Spiking GABAergic OPC tumor cells prolong survival in *IDH1* mutant glioma

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Abstract

Prior studies have shown that glioma cells form synapses with neurons to receive

synaptic inputs. To discern if glioma cells can send outgoing electrochemical signals in

the form of action potentials (APs), we employed Patch-sequencing on surgically-

resected human glioma slices. Results showed that half of patched cells in *IDH1* mutant

(IDH1^{mut}) tumors demonstrate select properties of both neurons and glia and fire single,

short APs. To define the transcriptional profiles of these hybrid cells (HCs) and discern if

they are tumor derived, we developed a computational tool, Single Cell Rule

Association Mining (SCRAM), to annotate features in each cell individually. SCRAM

revealed that HCs represent a heterogenous group of tumor and non-tumor cells that

are uniformly defined by both GABAergic neuron and oligodendrocyte precursor cell

(GABA-OPC) transcriptional signatures. We found that GABA-OPC tumor cells express

requisite voltage-gated ion channels needed to fire APs. We validated our findings in

human single cell and bulk RNA-seq datasets, confirming that GABA-OPCs represent

40% of IDH1^{mut} tumor cells, correlate with survival outcomes in IDH1^{mut} human patients

and are also found in select molecular subtypes of *IDH1* wild-type tumors. These

studies describe a new cell type in IDH1^{mut} glioma with unique electrophysiological and

transcriptomic   properties.

Introduction

Glioma is the most common central nervous system tumor with an estimated 20,000 cases diagnosed each year. These diffuse tumors include *IDH1* mutant (IDH1[mut]) and *IDH1* wildtype (IDH1[WT]) subtypes, each of which presents with unique clinical and histopathological correlates. Prognostic outcomes for IDH1[WT] tumors are poor, conferring a median survival of less than 14 months[1]. In contrast, IDH1[mut] tumors confer significantly better prognoses, with a median survival of 31 months after diagnosis[2]. Robust molecular and genomic studies conducted over the last two decades have revealed that the disparity in survival outcomes between glioma subtypes is primarily attributed to differences in tumor cell proliferative capacity and invasiveness[3], which are mediated by an intricate compendium of tumor intrinsic and extrinsic factors. Communication between tumor cells and their microenvironmental constituents has proven to be a critical mediator of glioma progression and is largely mediated by immunological and neural cellular components[4,5]. With regards to the latter, recent advances in the field of cancer neuroscience have revealed that glioma cells form functional synapses with peritumoral neurons and that heightened neuronal activity promotes glioma progression via increased proliferation and invasiveness[5–7]. While glioma cells have been shown to receive excitatory post synaptic potentials (EPSPs) from surrounding neurons[6], whether tumor cells can generate outgoing information in the form of propagating action potentials (APs) remains unclear.

One of the greatest impediments to successful treatment of glioma is the challenge of identifying tumor-specific attributes that can be exploited for therapeutic gain. Over the past decade, technological advances in single cell genomics have generated an

abundance of sequencing data, elucidating the robust transcriptional and genomic

heterogeneity that exists in glioma [8–10]. Moreover, Patch-sequencing (Patch-seq), which

couples whole cell recordings, morphological analysis and single cell RNA-sequencing

(scRNA-seq) permits characterization of both electrophysiological and transcriptomic

features from individual cells[11], and has been used to expound the extraordinary array

of neuronal subtypes present in the mammalian brain[12]. Despite these technological

advances, however, a computational framework capable of accurately distinguishing

glioma cells from non-tumor cells has yet to be developed cell annotation algorithms are

largely unable to assign integrated genomic and transcriptional profiles to single cells on

a cell-by-cell basis. Because glioma cells frequently share molecular profiles with their

non-tumor glial analogs, reliable identification of tumor cells within the brain is a difficult

but necessary task.

To address the aforementioned knowledge gaps, we performed *in situ* Patch-seq

studies in human glioma and developed a new single cell computational tool to

characterize the genomic and transcriptomic features of recorded cells. We found that

nearly half of patched cells in IDH1^{mut} tumors uniformly fire single, short APs and that

this unique electrophysiological phenotype is indicative of hybrid cells (HCs) possessing

select properties of both neurons and glia. Using our novel computational tool, Single

Cell Rule Association Mining (SCRAM) that is capable of accurately annotating tumor

cells and defining hybrid cellular states for individual cells, we found that HCs possess

transcriptomic features of both GABAergic neuron and oligodendrocyte precursor cells

(OPCs), which we term GABA-OPC cells. Prior work has documented shared features

between GABAergic neurons and OPCs, including the expression of GABA receptors

(GABARs)[13,14] and the ability to synthesize and breakdown GABA[15]. Using large scale

scRNA-seq datasets and immunostaining of human tumor samples, we confirmed that

GABA-OPC cells account for 40% of IDH1[mut] tumor cells and are less proliferative than

other tumor cell types. Importantly, IDH1[mut] tumor patients with more robust GABA-OPC

molecular signatures show significantly better survival outcomes as compared to those

without, and lower grade tumors were enriched for GABA-OPC profiles as compared to

higher grade tumors. These data suggest that the ability of tumor cells to generate

functional electrophysiological outputs is more prevalent in lower grade tumors and is

lost as tumor cells become more proliferative and progress to higher grades.

Collectively, our studies reveal a new tumor cell type in human glioma with important

clinically-relevant outcomes and highlight the utility of using our computational

framework for characterizing hybrid tumor cell types in glioma.

Results

IDH1[mut] glioma cells fire single action potentials and are distinct from neurons

and glia

To determine if glioma cells can fire APs, we performed whole-cell patch clamp

recordings followed by scRNA-seq (Patch-seq) on brain slices resected from the tumor

cores of two IDH1[mut] glioma specimens (**Fig. 1a**). We recorded from 53 total cells; 31

were used to recover high-quality RNA for scRNA-seq, and 20 were preserved with

biocytin for morphological analysis. For the 31 cells used for sequencing, an average of

4087 genes were identified per cell. Of the 53 total cells, 27 displayed

electrophysiological and morphological properties consistent with either glia or neurons

in non-tumor brain[16,17] (**Figs. 1b-d**). In contrast, 26 cells displayed select neuronal

electrophysiological properties but were morphologically consistent with glia (**Figs. 1e-**

g). These cells, which we assigned as hybrid cells (HCs) electrophysiologically

represented 49% of patched cells (**Fig. 1h**), had higher input resistances than neurons

or glia (**Fig. 1i**), and were all capable of firing single, small action potentials (**Figs. 1j-k**).

