been shown to predict patient survival independent of current clinical indicators. Additionally, the derivation of cell lines from such tumor tissue facilitates the development of clinically relevant patient-derived xenograft mouse models that can prospectively reform the tumor for further studies, yet have retrospective clinical history to associate bench and *in vivo* findings with clinical data. This represents a core capability of Precision Medicine where the focus is on understanding inter- and intra-tumor heterogeneity so as to best tailor therapies that will result in improved treatment outcomes.

Keywords
Glioblastoma • Histology • Glioma-propagating cells • TCGA • Patient-derived xenografts • Tumor resource • Precision medicine • Connectivity map • Patient stratification • Bioinformatics

Abbreviations

bFGF	Basic fibroblast growth factor
CMAP	Connectivity map
CNS	Central nervous system
EGF	Epidermal growth factor
GBM	Glioblastoma multiforme
GEMM	Genetically engineered mouse model
GPCs	Glioma-propagating cells
MRI	Magnetic resonance imaging
NIH	National Institutes of Health
PDX	Patient-derived xenograft
TCGA	The Cancer Genome Atlas

4.1 Introduction

Glioblastoma multiforme (GBM) remains a cancer with the worst prognosis. Patients often show a median survival period of 15 months, even with advanced surgical intervention, chemotherapy and radiation treatment [1]. Temozolomide, the standard of care drug in the clinic, has annual global sales of US$ 1 billion, yet it merely extends survival by 3 months. Among the reasons for the highly invasive and recurrent nature of the disease lies in the cellular and molecular heterogeneity of GBM. Recent deep molecular profiling efforts such as The Cancer Genome Atlas (TCGA) demonstrated that histologically identical GBM tumors are molecularly heterogeneous, further suggesting that their regulatory pathway networks determine each tumor's sensitivity to targeted therapeutic approaches [2, 3]. While these computational analyses reveal the putative mechanisms underlying tumor resistance and recurrence, biological or functional validation in preclinical animal models is lacking. In the past decade, the use of mouse xenograft models created from commercially procured serum-grown glioma cells has been challenged by studies demonstrating that such xenografted tumors fail to recapitulate the patient's original tumor morphology and transcriptomic profiles [4]. In addition, such *in vitro* serially passaged serum-grown cells

often contain genomic aberrations not found in the original primary tumor [5, 6]. Indeed, the widely used US National Cancer Institute NCI-60 panel of human cancer cell lines commonly passaged in serum-containing media will soon be decommissioned, with an aim to launch a rejuvenated repository of cancer models that are derived from fresh patient samples and tagged with details about their clinical past [7].

Glioma-propagating cells (GPCs) have been isolated from malignant brain tumors, and cultivated as spheroid structures in serum-free media supplemented with growth factors [8]. This media composition is similar to that used to passage neural stem cells, and helps promote self-renewal and tumorigenicity of GPCs [9]. In contrast, the addition of serum encourages differentiation of GPCs, resulting in cessation of cell proliferation with subsequent involution of tumor growth. We and others previously demonstrated the preservation of karyotypic hallmarks in these cells, similar to the original tumors [6, 10]. We developed a method to passage these spheroid structures by mechanical trituration, thus avoiding constant use of harsh enzymatic solutions that have been shown to alter karyotypic patterns in human embryonic stem cells grown as embryoid bodies [11]. We discuss vitrification, a technique adapted from *in vitro* fertilization procedures, and emphasize the importance of preserving these spheroid structures with reduced water content to prevent damage from ice crystals during the thawing process. Collectively, these methodologies preserve the integrity of GPCs and their ability to establish patient-derived xenograft (PDX) tumors that faithfully phenocopy the original tumor pathophysiology, cytogenetic and transcriptomic profiles. This capability reflects the importance of establishing a cell line and tumor tissue bank that presents the most clinically relevant resource to test and validate computational predictions generated from large patient glioma databases.

Crucial to establishing clinical relevance of our brain tumor resource, we discuss computational platforms such as the Connectivity Map to link molecular data acquired from *in vitro* and animal studies with multi-dimensional clinical information such as the patient's age, tumor grade, molecular information, Karnofsky score and magnetic resonance imaging (MRI) scans available in large clinical databases such as TCGA [12]. These computational advances have expanded the scope of studies made possible with our brain tumor resource [13–17]. Importantly, we now have a brain tumor biobank that facilitates studies in Precision Medicine where biological validation of patient-centric predictions is achievable.

