



**DECREASED EXPRESSION OF THE MITOCHONDRIAL BCAT
PROTEIN CORRELATES WITH IMPROVED PATIENT
SURVIVAL IN IDH-WT GLIOMAS**

Journal:	<i>Journal of Neurochemistry</i>
Manuscript ID:	Draft
Manuscript Type:	Short Communication
Date Submitted by the Author:	n/a
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Area/Section:	Clinical Studies, Biomarkers & Imaging
Keywords:	Glioblastomas, therapeutic target, branched-chain aminotransferase, biomarkers, hBCATm, hBCATc

SHORT COMMUNICATION**DECREASED EXPRESSION OF THE MITOCHONDRIAL BCAT PROTEIN CORRELATES WITH IMPROVED PATIENT SURVIVAL IN IDH-WT GLIOMAS**

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Abstract

Background and research question: Gliomas represent 43% of all solid intracranial tumours, of which glioblastomas have the poorest prognosis. Recently, the human cytosolic branched-chain aminotransferase protein (hBCATc), which metabolises the branched-chain amino acids (BCAA), was identified as a biomarker and therapeutic target for glioblastomas carrying wild-type isocitrate dehydrogenase (IDH-WT) genes. However, the clinical utility of the mitochondrial isoform, hBCATm, which also metabolises BCAAs, was not determined nor its potential role in predicting patient survival.

Methods: Glioblastomas, of grades II-IV, from 53 patients were graded by a neuropathologist, where the IDH and MGMT status were assessed. Tumours positive for hBCATm, hBCATc and BCKDC were characterised using immunohistochemistry and Western blot analysis using antibodies specific to these proteins.

Results: Here, we report that in IDH-WT tumours, the expression of hBCATm is significantly increased ($p=0.034$) relative to IDH mutation gliomas, and significantly correlates with patient survival, on Kaplan-Meier analysis, where low hBCATm expression is a positive prognostic factor ($p=0.003$). Moreover, increased hBCATm expression in these glioblastomas correlated with tumour grade indicating their role as a predictive biomarker of glioma progression. Multiple banding was observed for the branched-chain α -keto acid dehydrogenase complex, which catalyses the committed step in BCAA metabolism, but a significant change in expression was absent ($p=0.690$).

Conclusion: Until now, IDH-WT glioblastomas have a uniformly poor prognosis, however we demonstrate for the first time that relatively low hBCATm may select for a better performing subset within this group and may represent a therapeutic target in these hard to treat patients.

Keywords: Glioblastomas, therapeutic target, biomarkers, branched-chain aminotransferase, hBCATm, hBCATc

Introduction

Although histologically indistinguishable, glioblastomas vary in their clinical, genetic and epigenetic profiles (Ohgaki and Kleihues, 2013; Kreth *et al.*, 2014). Primary glioblastomas, which arise de novo, may show epidermal growth factor receptor (EGFR) amplification and PTEN mutations, compared with secondary glioblastomas that progress from lower grade astrocytomas, which occur in younger patients, and typically carry mutations including isocitrate dehydrogenase IDH1/2, which tend to be absent in primary glioblastomas (<5%) (Balss *et al.*, 2008; Guo *et al.*, 2011). Although glioblastomas with IDH mutations are associated with an improved prognosis and a hypermethylated phenotype, hypermethylation of the O⁶-methylguanine-DNA methyltransferase (MGMT) gene promoter can also be seen in primary glioblastomas in the absence of IDH mutations (Guo *et al.*, 2011; Esteller *et al.*, 2000; Esteller *et al.*, 2000). Conversely, IDH-wild-type (IDH-WT) tumours that exhibit an increased expression of the cytosolic human branched-chain aminotransferase protein (hBCATc) are linked with a poor prognosis (Tönjes *et al.*, 2013). Tönjes and co-authors showed that increased expression of hBCATc supports tumour growth and highlighted it as a new therapeutic target for glioblastomas.