When we attempted to record from an IDH1^{WT} glioblastoma core sample, very few cells

were patchable due to gliosis and necrosis; however, all three cells recorded were

electrophysiologically consistent with glia and unable to generate APs. PCA analysis of

scRNA-seq data showed that all HCs clustered together and most closely with glia

rather than neurons, suggesting that HCs are transcriptionally more similar to glia than

they are to neurons (**Fig. 1l**). Of the 31 cells used for Patch-seq, 15 had HC

electrophysiology profiles (**Fig. 1m**). The canonical *IDH1R132H* mutation was detected

in 5 of 31 cells, all 5 of which were HCs, confirming that IDH1^{mut} tumor cells are

excitable and can fire *bona fide* APs. Interestingly, of the cells that had coverage of the

IDH1 locus, one HC cell was not *IDH1R132H* mutant, suggesting that both tumor and

non-tumor cells can have HC electrophysiologies (**Fig. 1m**). These studies identified

HCs as an electrophysiologically unique group of cells in the human brain and confirm

that tumor and non-tumor cells in IDH1^{mut} gliomas possess HC electrophysiology

signatures.

SCRAM is a reliable annotation tool for human scRNA-seq datasets

To better define the HCs identified in our Patch-seq studies, we sought to create a new

computational platform that could annotate each cell from Patch-seq individually.

Because of the low cell numbers obtained using Patch-seq and the rarity of human

glioma samples for use in these experiments, our annotation tool needed to be able to

analyze each cell without a dependency on clustering methodologies, which requires

hundreds of cells for optimal analysis. Accordingly, we developed the Single Cell Rule

Association Mining (SCRAM) tool that can annotate each cell on a cell-by-cell basis,

independently of clusters. SCRAM uses a three-step orthogonal process to provide

detailed transcriptional and RNA-inferred genomic profiles for each cell: (1) cell type

transcriptional annotation using machine-learned neural network models (NNMs); (2)

single nucleotide variant (SNV) profiling using the XCVATR[18] tool; and (3) copy number

variant (CNV) calling using the CaSpER[19] and NUMBAT[20] tools (**Fig. 2a-d**). SCRAM

NNMs are trained from 11 previously published scRNA-seq (totaling ~1M cells) human

tumor and non-tumor datasets, including developing brain, immune, and glioma cell

atlases[9,10,21–28]. Each cell from Patch-seq is assigned a probability score for each cell

type in the training datasets **(Fig. 2e)**. Using this methodology, individual cells may be

annotated as more than one cell type, which permits for the characterization of hybrid

cell states like those of HCs from our Patch-seq experiments. SNVs and CNVs are also

considered for each cell and added to the cell annotation. Cells are assigned as "tumor"

if they have $\geq$ 2 tumor features (see *Methods*). We validated that SCRAM can reliably

discriminate between tumor and non-tumor tissue, which it does with >99% sensitivity

(true positive rate: TPR) in Allen Brain Atlas[21], Bhaduri et al.[23] , Aldinger et al. [27],

CoDEx[24] and >96% sensitivity in Human Protein Atlas (HPA)-Brain[28] (Allen Brain Atlas:

559 tumor cells out of 76,533 cells; HPA-Brain: 1333 tumor cells out of 76,533 cells;

Bhaduri et al. Developing Brain: 4237 tumor cells out of 404,218 cells, Aldinger et al.

Developing Brain: 586 tumor cells out of 69,174 cells, CoDEx Developing Brain: 559

tumor cells out of 75,974 cells). Additionally, we ran SCRAM on glioma scRNA-seq

datasets[8,10,22], in which tumor non-tumor annotations was reported in cluster resolution,

SCRAM achieved 100% specificity (true negative rate: TNR) for tumor cells in IDH1^{mut}

and IDH1^{WT} glioma datasets.

HCs express hybrid GABAergic neuron-OPC transcriptomes

We ran SCRAM on the 31 cells from Patch-seq experiments and found that cells with

pyramidal cell electrophysiologies were correctly annotated as glutamatergic neurons,

whereas those with interneuron electrophysiologies were appropriately annotated as

GABAergic neurons (**Fig. 2e**). Consistent with their electrophysiological profiles, HCs

showed concurrent annotation as tumor cells, OPCs, oligodendrocytes and GABAergic

neurons (**Fig. 2e**), suggesting that HCs are endowed with a hybrid of neuronal and glial

transcriptional features that may be responsible for the functional electrophysiological

properties of these cells. To validate these findings in larger datasets, we used SCRAM

to analyze our in-house scRNA-seq dataset consisting of 234,880 cells from 12 IDH1^{mut}

and IDH1^{WT} glioma patients[29] (**Fig. 2b-d**). SCRAM-assigned probability scores >0.9

were used for final cell annotations and probability scores used to generate a SCRAM

UMAP of our scRNA-seq glioma dataset, which clusters cells based on cell identity

rather than similarity of transcriptional features (**Fig. 3a**). Visualization of SNVs, CNVs

and tumor expression markers specific to glioma subtypes revealed that SCRAM

segregates the majority of IDH1^{mut} from IDH1^{WT} tumor cells (**Fig. 3b**). Given that the

HCs we observed in our Patch-seq experiment were found in IDH1^{mut} patients, we

focused on SCRAM clusters encompassing the majority of IDH1^{mut} tumor cells. These

cells were predominantly distributed amongst three discrete SCRAM clusters (Clusters

13, 18, and 19) that collectively contained less than 5% of IDH1^{WT} tumor cells (**Figs. 3c-**

e). Importantly, all IDH1^{mut} tumor patients had robust GABA-OPC expression profiles,

whereas only one IDH1^{WT} patient showed the same expression profile (**Figs. 3f-g**). An

analysis of tumor cells by patient revealed that on average 41.3% of IDH1^{mut} tumor cells

were GABA-OPCs, whereas only 2.2% of IDH1^{WT} tumor cells received the same

annotation (**Fig. 3h**). These SCRAM analyses revealed that HCs are defined by a

hybrid GABA-OPC transcriptome, which is found in nearly half of IDH1^{mut} tumor cells.

Having identified that HCs are defined by GABA-OPC transcriptomes and that these

cells are robustly present in most IDH1^{mut} patients, we sought to extract a GABA-OPC

molecular signature by identifying transcriptional markers. To this end, we used

SHapley Additive exPlanations (SHAP) analysis, which extracts essential features from

machine-learning NNMs. We identified SHAP markers from each cell type in our training

dataset (e.g. GABAergic PVALB neuron from Allen Brain Atlas; and compared these

SHAP genes to the differentially expressed genes (DEGs) obtained for GABA-OPC

tumor cells in our scRNA-seq dataset as compared to other non-GABA-OPC tumor

cells. The intersection of SHAP genes and DEGs produced a list of 61 genes (log$_2$FC >

1), which we termed the GABA-OPC gene set. The GABA-OPC gene set is comprised

of SHAP genes that are critical transcriptional features of cell types from tumor and non-

tumor training datasets (**Fig. 4a**). The training cell types that were most highly

represented by GABA-OPC SHAP features were GABAergic neuron and OPC,

demonstrating that the majority of GABA-OPC transcriptional features derive from

GABAergic neurons and OPCs. These data define a unique transcriptomic signature of

GABA-OPC tumor cells that can be used to identify these cells in other datasets.