4.2 Brain Tumor Resource

With the advance of TCGA efforts, several brain tumor banks containing low-passage cell lines have been established with a focus on acquisition of clinical history with deep content molecular information (genotype-phenotype databases). The goal of such tumor banks is to facilitate studies requiring capability to remodel the disease accurately so as to prospectively test computational predictions based on patient information [18]. Central to this biobank creation, we previously demonstrated three important criteria: (1) Growth of patient-derived glioma cells in serum-free media supplemented with growth factors, (2) Vitrification as a cryopreservation method, and (3) Ability to establish orthotopic PDX models that recapitulate the patient's original tumor pathophysiology. As such, genotype–phenotype databases previously needed only simple computing technologies, including very basic data fields relating to pathogenicity, but did not capture the process of pathogenicity interpretation. Going forward, this approach will have to change, especially if we wish to deliver truly Precision Medicine-based findings, which will require mechanistic in addition to probabilistic modeling, and hence even more sophisticated sources of input information and tools for the recording of results.

GPCs can be maintained and propagated as tumor neurosphere cultures in defined serum-free condition supplemented with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), a paradigm that is adopted from the traditional neurosphere culture [19, 20]. Furthermore, Lee and colleagues have shown that tumor stem-like cells grown in serum-free condi-

tion closely mimic the genotype, transcriptomic profile and morphological features of their parental tumors [6]. Thus, the establishment of a tumor neurosphere repository with preservation of essential features of tumor heterogeneity would provide a clinically relevant resource to investigate the disease. Such a method would also allow us to return to the same experimental cell line passages to reduce variability in experimental replication. Recent efforts by the Roadmap Epigenomics Project spearheaded by the National Institutes of Health (NIH) highlighted epigenetic silencing differences of *in vitro* cultured cells when compared to similar cell lineages in primary tissue, thus underscoring the importance of cryopreserving low-passage GPCs with retention of its molecular fingerprint [21]. In many studies involving the prospective isolation of tumor-propagating cells, only small amounts of clinical material are available, and this limitation is compounded by lack of appropriate methods to preserve such cells at convenient time points. Although *in vivo* serial passage of GPC-derived tumors has been described as the most reliable method to preserve the cells, practically, it is not always feasible to have access to suitably-aged immunocompromised mice [8]. We developed mechanical trituration as a method to passage three-dimensional spheroid cultures for the reason that acute dissociation of such structures into single cells promotes cellular senescence over an extended period [9]. In addition, induction of cellular differentiation, such as by the presence of serum, results in loss of tumorigenicity and consequent involution of tumor growth [22].

We adapted a method used in cryopreservation of embryos from *in vitro* fertilization procedures. Vitrification is a process of glass-like solidification in which an aqueous solution is prevented from crystallization by rapid cooling [23]. This method has been commonly used for the cryopreservation of embryos at different developmental stages from various species such as murine, rabbit, sheep and bovine [24–29]. Furthermore, human and mouse multi-cell embryos have been successfully cryopreserved using this strategy [30]. This highlights the feasibility of cryopreserving cellular aggregates. In addition, it has been demonstrated that vitrified embryonic stem cells retained their pluripotency, cytogenetic profile and viability upon thawing [31]. Taken together, vitrification could provide an effective means of storage of brain tumor-propagating cells cultured as spherical structures. Although adherent GPC cultures using laminin have been proposed, these growth conditions resulted in transcriptomic shift of the cells [32]. In support, Dirks and colleagues showed that a chemical genetics screen utilizing GPC spheroid cultures identified small molecules affecting neurotransmission in the adult central nervous system (CNS), thus suggesting that clinically approved neuromodulators may remodel the mature CNS and find application in the treatment of brain cancer [33].

To facilitate testing of patient stratification methods and identification of molecular mechanisms contributing to disease progression, a genomic roadmap is created to characterize the cells, tumor xenografts and primary tumors. The pivotal goal of molecular profiling of patient-derived cells aims to stratify patient cohorts and identify amenable therapeutic strategies. The content-rich patient tumor molecular profiles need to be systematically archived for efficient data mining to evaluate proof-of-concept studies. Initial steps at identifying potential cancer-specific biomarkers require patient-centric bioinformatics interrogation, information from which can then be used for the analysis of samples stored in tissue banks. In TCGA, the quality of samples acquired was assessed from several participating tissue banks that surprisingly showed only one percent of the samples being reliable for downstream molecular data acquisition [2, 34, 35]. Sample quality and the associated clinical information are important factors in tissue banking. The importance in having expertise at tissue sampling and culturing of patient-derived cells, with preservation of cytogenetic and transcriptomic hallmarks found in the original primary tumor has previously been reported by our colleagues [6, 10]. Precision Medicine-driven studies, dependent on this molecular heterogeneity, warrant a preclinical mouse model that recapitulates the patient's original tumor pathophysiology and aids at advancing chemotherapeutic candidates into clinical trials.

