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Research Article

The Human Glioblastoma Cell Culture Resource: Validated Cell Models Representing All Molecular Subtypes

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ABSTRACT

Glioblastoma (GBM) is the most frequent and malignant form of primary brain tumor. GBM is essentially incurable and its resistance to therapy is attributed to a subpopulation of cells called glioma stem cells (GSCs). To meet the present shortage of relevant GBM cell (GC) lines we developed a library of annotated and validated cell lines derived from surgical samples of GBM patients, maintained under conditions to preserve GSC characteristics. This collection, which we call the Human Glioblastoma Cell Culture (HGCC) resource, consists of a biobank of 48 GC lines and an associated database containing high-resolution molecular data. We demonstrate that the HGCC lines are tumorigenic, harbor genomic lesions characteristic of GBMs, and represent all four transcriptional subtypes. The HGCC panel provides an open resource for *in vitro* and *in vivo* modeling of a large part of GBM diversity useful to both basic and translational GBM research.

1. Introduction

The prognosis for glioblastoma (GBM), the commonest primary malignant brain tumor in adults (Dolecek et al., 2012) is poor with a 1-year survival rate of 36.5% (Ostrom et al., 2014). Standard treatment involves surgery to remove as much of the tumor as possible, followed by combined radiation and chemotherapy with temozolomide (Stupp et al., 2005). GBM is characterized by pronounced invasiveness, as well as extensive intra- and inter-tumor heterogeneity (Patel et al., 2014; Sottoriva et al., 2013; Verhaak et al., 2010).

Transcript profiling in combination with analysis of genomic aberrations have revealed distinct molecular subtypes of GBM (Brennan et al., 2013; Phillips et al., 2006; Verhaak et al., 2010). The most commonly used classification is that presented by The Cancer Genome Atlas Research Network (TCGA), which describes four subtypes (Verhaak et al., 2010), *i.e.* Proneural, Classical, Mesenchymal and Neural GBM. These are defined primarily on the basis of their particular transcriptional signatures but can be associated, at least statistically, with distinctive genetic aberrations. Proneural tumors exhibit a higher frequency of *PDGFRA* or *IDH1* mutations, as well as a G-CIMP$^+$ (glioma-CpG island methylator phenotype) subgroup that displays global hypermethylation, which overlaps with *IDH1* mutations. Patients with G-CIMP$^+$ tumors are younger at the time of diagnosis and have a survival advantage (Brennan et al., 2013; Noushmehr et al., 2010). Classical tumors demonstrate high rates of *EGFR* amplification and homozygous deletions of *CDKN2A*; mesenchymal samples often harbor hemizygous deletions of *NF1*; whereas no distinctive mutations have yet been found in neural GBMs. Subtyping is complicated by the pronounced intratumor heterogeneity and different regions of one and the same tumor can be classified differently (Sottoriva et al., 2013). In addition, single-cell sequencing of cells from five patients has revealed a mixture of subtypes even at the cellular level in each individual patient (Patel et al., 2014).

There is a high demand for readily available and relevant cell models of GBM. For 30 years, several GC lines, including U87 (>1900 citations in PubMed), U251 (>1100 citations) and T98G (>900 citations), have been employed extensively in this context, providing valuable knowledge about this type of tumor. However, these models are imperfect for several reasons. First, the serum-containing medium in which these

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standard cell lines are cultured alters both their genomes and transcriptomes and causes depletion of stem cell-like tumor cells (Lee et al., 2006). Secondly, tumors formed by injection of such cell lines into the brains of mice fail to develop the defining morphological features of GBM, such as diffuse infiltration into surrounding healthy tissue and microvascular proliferations (Lee et al., 2006; Mahesparan et al., 2003; Pontén and Macintyre, 1968; Westermark et al., 1973). And third, the lack of systematic clinical characterization of the tumors from which the current GC lines derive makes it impossible to correlate findings with these models to patient parameters.

There is ample evidence that a minor subset of GBM cells (GCs), denoted glioblastoma stem cells (GSCs) are likely responsible for relapse because they possess a unique capacity for growth and progression (Beier et al., 2007; Galli et al., 2004; Lathia et al., 2010; Ogden et al., 2008; Singh et al., 2004; Son et al., 2009) and are particularly resistant to therapy (Bao et al., 2006; Bleau et al., 2009; Chen et al., 2012). Research designed to improve GBM culture conditions has shown that GSCs can be readily cultured as spheres, utilizing the same conditions as for normal neural stem cells (Hemmati et al., 2003; Ignatova et al., 2002; Singh et al., 2003). Moreover, orthotopical transplantation of such spheres into mice generated secondary tumors that retained the features of the primary tumor (Galli et al., 2004), which was also the case after injection of adherent cultures of GSC (Fael Al-Mayhani et al., 2009; Pollard et al., 2009). Recent extensive work characterizing orthotopic xenograft models showed that acutely transplanted patient-derived GCs mimicked well the histopathology, genomics and phenotypic properties of the corresponding patient's primary tumor (Joo et al., 2013). This study provides an important platform for accurate *in vivo* modeling of GBM but cannot fully meet the need for cell-based models.