Strong GABA-OPC signatures are correlated with better survival in IDH1^{mut}

patients

To validate our observation that GABA-OPC signatures were more prominent in our

IDH1^{mut} cohort as compared to IDH1^{WT}, we used the GABA-OPC-like glioma geneset to

score 225 IDH1^{WT} and 441 IDH1^{mut} bulk RNA-seq samples from TCGA (**Fig. 4b**).

Consistent with our internal scRNA-seq dataset, we found that nearly all IDH1^{mut}

samples had high GABA-OPC scores, whereas IDH1^{WT} samples were distributed more

broadly across low and high scores. An analysis of scores by IDH1^{WT} molecular

subtypes revealed the highest GABA-OPC scores belonged to OPC-like and NPC-like

molecular subtypes (**Fig. 4c**). Consistent with these observations, examining the

percentage of GABA-OPC tumor cells in Neftel et al.'s IDH1^{WT} scRNA-seq dataset[9]

revealed that OPC-like samples had significantly higher GABA-OPC tumor percentages

than the other molecular subtypes (**Fig. 4d**). Reexamining our internal scRNA-seq

dataset revealed that the only IDH1^{WT} patient with a strong GABA-OPC signature was

an OPC-like subtype. Interestingly, analysis of the TCGA IDH1^{mut} samples by

histopathological subtype showed that GABA-OPC scores were higher in low-grade

glioma (LGG) than high-grade glioma (HGG), suggesting that stronger GABA-OPC

phenotypes are associated with lower grade tumors (**Fig. 4e**). RNAvelocity pseudotime

analyses of three IDH1^{mut} and one OPC-like IDH1^{WT} samples revealed that GABA-OPC

tumor cells emerge from more primitive tumor cells to become the largest population of

tumor cells in tumors that bear them, demonstrating that select tumor cells from

divergent genetic backgrounds of IDH1^{mut} and IDH1^{WT} converge at a shared

transcriptional phenotype (**Figs. 4f-g**). We assigned GABA-OPC scores to bulk RNA-

seq IDH1^{mut} glioma samples for which long-term survival follow up was collected[30].

Samples were split into low (n=41) and high (n=41) GABA-OPC groups based on

median expression. Kaplan-Meier survival analysis showed that low GABA-OPC tumor

patients had a median survival of 84.5 months whereas high GABA-OPC tumor patients

showed a median survival of 156.9 months (**Fig. 4h**). Survival outcomes for HGG

IDH1^{mut} patients alone were also significantly different, with a median survival of 80.5

and 182.5 for low and high GABA-OPC groups in, respectively (**Fig. 4i**). Collectively,

these analyses confirm that GABA-OPC tumor cells are a defining feature of IDH1^{mut}

glioma and the OPC-like subtype of IDH1^{WT} glioma and demonstrate that a decreased

GABA-OPC transcriptional signature confers significantly worse survival outcomes in

IDH1^{mut} glioma patients **(Fig. 4h-i,)**.

GABAergic neurons and OPCs share transcriptional profiles in non-tumor human

brain

To discern whether AP-firing GABA-OPC tumor cells represent a population of hybrid

neuronal-glial cells unique to tumor, we examined expression data of the non-tumor

adult human brain scRNA-seq cell datasets from Allen Brain Atlas and the Human

Protein Atlas (HPA). We examined pan- and subtype-specific GABAergic neuron

markers as well as established OPC markers, finding that 6 of 30 GABAergic neuron

markers were similarly expressed by OPCs (**Fig. 5a**). Conversely, and unexpectedly,

PDGFRA, a widely-used OPC-specific marker, is highly expressed by GABAergic

LAMP5+ neurons both in Allen Brain Atlas and the HPA, demonstrating a transcriptional

overlap that is uniquely shared by OPCs and GABAergic neurons. To determine

whether GABA-OPC glioma features are also present in non-tumor cells, we examined

the percentage of each cell type that express the GABA-OPC transcriptional signature.

We found that on average 48% percent of OPCs from non-tumor brain atlases

possessed GABA-OPC molecular profiles (**Fig. 5b**), suggesting that GABA-OPC tumor

cells are not hybrid neuronal-glial tumor cells, but are malignant manifestations of an

OPC subclass that is found in non-tumor brain.

GABA-OPC tumor cells express voltage-gated ion channels and GABA-related

genes

Given prior reports documenting the existence of GABARs and GABA synthesis genes

in non-tumor OPCs, we investigated the prevalence of these gene sets in non-tumor

developing and adult scRNA-seq datasets from the human brain[31,32], finding that they

were more enriched in non-tumor adult OPCs than in development (**Fig. 5c**). We found

that GABA metabolism genes are also widely expressed by GABA-OPC HCs from our

Patch-seq studies, including high expression of the GABAergic neuron marker and

GABA synthesis gene *glutamate decarboxylase 1 (GAD1)*, which was also found in

GABAergic interneurons but not detected in glutamatergic pyramidal cells (**Fig. 5d**).

Notably, *GABA transaminase (ABAT)*, which is a crucial enzyme in GABA catabolism,

was also detected at high levels in GABA-OPC HCs. Analysis of these genes in our

larger scRNA-seq cohorts confirmed that *ABAT* and *GAD1* are highly expressed by

GABA-OPC tumor cells (**Fig. 5c**), the latter of which we confirmed through

immunostaining of human IDH1^{mut} tumor samples (**Fig. 5e**). GABARs were also robustly

expressed in GABA-OPC tumor cells as compared to other non-GABA-OPC tumor

cells, suggesting that GABA-OPC tumor cells are transcriptionally equipped to receive

electrochemical signals from GABAergic neurons (**Figs. 5c-d**). Importantly, previous

work has shown that quantal GABA release from interneurons to OPCs at interneuron-

OPC synapses can elicit miniature depolarizations in OPCs[33]. Given this precedent, our

data suggest that the electrophysiological activity of GABA-OPC tumor cells may be in

part conferred through the expression of GABARs and GABA metabolism genes that

enables these cells to respond to signals from GABAergic neurons of other GABA-OPC

tumor cells.