The hBCATc protein is however one of two isoforms (the other, mitochondrial (hBCATm)) that can catalyse the transamination of the branched-chain amino acids (BCAA) followed by complete oxidation by the branched-chain α -keto acid dehydrogenase complex (BCKDC) (Conway and Hutson, 2015). Like BCAT1 (gene encoding hBCATc), a significant up regulation of BCAT2 (gene encoding hBCATm) was reported in IDH-WT tumours (Tönjes *et al.*, 2013). However, hBCATm expression was not studied at the protein level nor assessed as a prognostic marker for patient survival. It is important to consider hBCATm expression and indeed the expression of the BCKDC in IDH-WT tumours because their existence will also contribute to BCAA metabolism promoting tumour survival. This is of relevance because, if hBCATc was considered the only contributor to BCAA metabolism and thus the sole therapeutic target, then if hBCATm expression was found to be increased in

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3 glioblastomas it could potentially attenuate a hBCATc inhibitor. Therefore, in light of this and
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5 other studies where expression of hBCATm is raised under proliferative conditions (Pérez-
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7 Villaseñor *et al.*, 2005) and our studies which show that hBCATm expression, more so than
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9 hBCATc, is significantly increased and correlates with Braak stage in Alzheimer's disease
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11 (Hull *et al.*, 2015;Hull *et al.*,2015) we examined the differential expression of hBCATm and
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13 BCKDC in IDH-WT and IDH mutation glioblastomas and determined if their expression
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15 correlated with patient survival. Our interest also extends to the potential for these metabolic
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17 proteins to show value as biomarkers between tumour grades, offering increased clinical
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19 utility for these proteins.
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Materials and methods

Patient Samples

The study was approved by the Brain Tumour Bank South West Research Ethics Committee, Bristol, UK. For Western blot analysis, 64 glioma tumours with a range of subtypes were compared for BCKDC, hBCATc, and hBCATm expression (Supplementary Table 1). For IHC 53 glioma cases were graded by Dr. Kurian, consultant neuropathologist at Southmead Hospital Neuropathology Department, Bristol, UK. All glioma cases had a diagnosis from grade II to IV.

IDH and MGMT analysis

DNA was extracted from fresh, frozen tissue using the BioRobot EZ1 workstation (Qiagen). Sanger sequencing of the p.Arg132 region of IDH1 (codons 55 – 138) and p.Arg172 region of IDH2 (codons 151 – 179) was completed using a 3730 DNA analyser (Applied Biosystems). Immunohistochemistry (IHC) was performed using standard techniques with the antibody clone H09 (Dianova), which specifically reacts with the isocitrate dehydrogenase 1 (IDH1) R132H point mutation (1:10 dilution for 30 min at room temperature) in formalin-fixed tissue. MGMT analysis was assessed by methylation sensitive PCR. DNA samples underwent bisulphite modification, followed by PCR using the primers described by Estellier *et al* 2000 in a duplex reaction. PCR products were separated on the Beckman Coulter CEQ 8000 system.

Immunohistochemistry

Sections (3 µm in thickness) were taken onto 3-aminopropyltriethoxysilane coated adhesive slides and prepared for incubation with antibodies as previously described.^{10,12} Primary antibodies included: anti-hBCATm, 1:1000; anti-hBCATc, 1:6000; anti-BCKDC (ab126173, raised to the E1α subunit), 1:6000, incubated overnight (~20 hours) at room temperature. Standard IHC was performed as per Vectastain ABC and DAB substrate protocol, prior to immersion in copper sulphate (16 mM CuSO₄.5H₂O, 123 mM NaCl) for 4 minutes and

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3 counterstained with Harris's haematoxylin (25% Gill haematoxylin). Sections were viewed,
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5 scored and imaged on a Nikon Eclipse 50i and 80i microscope.
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8 **Scoring protocol**

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10 Each image was scored based on the degree of DAB staining. The sections were examined
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12 under a 20X objective and the degree of labelling designated on a semi-quantitative scale: 0
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14 = no staining, 1 = mild, 2 = moderate and 3 = intense staining. Scoring of each slide was
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16 independently performed by 3 individuals, with the median score used to obtain consensus.
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18 The analysis was conducted blind to mutation status, grade and age at diagnosis of each
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20 patient. The corresponding area of tissue was found with each of the antibodies in order to
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22 allow direct comparison of tumour cells. Negative antibody controls, where primary antibody
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24 was replaced with PBS, were supplied by Frenchay Neuropathology Department.
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27 **Western blot analysis**