Since GSCs cultured under stem cell conditions more accurately mirror GBM biology and because such models are increasingly in demand, we have created a novel library of well-characterized GC cultures that we make publicly available here. We describe the establishment and characterization of 48 sustainable GC lines, derived from Swedish patients during the period of 2009–2012, and including all four molecular subtypes, a biobank we refer to as the Human Glioblastoma Cell Culture (HGCC) resource. This information, along with clinical variables, is also available online (www.hgcc.se). The utility of this database is reflected in the fact that several of these cell lines have already been shared and used to discover a novel potent candidate drug for treatment of GBM (Kitambi et al., 2014), as well as in a number of other studies (Wee et al., 2014; Babateen et al., 2015; Schmidt et al., 2013; Savary et al., 2013; Yu et al., 2013).

2. Materials & Methods

2.1. GBM Patients and Glioma Cell Cultures

Surgical specimens and clinical records for 102 adult patients with glioma were obtained from Uppsala University Hospital in accordance with protocols approved by the regional ethical review board and after obtaining written consent by all of the patients. Most of the tumor specimens were obtained directly from the operating theater, but in some cases from Clinical Pathology. Following World Health Organization (WHO) guidelines (Louis et al., 2007) neuropathologists classified the tumors as grades II–IV. The surgical samples were rendered anonymous and coded. A piece of each was stored at − 70 °C for later RNA extraction and another piece fixed with formalin and embedded in paraffin for histological analysis. The remainder of the specimen was explanted as described in detail in the Extended Experimental Procedures.

2.2. Analysis of Global Gene Expression and Classification of the Molecular Subtype of the GC Lines

Total RNA extracted from 48 GC lines using the RNeasy Mini kit (Qiagen) was labeled and hybridized on Affymetrix GeneChip Human Exon 1.0 ST arrays. Expression levels were RMA-normalized employing the Affymetrix Expression Console software. The GC lines were classified with the *k*-nearest neighbor approach, and bootstrap aggregation in which the classification was repeated 1000 times, each time using a subsampled version of the TCGA dataset (529 randomly selected cases, sampled with replacement from the original dataset). Isomap analysis was applied to visualize the GC lines and TCGA samples in two dimensions. The details of data analysis are provided in the Extended Experimental Procedures. Expression data is made available *via* NCBI Gene Expression Omnibus (GSE72217) and hgcc.se.

2.3. Analysis of Gene Expression by NanoString Technology and Assignment of a Subtype to the Surgical Samples and GC Lines

To determine molecular subtypes, RNA extracted from 22 specimens of fresh frozen human glioma using TRIzol and from the corresponding GC lines in the same manner as described above, was used in a custom-made assay by NanoString Technology. For further details, see the Extended Experimental Procedures.

2.4. Proliferation Assay

The proliferation of 13 GC lines was assessed by the AlamarBlue assay (Invitrogen) and that of 18 other lines by Trypan blue exclusion on Countess Cell Counting Chamber Slides (Invitrogen). See the Extended Experimental Procedures for additional details.

2.5. Analysis of the In Vivo Tumorigenicity of the GC Lines

All animal experiments were performed in accordance with the rules and regulations of Uppsala University and approved by the local animal ethics committee. Neonatal non-obese diabetic-severe combined immunodeficiency (NOD-SCID) mice (P1–3) were injected intracerebrally with 1.0×10^5 human GCs, as summarized in Table S6. The mice were sacrificed when they showed symptoms or otherwise 20 weeks after injection and their brains were analyzed for xenograft tumors. See the Extended Experimental Procedures for additional details.

2.6. Analysis of Aberrations in DNA Copy Number

DNA isolated from 48 GC lines using the DNeasy blood and tissue kit (Qiagen) was profiled on Affymetrix Cytoscan arrays at the Uppsala Academic Hospital Array and Analysis facility, in accordance with the manufacturer's instructions. Identification of segments carrying altered numbers of copies was achieved with the Patchwork R package (Mayrhofer et al., 2013), which quantifies the log-relative change in DNA content for each chromosomal region. CNA data is made available *via* NCBI Gene Expression Omnibus (GSE72209) and hgcc.se. See the Extended Experimental Procedures for additional details.

2.7. Subtype Stability In Vitro and In Vivo

RNA was prepared from cells and tumor tissue and analyzed on the Affymetrix HTA 2.0 platform. See the Extended Experimental Procedures for additional details. Expression data is made available *via* NCBI Gene Expression Omnibus (GSE72218) and hgcc.se.

2.8. Statistical Analyses

The unpaired t-test was used for comparison of two groups, a one-way ANOVA for comparison of more than two groups; and the log-rank test for survival curves. Statistical significance was defined as $p \leq 0.05$. Cox regression was performed using the package *rms* in R 3.1.2. All statistical analyses were carried out with the GraphPad Prism6.0 software.