Having confirmed that GABARs and GABA metabolism genes are expressed by GABA-

OPC tumor cells, we next sought to elucidate the molecular constituents that may be

responsible for AP firing in HCs. Importantly, previous work demonstrated that a

subgroup of white matter OPCs in the rat brain fire single[34], short APs that are similar to

those observed in GABA-OPC HC cells from our Patch-seq studies. The AP capacity of

these spiking OPCs is dependent on voltage-gated ion channels, particularly voltage-

gated sodium channels (Na_vs) and voltage-gated potassium channels (K_vs), which we

confirmed were highly expressed by human non-tumor OPCs (**Fig. 5f**). We found that

the majority of GABA-OPC tumor cells have high expression of Na_vs and but that high

expression of K_vs is restricted to a smaller population of cells. These findings were

confirmed in our Patch-seq dataset, which revealed that Na_vs were broadly expressed

by GABA-OPC HC cells, whereas high expression of K_vs was detected in fewer of these

cells (**Fig. 5g**). Immunostaining for the voltage-gated sodium channel $Na_v1.1$, encoded

by the *SCN1A* gene, in a human IDH1^{mut} tumor confirmed GABA-OPC tumor cells

express Na$_v$s, which are essential for the rising phase of APs[34,35]. These data suggest

that GABA-OPC tumor cells are endowed with the requisite machinery that can

mechanistically elicit and sustain the APs observed in these cells (**Fig. 5h**).

Discussion

In recent years, studies in the field cancer neuroscience have elucidated how glioma

cells interact with surrounding neural networks to direct disease progression[36,37]. These

reports demonstrate that human glioma cells receive synaptic inputs from the

surrounding excitatory glutamatergic neuronal circuitry that can evoke EPSPs in glioma

cells[6,7,38]. Whole cell patch clamp experiments from the 1990s documented that cells

firing short, single APs were the majority in human glioma slices; however, conclusively

showing that these cells were tumor in origin was not possible without the advent of

single cell transcriptomics[39–41]. These early electrophysiology studies described cells

that fire single, short APs that were dependent on voltage-gated sodium currents.

Separately, the identification of a spiking class of OPCs in rats revealed that neurons

are not the only cells capable of firing APs and suggested that an analogous population

of spiking OPCs exists in human[34]. Given these precedents, their electrophysiological

similarities to our Patch-seq HCs and the enrichment of voltage-gated ion channels that

are expressed transcriptionally by both adult OPCs and GABA-OPC tumor cells, we

believe that GABA-OPC tumor cells are likely the malignant analogs of spiking OPCs

and constitute the major tumor cell population in IDH1^{mut} patients. To this point, while

five of the HCs from Patch-seq had the *IDH1R132H* canonical mutation, a single HC

that was confirmed *IDH1* wild-type showed the same electrophysiological profile as

those *IDH1R132H* mutant HCs and likely represents a non-tumor spiking OPC. There is

mounting evidence to support the OPC as a cell of origin in glioma[42–44], which

represents the largest proliferative neural cell population in the adult brain[45]. Our

studies further support the notion that OPCs undergo malignant transformation to

become glioma cells and adds to our understanding of cancer neuroscience by

implicating GABAergic metabolism and signaling as an important mediator of tumor cell

neurophysiology. Interestingly, GABARs, which are highly expressed by GABA-OPC

tumor cells, function to limit neuronal excitability in neurons by triggering an influx of

chloride anions into the cell. The accumulating negative charge decreases the resting

membrane potential, making subsequent depolarizations more difficult to achieve[46],

raising the question of whether GABARs restrict tumor cells from firing more than one

AP. Our analyses revealed that the GABA-OPC molecular profile that supports AP

activity in tumor cells are lost in the process of malignant transformation from LGG to

HGG and concordantly, that diminished GABA-OPC scores correlate with significantly

worse survival outcomes in IDH1[mut] patients. These findings suggest that GABA-OPC

tumor cells can serve as a biomarker of premalignant brain tumors and may serve as

the target of tumor-specific treatments in future endeavors.

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Data availability

350
351 In our previous study, we published the scRNA-seq datasets of 12 samples under the

352 accession number GSE221534[29]. The Patch-seq and scRNA-seq datasets generated

353 during this study will be made available through the NCBI Gene Expression Omnibus

354 (GEO) website. All other study data are included in the article and/or supporting

355 information.

Author contributions

356
R.N.C. and A.S.H. are responsible for conception of this project, the study and pipeline

design, and interpretation of the results. R.N.C. devised all experimental set ups,

performed immunostaining and statistical analyses, and prepared all scRNA-seq

samples from with assistance from M.F.M. A.S.H. wrote the code for the SCRAM

pipeline. Q.M. and J.J. performed all human electrophysiology and Patch-seq

experiments. R.N.C., A.O.H., S.W. and A.S.H. performed bioinformatics analyses on

human scRNA-seq datasets. B.L., E.L.F., and A.R. provided experimental support for

immunohistochemistry. G.R. identified and obtained consent from patients for the study.

R.N.C. and A.S.H prepared the manuscript. X.J., B.D., and G.R. contributed to the

manuscript with feedback from all authors.

Declaration of Interests

367
368 The authors declare no competing interests.

Contact for reagent and resource sharing

370   Dr. Akdes Serin Harmanci (akdes.serinharmanci@bcm.edu) is the lead contact for

371   reagent and resource sharing. All published reagents will be shared on an unrestricted

372   basis; reagent requests should be directed to the corresponding author.

Figure 1

Figure 1. Patch-seq of human glioma samples reveals tumor cells fire single action potentials. (a) Experimental workflow for whole-cell recordings and whole-cell patch clamp recordings, followed by singe-cell RNA-sequencing (Patch-seq) assays. (b) Exemplary membrane responses from patched *IDH1*-mutant (IDH1^{mut}) neuronal and glial cells to a 600-ms hyperpolarizing current step (black) and suprathreshold depolarizing current step (coloured). (c) Matched traced cell morphologies are shown for recorded neuronal and glial cells. (d) Matched images of biocytin-filled cell morphologies for patched neuronal and glial cells; scale bar: 50 μm. (e) Exemplary membrane responses from IDH1^{mut} HCs to a 600-ms hyperpolarizing current step (black) and suprathreshold depolarizing current step (coloured). (f) Matched traced cell morphologies are shown for recorded HC cells. (g) Matched images of biocytin-filled cell morphologies for patched HC cells; scale bar: 50 μm. (h) Pie chart showing percentage of PC, IN, GL and HC patched cells. (i) Bar graph showing HC have higher input resistance compared with non-tumor neurons; *p*-values for pairwise comparisons are noted in the figure. (j) Bar graph showing HC cells fire fewer spikes compared to neurons; *p*-values for pairwise comparisons are noted in the figure. (k) Bar graph showing HC cells have AP amplitudes compared to neurons; *p*-values for pairwise comparisons are noted in the figure. (l) Principal component analysis (PCA) plot of 31 Patch-seq cells shows clustering of cells based on electrophysiological properties. HC cells are transcriptionally like each other and glia. Cells are coloured according to electrophysiological properties. (m) SNV profiling for the *IDH1R132H* mutation is shown for 31 Patch-seq cells. For Patch-seq, two voltage traces are shown: the hyperpolarization trace obtained with injected currents (black) and the depolarization

400 trace showing maximal AP firing rate; injected current: −100 pA. AP: action potential;

401 GABA: γ-aminobutyric acid; GL: glia; GSC: glioma stem cell; IN: interneuron; OPC:

402 oligodendrocyte   precursor   cell.   PC: pyramidal   cell.