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29 For Western blot analysis, 250 mg of tissue was collected in RNase free tubes and
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31 homogenised as described in Hull *et al.*, 2015a. Primary antibody (anti-BCKDC, 1/5000;
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33 anti-hBCATc, 1/5000; anti-hBCATm, 1/5000; anti-tubulin 1/000, 1/10 000 anti-GAPDH, 1/10
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35 000) was prepared in 5% non-fat milk powder in TBST and incubated overnight (20 hours,
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37 4°C) prior to TBST washes. Secondary antibody (1/5000) linked with HRP, was added for 1
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39 hour and then washed with TBST. The positive bands were visualized using
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41 chemiluminescent HRP substrate. GBX developer and fixer were used for 40 and 20
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43 seconds respectively. Integrated area densitometry was carried out with ImageJ. For re-
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45 probing, membranes were incubated with 1 M NaOH (7 minutes) and washed in TBST.
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48 **Statistical analysis**

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50 Data validity checks and descriptive analyses were performed prior to inferential analyses.
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52 These preliminary analyses indicated good quality data, free from unusual or aberrant
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54 observations, on model fit in a Cox proportional hazards regression. The strength of the
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56 association between BCKDC, hBCATc and hBCATm was quantified using Pearson's
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3 product moment correlation coefficient for linear association and for monotone association
4 using the nonparametric Spearman's rank correlation coefficient. Both approaches gave the
5 same statistical conclusions. Differences in mean levels for each of BCKDC, hBCATm and
6 hBCATc between IDH+ and IDH- was assessed using the robust independent samples t-test
7 without assuming equality of variances (the Welch-Aspin test) and was triangulated using
8 bootstrapping. Both approaches gave the same statistical conclusions. A two-group
9 Kaplan-Meier analysis of overall survival (OS) using the Mantel Cox log-rank test to judge
10 statistical significance was conducted using a median cut-point on each of BCKDC (median
11 = 0.6), hBCATm (median = 0.40), and hBCATc (median = 0.40). A two-group Kaplan-Meier
12 analysis is robust to the presence of potentially influential observations. In addition, a
13 sensitivity analysis was also performed to ensure conclusions were robust to the choice of
14 cut-point.
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RESULTS

All IDH-WT tumours examined have increased expression of the BCAA metabolic proteins relative to normal brain tissue (Hull *et al.*, 2012). Western blot analysis has demonstrated that in IDH-WT tumours there was a significant increase in the expression of hBCATm ($p=0.036$) and hBCATc ($p=0.007$) relative to IDH mutation glioblastomas (Figure 1A and B). This increase in hBCAT expression was supported using IHC analysis (Figure 1C). Although several banding patterns for BCKDC are evident in these gliomas, where combinations of 50 and 70 kDa proteins existed, the overall expression between IDH-WT and IDH1 did not show a significant difference (Figure 1A, B and C). Interestingly, substantive expression of BCKDC is evident in IDH1 mutation tumours even though hBCAT expression is largely absent.

Statistically, hBCATm and hBCATc have a high positive correlation coefficient ($r = 0.625$, $p < 0.001$). However, hBCATm is more specific than hBCATc, when considering Overall Survival. A Kaplan-Meier survival curve (Figure 2) and log-rank tests demonstrated that the predominant factor effecting overall survival is IDH status (Figure 2A), with IDH mutation positive gliomas associated with significantly better survival ($p=0.005$). Additionally, for all combinations of IDH-WT gliomas (Grade 2, 3 and 4), those expressing low hBCATm (median split) are associated with better overall survival (Figure 2B $p=0.003$). Moreover, in the Grade 4 only population, and exclusively considering those IDH-WT, a similar Kaplan-Meier curve (Figure 2C) and log-rank analysis shows that low hBCATm is significantly associated with better overall survival ($p=0.043$). The overall summary from these analyses is that hBCATm retains clinical utility across all data. Neither hBCATc nor BCKDC had a statistical effect on patient survival ($p=0.971$ and 0.642 , respectively).