4.3 Animal Model Established from the Biobank: An Informative Preclinical Mouse Model

Modeling brain tumors in animals reveals genetic events and molecular mechanisms that contribute to oncogenesis. The mouse shares extensive molecular and physiological similarities to human beings and is a powerful tool for studying cancer [36]. Unlike invertebrate model systems, tumor development in mice is accompanied by other complex processes such as angiogenesis and metastasis, similar to those in human cancer [37]. More importantly, mouse tumor models provide a temporal perspective and genetically-controlled system for studying the tumorigenic process, as well as response to specific therapies.

Genetically engineered mouse models (GEMMs) are a popular model to study tumor biology [37]. There are several limitations to using GEMMs as more than one driver mutation is required to initiate tumorigenesis [38, 39]. The expression of the transgene is often elevated to levels that exceed those in patients. Tumors that arise in this model are often sporadic, resulting in difficulty of study designs that require significant animal numbers for reproducibility. TCGA efforts have also demonstrated that the spectrum of driver mutations differs significantly among patients, thus the relevance of a particular GEMM may be limited [2, 40]. Nevertheless, GEMMs will likely provide useful insight into the tumor cell-of-origin and initiating events.

Xenograft models established from commercially procured serum-grown cell lines date back to the late 1960s. However, several studies demonstrated that such xenografted tumors exhibit significant morphological and molecular features not found in the original primary tissue [4, 6]. Such issues have subsequently been overcome through the development of PDX models. Several studies revealed that the orthotopic xenograft model established from patient-derived glioma cell lines or tumor explants bear more clinical relevance [41–43]. This is due to the presence of the microenvironment provided by the normal brain parenchyma, where better measurement of drug delivery and clearance kinetics can be evaluated. The orthotopic PDX model is useful as tumor formation with high incidence and the ability to generate large cohorts of animals in preclinical studies are attainable. Several investigators have provided evidence that PDX tumors phenocopy the pathophysiology and molecular features of their parental tumor [6, 44]. Importantly, this model allows assessment of therapeutic responses using stratified clinical material, a core capability of Precision Medicine. We and others previously integrated the use of such an in-house brain tumor resource to interrogate lab findings in clinical glioma databases [7, 10, 14–17]. We showed that transcriptomic patterns derived from *in vitro* drug-treated or genetically manipulated cells mapped to patient clinical databases, and dictated primary tumor phenotype.

Despite the frequent use of the PDX model in studying drug therapy response, we recognize that immunogenic and microenvironmental factors may not be fully represented. Additionally, engraftment inefficiency can be as high as 90 %, depending on the type of cancer [45]. Such limitations suggest that the use of mouse models should be carefully considered to provide maximum information about the study question.

4.4 Enabling Precision Medicine-Based Studies: Highlighting the Importance of a Biobank

A brain tumor biobank that merges multi-platform data from patient material (cells, xenograft and primary tumors) and seeks to address clinical and imaging phenotypes with molecular data will advance studies in stratified medicine. The development and preclinical validation of novel anti-cancer drugs require low-passage cell lines that are representative, scalable and reproducible in experimental models. In the absence of an integrated human brain biobank, research findings from *in vitro* and *in vivo* models of neurological disorders cannot be functionally validated in the actual disease context. TCGA efforts

revealed that gene expression drives GBM disease progression and survival outcome [3, 46–48]. Although an important prognostic factor of glioma progression relies on the World Health Organization (WHO) grading scheme, the wide differences in treatment response and survival suggest that the aggressiveness of treatment cannot be decided just by histology alone. These findings underscore the limitation of relying solely on morphological criteria to diagnose patients. Future brain tumor classification must now include molecular information which in turn guides diagnosis and subsequent treatment decision [49]. Development and maintenance of biobanks as an international resource for the study of human diseases provides the scientific community with well-characterized cells and rich phenotypic data. Such resources facilitate prospective remodeling of the disease in mouse models, with retrospective clinical information to evaluate correlation patterns and directly validate the mechanism.

Targeted therapy is an attractive approach to overcome the highly infiltrative and recurrent nature of GBM. Recent characterization of the epigenome, somatic mutation profile and transcriptome of tumor tissue has now provided a deeper understanding of the alterations underlying the disease phenotype [2, 3, 50, 51]. Tumor cells are assessed for the underlying pattern using unsupervised computational approaches to discern their molecular heterogeneity. An important evaluation is to computationally identify regulatory pathways that can be targeted with small molecule drugs. These predictions are then functionally validated in PDX models. Transcriptomic resources of xenograft and primary tumors are scrutinized for highly variable genes which can reflect a common bias present among primary tumors and xenografts established from GPCs. This common and systematic bias can be controlled and nullified by statistical algorithms such as Anova-based batch effect removal and principal component based analysis [52, 53]. A scatter plot accounting for major principal components across matched primary and orthotopic tumors demonstrates close proximity of matched samples, indicating that GPCs can recreate the original tumor molecular profile *in vivo* (Fig. 4.1).