Figure 2

Figure 2. SCRAM reveals HC cells are GABA-OPC tumor cells. (a) Schematic of the SCRAM pipeline. Briefly, 11 scRNA-seq datasets are used to train cell type neural network models (NNMs). Each cell from scRNA-seq is then assigned cell type annotation independently of all other cells using NNM trained models. CNVs are added for each cell using CaSpER and NUMBAT. SNVs are added for each cell using XCAVTR. (b) Seurat clusters are shown for 234,880 cells from our in-house glioma scRNA-seq dataset. (c) Zoom-in of Seurat clusters 3, 12, 16 and 22 colored by Seurat clusters. (d) Zoom-in of Seurat clusters 3, 12, 16 and 22 coloured by SCRAM cell-by-cell annotations. (e) Heatmap showing SCRAM cell type probability scores for 31 Patch-seq cells.

Figure 3

Figure 3. GABA-OPC tumor cells in human glioma. (a) SCRAM UMAP of 234,880

scRNA-seq cells. (b) Commonly used IDH1^{mut} (top row) and IDH1^{WT} (bottom row)

tumor features are shown. Top (from left to right): chromosome 1p deletion feature plot,

IDH1R132 mutation density plot, *PDGFRA* expression feature plot. Bottom (from left to

right): chromosome 7p amplification feature plot, *EGFR* mutation density plot, *EGFR*

expression feature plot. (c) SCRAM tumor and non-tumor cell annotation. (d) Zoom-in of

inset from *(c)* showing SCRAM cell type annotations for each cell. (e) Bar graph

showing the majority of cells in *(d)* are from IDH1^{mut} tumor patients. (f) SCRAM

probability scores are shown for cell types of interest. (g) Heatmap of SCRAM cell type

annotations for IDH1^{mut} (n=5) and IDH1^{WT} (n=7) glioma patients. (h) Bar graph showing

the percentage of tumor cells with GABA-OPC annotations; *p*-value is noted in the

figure.

 Figure 4

Figure 4. GABA-OPC tumor cell signatures are most highly expressed in IDH1^{mut} LGG and are reduced in both IDH1^{mut} and IDH1^{WT} HGG. (a) Circos plot showing the 61 genes (log$_2$FC > 1) comprising the GABA-OPC tumor cell signature. DEGs from GABA-OPC tumor cells versus all other tumor cells were extracted from our scRNA-seq dataset and crossmatched with SHAP genes from our trained NNMs. (b) Density plot of GABA-OPC tumor scores is shown for IDH1^{mut} (n=414) and IDH1^{WT} (n=225) TCGA bulk RNA-seq glioma samples; *p*-value is noted in the figure. (c) Box and whiskers plot showing GABA-OPC scores for TCGA samples by IDH1^{WT} tumor subtype: MES-like (n=149); AC-like (n=157); NPC-like (n=12); OPC-like (n=9); *p*-values for pairwise comparisons are noted in the figure. (d) Bar graph showing the percentage of tumor cells that are GABA-OPC tumor from Suva's IDH1^{WT} SMART-seq dataset. (e) Density plot of GABA-OPC tumor scores is shown for IDH1^{mut} TCGA bulk RNA-seq glioma samples by histology subtype: oligodendroglioma (n=150); astrocytoma (n=111); diffuse astrocytoma (n=8); *p*-values for pairwise comparisons are noted in the figure (f) RNAvelocity pseudotime analyses are shown for one IDH1^{WT} and three IDH1^{mut} samples. (g) Bar graphs showing percentage of tumor cells by cluster over pseudotime. (h) Kaplan-Meier survival analysis in IDH1^{mut} patient cohort. (i) Kaplan-Meier survival analysis in HGG IDH1^{mut} patient cohort.

Figure 5

Figure 5. GABAergic neurons and OPCs share transcriptional profiles in non-

tumor human brain (a) Heatmap showing subtype-specific GABAergic neuron markers

as well as established OPC markers in Allen Brain Atlas and the Human Protein Atlas

(HPA). White dotted box outlines OPCs. (b) Percentage of OPCs that express the

GABA-OPC transcriptional signature in non-tumor brain atlases. (c) DotPlot showing the

average expression of GABARs and GABA metabolism genes in human non-tumor

OPCs and in GABA-OPC tumor cells. (d) Heatmap showing the expression GABARs

and GABA metabolism genes in Patch-seq data. (e) IHC staining for GAD1 (red),

IDH1R132H (green), OLIG2 (pink) and Hoechst (blue) in a human IDH1^{mut} tumor

sample. (f) DotPlot showing the average expression of voltage-gated sodium channels

(Na$_v$s) and voltage-gated potassium channels (K$_v$s) genes in human non-tumor OPCs

and also in GABA-OPC tumor cells (g) Heatmap showing the Na$_v$s and K$_v$s genes in

Patch-seq data. (h) IHC staining for Na$_V$1.1 (SCN1A; red), IDH1R132H (green), OLIG2

(pink) and Hoechst (blue) in a human IDH1^{mut} tumor sample.

Experimental Methods

Human data

Adult patients at St. Luke's Medical Center and Ben Taub General Hospital provided preoperative informed consent to participate in the study and gave consent under Institutional Review Board Protocol H35355. Patients included males and females. Clinical characteristics were maintained in a deidentified patient database and are summarized in **Table S4**.

Tumor samples were collected during surgery and immediately placed on ice. Tissue was divided for use in subsequent transcriptomic, histopathological, proteomic, or biochemical studies. Patient samples were collected separately for pathology and molecular subtyping. Histopathology and molecular subtyping of *IDH1* and 1p19q deletion status were confirmed by board-certified pathologists. Samples for scRNA-seq and immunoprecipitation assays were fixed in LN$_2$ and kept at –80°C.