Computational evaluation of matched molecular data from biobanked patient tumors and GPCs requires systematic validation of molecular profiles using bioinformatics approaches. A computational pipeline is required to interrogate gene signatures (transcriptomic classifiers) derived from GPCs and a large set of independent predictive database collection from patients' molecular data [12, 54, 55]. Molecular perturbation experiments on GPCs have demonstrated promising evidence in conferring the patient's prognosis in predictive databases and *in vivo* experiments [13, 15]. The most comprehensive glioma patient's database was established by TCGA, where key components of clinical phenotype and genomics information were catalogued into a multi-tier organizational structure for 33 different tumor types [56]. Each tier is confined with a data structure from genomic platforms including somatic mutation, copy number, methylation, transcriptomic and proteomic technologies. Importantly, we have merged our cell and xenograft tumor

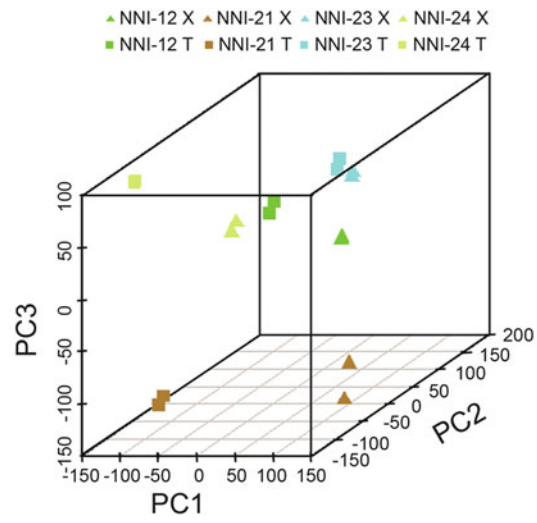

Fig. 4.1 Recapturing molecular portrait of primary tumors in orthotopic xenograft tumors derived from GPCs. Principal Component Analysis (PCA) map demonstrates similar transcriptomic profiles between matched xenograft and primary tumors. Each *color* denotes similar patient material with corresponding xenograft tumor. *Triangle*, xenograft tumor; *square*, patient tumor

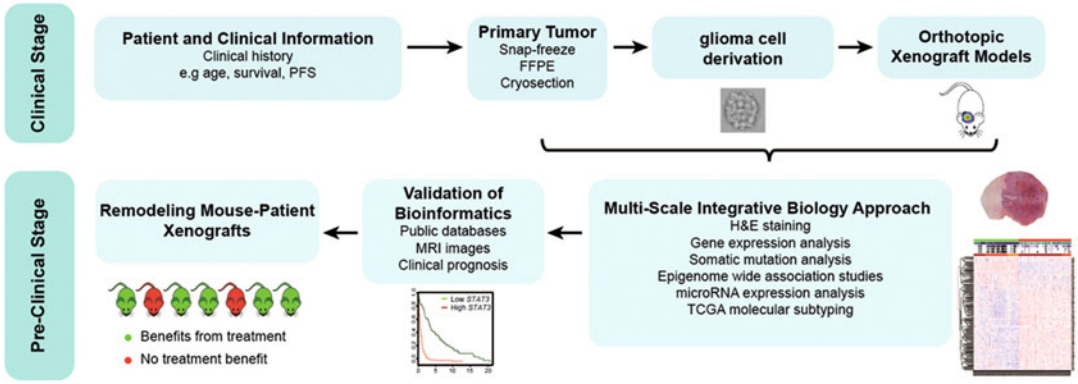

Fig. 4.2 Graphical summary for brain tumor resource. A brain tumor resource allows orthotopic xenograft tumors to be re-established from clinical material, thus facilitating future testing of small molecules identified through molecular stratification approaches

molecular patterns with international collections by adapting the statistical framework to control for systematic batch effects across different collections [52, 53]. This integrated evaluation also confirms the consistency across tumor cell types and provides greater statistical power [57]. We have successfully adapted a qualitative enrichment pipeline, the Connectivity Map (CMAP) to interrogate active pathway programs coded as gene signatures in our biobanked cells with our patients' transcriptomic patterns [12, 13, 17]. These patients' prognoses can then be retrospectively predicted by mapping survival and clinical response parameters with enrichment scores from the CMAP pipeline. Thus, molecular perturbation experiments tapping into our brain tumor biobank serve as effective tools to biologically validate these patient-centric computational predictions.

4.5 Conclusion

Biobanking coupled with deep molecular characterization is a core capability for Precision Medicine-based studies (Fig. 4.2). The ability to remodel brain tumors in mice facilitates biological and functional validation of computationally predicted pathway networks that should be therapeutically targeted.

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