Single-cell RNA-sequencing

Human tumors were prepared as single-cell suspensions. Briefly, samples were coarsely chopped with surgical scissors and enzymatically digested with Papain supplemented with DNase I (Worthington Biochemical Corporation, LK003150). Samples were incubated for 15 minutes at 37°C on a thermocycler kept at 1400×*g* and briefly pipetted every 5 minutes. Cells were pelleted at 13,000×*g* for 10 seconds and resuspended in phosphate-buffered saline (PBS) before processing for debris and dead cell removal. Cell suspensions were processed using the MACS Debris Removal Kit (Miltenyl, 130-109-398) and MACS Dead Cell Removal Kit (Miltenyl, 130-090-101), according to the manufacturer's instructions. Live cells were collected through negative

selection using an MS Column in the magnetic field of a MiniMACS Separator (Miltenyl,

130-042-102). Eluted cells were spun at 300×g for 5 minutes and resuspended in Gibco

Dulbecco's Modified Eagle Medium with GlutaMAX (DMEM; ThermoFisher, 10566016)

supplemented with 10% foetal bovine serum (FBS; ThermoFisher, 16000044). Single

cells were processed with the 10X Chromium 3′ Single-Cell Platform using the

Chromium Single-Cell 3′ Library, Gel Bead, and Chip Kits (10X Genomics) following the

manufacturer's protocol. Briefly, approximately 5,000–15,000 cells were added to each

channel of a chip to be partitioned into Gel Beads in Emulsion (GEMs) in the Chromium

instrument, followed by cell lysis and barcoded reverse transcription of RNA in droplets.

GEMs were broken, and cDNA from each single cell was pooled. Clean-up was

performed using Dynabeads MyOne Silane Beads (ThermoFisher, 37002D).

Subsequently, the cDNA was amplified and fragmented to optimal size before end

repair, A-tailing, and adaptor ligation. Libraries were run individually using a NextSeq

500/550 High Output Kit v2.5 (75 Cycles) (Illumina, 20024907) and sequenced on an

Illumina NextSeq550 instrument.

Human tumor slice preparation

Fresh tumor samples were immediately placed into a cold (0–4°C) oxygenated N-

methyl-d-glucamine (NMDG) solution (93 mM NMDG, 93 mM HCl, 2.5 mM KCl, 1.2 mM

NaH_2PO_4, 30 mM $NaHCO_3$, 20 mM HEPES, 25 mM glucose, 5 mM sodium ascorbate, 2

mM thiourea, 3 mM sodium pyruvate, 10 mM $MgSO_4$, and 0.5 mM $CaCl_2$, pH 7.35).

Slices were cut at 300-μm thickness with a microslicer (Leica VT 1200) and kept at

37.0±0.5°C in oxygenated NMDG solution for 10–15 minutes before being transferred to

artificial cerebrospinal fluid (ACSF, 125 mM NaCl, 2.5 mM KCl, 1.25 nM NaH_2PO_4, 25

mM NaHCO$_3$, 1 mM MgCl$_2$, 25 mM glucose, and 2 mM CaCl$_2$, pH 7.4) for 1 hour before

recording.

Single-cell processing

We ran samples on the 10X Chromium platform to produce next-generation sequencing

libraries. We performed standard procedures for filtering, mitochondrial gene removal,

and variable gene selection using the Seurat pipeline. The criteria for cell/gene inclusion

were as follows: genes present in more than three cells were included, cells that

expressed >300 genes were included, the number of genes detected in each cell was

>200 and <5000, and the mitochondria ratio was 10. We integrated cells from different

patients using the Harmony algorithm[47]. Next, we visualized clusters using a uniform

manifold approximation and projection constructed from the Harmony-corrected PCA.

We also performed lineage tracing, trajectory analysis, and RNA velocity assessments

to create developmental hierarchies and lineage histories of glioma cells using the

scvelo R package[48] and IntrExtract[49].

SCRAM pipeline and methodology

SCRAM input consisted of aligned scRNA-seq reads and our neural network model

trained on 11 diverse single-cell RNA-Seq datasets encompassing 1 million cells of

publicly available data from healthy adult and developing brain samples, as well as

brain tumor samples. Tumor and normal cells were annotated independently for two

reasons. (1) Significant overlap exists between tumor and non-tumor expression

markers. For example, *EGFR* and *PDGFRA* are often used to denote tumor cells[9] but

are also cell type markers for OPCs and ependymal host cells, respectively (2) We

hypothesize that by separating tumor-specific and normal-specific features, we can

achieve more robust identification of hybrid cells (HCs). This hypothesis is supported by

our observation that existing cell type assignment methods, which typically classify both

tumor and normal cells together, fail to accurately characterize HCs. These

tumor/normal features were systematically employed in our pipeline as follows:

Step 1. Annotation of non- tumor cells:

556
Training Neural Network Models (NNMs). We trained our neural network model (NNMs)

on 11 diverse single-cell RNA-Seq datasets, which collectively contain 1 million cells.

These datasets comprise publicly available data sourced from various datasets,

including healthy adult and developing brain samples, as well as brain tumor samples[8–

10,21–28]. We trained our model using a deep neural network (DNN), with an input layer of

around 20K genes, three intermediate layers (256, 64, 32), and an output layer of size

16 or 21, depending on the number of referenced cell types. Following the dense

connection within each hidden layer, there are batch normalization, activation, and

dropout functions. We use the popular Rectified Linear Unit (ReLU) for hidden layer

activation and set dropout rate to be 0.1. The output layer uses Softmax activation

function so that each node outputs a non-negative value smaller than 1 and all the

values sum up to 1. Therefore, each output corresponds to the probability of one cell

type. We compile the model using categorical crossentropy as loss function, Adam as

optimizer, and accuracy as metrics. In order to achieve a more balanced class

distribution, we opted to subsample cell types within our training model. We train one

neural network-based classifier on each developmental-like, normal, tumor cells

datasets and save the model in repository. We predicted the developmental-like, normal

and tumor cells, in our glioma scRNA-Seq data using our trained NNMs. Model

probability scores >0.9 were used for final cell annotations. In building our NNMs, we

utilized the Python packages TensorFlow and Keras. Additionally, we used the Python

Scipy package for processing the scRNA-Seq data. Prerequisite packages for data

preprocessing and model training include Numpy 1.19.5, Pandas 1.1.5, Scanpy 1.7.2,

Anndata 0.7.8, Scipy 1.5.4, and Scikit-learn 0.24.2.

In order for a single cell to be classified as "immune" within our framework, it

needs be annotated as immune with a probability score above 0.9 in three or more

trained datasets.

SHapley Additive exPlanations (SHAP) analysis.

SHAP analysis was employed to gain insights into the main features that affect the

output of the NNMs, we used SHAP method, to explain how each feature affects the

NNMs in inhouse glioma single-cell dataset. To perform the SHAP analysis, the model

predictions were decomposed into contributions from individual features, allowing us to

assess their impact on the final outcome using the *shap* python package.

Step 2. Annotation of tumor cells:
The SCRAM pipeline integrates multiple orthogonal tumor features to identify tumor

cells at a single-cell resolution. These features include:

Module 1. Neural Network Model-Based Tumor Cell Prediction: Above explained NNMs

is used to predict tumor cells based on the probability score. Cells with a probability

score above 0.9 are classified as tumor cells.

Module 2. Marker based Expression Modeling: SCRAM employs finite Gaussian mixture

modeling to model marker expression of three tumor marker genes: *SOX2, EGFR*

PDGFRA. This approach helps to distinguish tumor cells based on their specific marker

gene expression profiles (details explained below section "*Marker Expression Modeling*

for tumor annotation").

Module 3. RNA-Inferred Genotyping of Chromosome Alterations: A modified version of

our CNV-calling algorithm, CaSpER[19], another state of art CNV calling method

numbat[20] is used in SCRAM to perform RNA-inferred genotyping of large-scale

chromosome alterations.

Module 4. RNA-Inferred Mutational Profiling: SCRAM utilizes our XCVATR[18] tool, a

recently developed tool, to deduce rare deleterious single-nucleotide variants (SNVs)

present in the tumor cells. This analysis involves considering SNVs that are reported in

the COSMIC[50]- database and have a frequency of less than 0.1% in the

dbSNP[51]database.

These orthogonal tumor features are called separately in the SCRAM pipeline. By

combining these different approaches, SCRAM aims to accurately identify tumor cells at

a single-cell resolution.

In order for a single cell to be classified as "tumor" within our framework, it needs to

meet two or more of the following criteria:

1. Neural Network Model-Based Tumor Cell Prediction (Module 1): The cell is

annotated as "tumor" when it receives a probability score greater than 0.9 from

the trained Neural Network Models (NNMs) in Richards et al.[10], or Venteicher et

al.[22] or Neftel et al.[9] or Tirosh et al.[8] datasets.

2. Marker Expression Modeling (Module 2): The expression levels of at least two

tumor cell markers, (*SOX2*, *EGFR*, or *PDGFRA*) should surpass a predetermined

threshold. This threshold is established using finite Gaussian mixture modeling

(details explained below section "*Marker Expression Modeling for tumor*

annotation").

3. RNA-Inferred Genotyping of Chromosome Alterations (Module 3): The presence

of large-scale copy number variations (CNVs) is considered a tumor cell.

4. RNA-Inferred Mutational Profiling (Module 4): Tumor cells that have SNVs in

genes *IDH1* (R132H/R132C) or EGFR.

Module 2. Marker Expression Modeling for tumor annotation: Given the expressional

heterogeneity of tumor markers in normal cells, we used previously published tumor and

non-tumor cell datasets to establish a marker expression–based tumor classification

model (i.e., thresholding requirements for "high expression" annotation) for the tumor

markers *PDGFRA, EGFR,* and *SOX2*. For each tumor marker, an independent classifier

model was built using (1) Allen Brain Atlas human scRNA-seq data, which represent the

largest compendium of healthy brain data as a training set for normal cells; and (2) a

compendium of publicly available brain tumor scRNA-seq datasets as a training set for

tumor cells[9]. The following statistical models were used to infer the class (normal vs

tumor) of our in-house tumor scRNA-seq data.

We modelled expression as a mixture of Gaussian distributions to identify and classify

normal and tumor cells:

Let $X_j = \{\, x_1, x_2, \ldots, x_i, \ldots, x_n \}$ be the training expression vector of normal and tumor cells

for gene j, where x_i is the expression value of cell i. The distribution of every expression

value is specified by a probability density function through a finite mixture model of *G=2*

classes (normal vs tumor):

$$f(x_i; z) = \sum_{k=1}^{G} \pi_k f_k\,(x_i;\ \theta_k)$$

where $z = \{\pi_{1,\ldots,}\pi_G, \theta_1, \ldots \theta_G\}$ represents the parameters of the mixture model and

$f_k(x_i; \theta_k)$ is the kth component density, which is assumed to follow a Gaussian

distribution $f_k(x_i; \theta_k) \sim N(\mu_k, \sigma_k)$. $\{\pi_{1,\ldots,}\pi_G\}$ is the vector of probabilities, non-negative

values that sum to 1, known as the mixing proportion. The mixing proportion, π, follows

a multinomial distribution.

We used the above model to predict normal vs tumor class in our in-house glioma cells.

For each gene, j, z parameters were estimated by maximizing the log-likelihood function

via the expectation-maximization algorithm. The log-likelihood function is formulated as:

$$l(z; x) = \sum_{i=1}^{n} \log f_k(x_i; z)$$

For each tumor marker, we generated a matrix, with genes indicated by rows and cells

indicated by columns, and the cell value index was 1 if the cell had a high "tumor class"

probability for the corresponding gene. A cell was classified as "tumor" if at least two

markers had high "tumor class" probabilities. We used the mclust R package for

Gaussian mixture model implementation[52].

Module 3. RNA-Inferred Genotyping of Chromosome Alterations: CNVs are hallmark

features of tumor cells that can be used to classify tumor vs non-tumor cells with or

without expression markers. However, CNV detection from scRNA-seq data is

inherently noisy due to dropouts and unmatched control sets, among other factors,

requiring a set of known tumor cells. To estimate a "clean" set of CNV calls to provide

reliable CNV-based tumor scores, we used a *pure tumor pseudobulk* sample.

Estimation of CNV profiles using patient-specific pure tumor pseudobulk samples: We

first used our marker expression–based and NNMs models from Module 1 and Module

2 to identify tumor cells. Cells assigned as "tumor" cells using Module 1 and 2 were

treated as a pure tumor cell cohort. Cells assigned as "immune" cells using our NNMs

are considered control cells.

CNV calling of patient-specific pure pseudobulk samples: We hypothesized that the

pseudobulk sample contained representative sets of CNVs with high probability and,

therefore, should be useful to identify a clean CNV call set. CNV calling of the

pseudobulk samples from each patient was performed using our CNV-calling algorithm,

CaSpER. CaSpER CNV calls were used as the ground truth, large-scale CNV calls for

each patient.

Genotyping of CNVs of all cells: After CNVs were identified from the pseudobulk

sample, we genotyped the CNVs in all cells and generated a binary matrix that

represents the existence of CNVs in cells, i.e., $CNV_{i,j}$.

Module 4. RNA-Inferred Mutational Profiling: We performed RNA-inferred rare

deleterious (COSMIC[50]-reported and dbSNP[51], <0.1% frequency) mutational profiling

via our recently developed XCVATR[18] tool. We detected mutations in *IDH1*

(R132H/R132C), *EGFR* and annotated cells harbouring these mutations as tumor cells.

Visualization and clustering of single-cells

We used the probability score output from NNMs instead of relying solely on the most variable

genes for clustering and visualization of our in-house single-cell data. This, named as SCRAM

UMAP, involved applying UMAP and clustering techniques to the model probability scores using

the Seurat package's runUMAP, FindNeighbors, and FindClusters functions. Additionally, we

employed the most variable genes for cell data clustering and visualization, referring to this

UMAP representation as the original/Seurat UMAP.

Summarizing co-occurring cell types using maximally frequent gene set identification

We summarized co-occurring cell types using a frequent itemset rule mining approach. CNV and SNV calls were added to provide an integrated transcriptomic and genomic summary for each cell. An example SCRAM output for a single cell is given as "glioma stem cell, oligodendrocyte precursor cell, chr1p_deletion, chr19q_deletion + *IDH1:2:208248389* mutation". We used the tumor and normal cell assignments of Step 1 and Step 2 to integrate co-occurring tumor and normal cell features.

The simplest method for detecting maximally frequent tumor and host feature sets is a brute force approach in which each possible subset of features is a candidate frequent set. The *a priori* algorithm is an efficient implementation for finding maximally frequent sets with support above a given threshold. In the *a priori* algorithm, the minimum *support* threshold is set to min (50, number_cells_in cluster*0.1), and the maximum number of genes in a gene set is set to 50. Using the *a priori* algorithm, we identified co-occurring gene sets expressed concurrently within each cell and provided annotation of high-resolution cellular identities using a three-step co-occurrence analysis. We performed our co-occurrence analysis at multiple levels: 1) cell type level (example output of this step: *tumor* AND *radial glia* AND *astrocyte*); 2) cell class level (example output of this step: *tumor* AND *neural cells* are commonly upregulated).

Maximally frequent cell type (or cell class) co-occurrence analysis: Within each cluster, *m,* we calculated the maximally frequent cell types (or cell lineage or cell class) using the *a priori* algorithm. The input was the binarized matrix E^m, where the cell types (or cell lineage or cell class) were the rows, and the cells in cluster *m* were the columns.

$$e_{ij}{}^m = \begin{cases} 1, if\ cell\ type\ (or\ cell\ lineage\ or\ cell\ class)\ i\ is\ annotated\ in\ cell\ j\ in\ cluster\ m \\ 0, \text{otherwise} \end{cases}$$

Analyzing bulk expression data and survival analysis

TCGA-GBM (high grade glioma), TCGA-LGG (low grade glioma) raw read counts and

accompanying clinical data are downloaded using TCGAbiolinks R package[53]. TCGA-

GBM, TCGA-LGG and our bulk RNA-Seq data of the IDH Mutant cohort were both

normalized and applied variance stabilizing transformation using the DESeq2

package[54]. Single sample gene set enrichment analysis (ssGSEA) was performed using

GSVA R package. We used our GABA-OPC tumor gene sets and also the MES-like,

AC-like, NPC-like and OPC-like gene sets reported in a previous study[9]. SsGSEA

GABA-OPC scores were split by median to assign high-OPC-GABA and low-OPC-

GABA scored samples. Those groups are compared against "overall survival" in a Cox

Proportional Hazards (Cox) survival model. used in survival analysis and compared

using a Log-rank test P-value. We used survminer and survival R package for the

survival analysis.

Patch-seq recording procedures

Electrophysiological, morphological, and transcriptomic data from the same cell were

obtained simultaneously using the Patch-seq protocol described previously[11,55]. Briefly,

patch pipettes (5–7 MΩ) were filled with RNase-free intracellular solution (111 mM

potassium gluconate, 4 mM KCl, 10 mM HEPES, 0.2 mM EGTA, 4 mM MgATP, 0.3 mM

Na_3GTP, 5 mM sodium phosphocreatine, and 13.4 mM biocytin). Whole-cell recordings

were performed using a Quadro EPC 10 amplifier (HEKA Electronic). After 5–10

minutes of whole-cell recording of firing patterns, the nucleus was extracted using

gentle and continuous negative pressure. The contents in the pipette were ejected into

a 0.2-mL PCR tube containing 4 mL lysis buffer[55]. RNA in the lysis buffer was

denatured, reverse transcribed, amplified, and purified following the Smart-seq2-based

protocol[56]. Only high-quality cDNA samples (yield ≥2 ng, average length ≥1500 bp)

were sequenced.

Sequencing libraries were constructed from the cDNA using the Illumina Nextera XT

DNA Library Preparation Kit (Illumina, FC-131-1096). The cDNA library was sequenced

on a NovaSeq 6000 instrument using 150-bp paired-end reads.

Biocytin staining and morphological reconstruction

Following slice recording, slices were fixed by immersion in the fixation solution at 4°C

for at least 48 hours and processed with an avidin-biotin-peroxidase method to reveal

the cell morphology. The morphology of the cells was reconstructed and analysed using

a 100× oil-immersion objective lens and camera lucida system (Neurolucida,

MicroBrightField).

Histology

Human samples were retrieved from the operating room on ice and then fixed in 4%

paraformaldehyde in PBS for 12 hours at 4°C before being transferred to 70% EtOH.

Paraffin embedding was performed by the Breast Cancer Pathology Core at Baylor

College of Medicine.

Immunohistochemistry

For immunohistology, 10 μm paraffin-embedded human glioma sections were cut,

deparaffinized and subject to heat-induced epitope retrieval (HIER) using antigen

retrieval buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) when needed.

Sections were blocked for 1 hour at room temperature and kept in primary antibody

incubation overnight at 4°C. The following primary antibodies were used: mouse anti-

IDH1R132H (1:50; Dianova, DIA-H09), rabbit anti-OLIG2 (1:100; R&D, AF4788), rabbit

anti-GAD1 (1:200; Synaptic Systems, 198013), rabbit anti-SCN1A (Na$_V$1.1) Antibody

(1:200; Alomone Labs, ASC-001). Species-specific secondary antibodies tagged with

Alexa Fluor corresponding to emission spectra 488 nm, 568 nm, or 647 nm (1:1,000,

ThermoFisher) were used for immunofluorescence and Hoechst nuclear counter

staining (1:50,000; ThermoFisher, H3570) was performed before coverslipping with

Vectashield antifade mounting medium (Vector Laboratories, H-1000).

Patch-seq data processing

The Patch-seq reads were mapped using STAR[57] to hg38 assemblies for humans.

Read count matrices were generated using FeatureCounts[58] with the latest gene

annotations from GENCODE[59] consortia. DEGs and transcripts were identified using

DESeq2[54] and limma[60]. Cells were clustered and visualized using PCA methods. Cell

type enrichment analyses were performed with enrichR[61] using the

PanglaoDB_Augmented_2021 and *CellMarker_Augmented_2021* cell type marker sets.

IDH1 mutations were identified using our variant detection tool, XCVATR[18], and visually

confirmed using the Integrative Genomics Viewer[62].

Statistical analysis

For electrophysiology analyses, a Kruskal–Wallis test or two-way ANOVA was used,

followed by unpaired t-tests with a two-stage step-up (Benjamini, Krieger, and Yekutieli).

For RT-qPCR, a two-tailed Student's t-test was used. Significant differences are

denoted by asterisks in associated graphs. Data are presented as the mean±standard

error of the mean. Levels of statistical significance are indicated as follows: ns: not

significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$.

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