Moreover, between tumour grades we observed a significant increase in hBCATm expression ($p=0.010$), indicating that as expression of hBCAT increases prognosis is poorer (Figure 3). Multiple banding patterns for hBCATm are also reported for anaplastic

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3 oligodendroglioma indicating that different isoforms of hBCATm are prominently expressed
4 in these tumour types (Figure 4). In some IDH1 mutation tumours positive hBCATc
5 expression was observed, which was previously considered to be absent in these tumours
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9 (data not shown). Also, less expression of the BCKDC upper band at 70 kDa is observed
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11 between the stage III AO tumours and the stage IV GBM tumours (observation).
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DISCUSSION

Despite aggressive treatment with combined radiochemotherapy and cytoreductive surgery, glioblastomas still have a poor prognosis, highlighting the need for new therapeutic targets and prognostic indicators. However, there is a large degree of variation, with marked differences in survival time attributed to the mutation status of key genetic markers, two of which are IDH and MGMT status (Nikiforova *et al.*, 2011). IDH status and methylation of genes is strongly associated with prognosis. Hypermethylation of the MGMT repair enzyme leads to epigenetic gene silencing, preventing the transcription of MGMT, and increasing tumour susceptibility to DNA damage by alkylating agents such as temozolomide, thus improving patient outcome (Esteller *et al.*, 2000; Esteller 2000). Improved prognosis is also reported for patients with a mutation in the IDH1 isoform. There are three isoforms of IDH, IDH1 is cytosolic and IDH2 and IDH3 are mitochondrial (Guo *et al.*, 2011). IDH1 and IDH2 are NADP⁺-dependent enzymes that, under normal physiological conditions, catalyse the oxidative carboxylation of isocitrate to α -ketoglutarate as part of the TCA cycle (Reitman *et al.*, 2011). In some gliomas, IDH has a point mutation resulting in a single amino acid substitution at arginine-132 in IDH1 and arginine-140 in IDH2 (Gross *et al.*, 2010). Both point mutations result in a functional enzyme that produces 2-hydroxyglutarate (2HG) (Gross *et al.*, 2010; Dang *et al.*, 2010) (Figure 5). Accumulation of 2HG causes methylation of histones within chromatin, preventing uncoiling and can prevent growth by impairment of cellular differentiation (Lu *et al.*, 2012). In gliomas with IDH-WT profiles there is frequently an amplification of EGFR (Hartmann *et al.*, 2010), which is associated with increased proliferation and radiotherapy resistance (Nakamura 2007). For survival, these tumours have hypermetabolic rates where glucose and glutamine are key metabolic fuels. More recently, an increased expression of hBCATc, a key metabolic protein, was associated with IDH-WT tumours (Tonjes *et al.*, 2013). In this study, hBCATc metabolism was shown to contribute to cell proliferation and in IDH1 mutation models, BCAT1 was shown to be hypermethylated resulting in decreased expression, indicating a direct role for hBCATc in tumorigenesis, and a potential therapeutic target (Tonjes *et al.*, 2013).

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3 Under normal physiological conditions, the hBCAT isoforms show both tissue and cell
4 specific expression (Hull *et al.*, 2012), which makes their co-expression in gliomas most
5 unusual, highlighting new regulatory mechanisms for the BCAT genes under pathogenic
6 conditions. Here, we report that the mitochondrial isoform, hBCATm, was also significantly
7 ($p=0.036$) increased in IDH-WT tumours and more importantly is a negative predictor of
8 patient survival in IDH-WT glioblastomas ($p=0.003$) and also in grade IV tumours alone
9 ($p=0.043$). This is significant because if hBCATc alone was inhibited i.e. the sole BCAA
10 metabolic target, then the activity of hBCATm might hypothetically supersede that of
11 hBCATc attenuating the impact of a targeted therapeutic. As expression of both hBCATs is
12 significantly increased in IDH-WT tumours, we predict that this will generate high
13 concentrations of BCKAs and glutamate, supporting the hypothesis that increased BCKA
14 production contributes to tumour growth and proliferation, dependent on the activity of the
15 BCKDC. Glutamate, also a product of BCAA metabolism, is a precursor for glutamine
16 synthesis, a major nutrient for tumorigenesis. Numerous metabolic studies have supported
17 a BCAA/BCKA shuttle between neuronal and astrocytic cells in rat and cell culture models
18 (Leith *et al.*, 2001; Sweatt *et al.*, 2004), indicating that in IDH1-WT tumours, a role exists for
19 glutamate and glutamine metabolism, which will be critical for brain neurotransmitter
20 metabolism and detoxification. Increased concentrations of glutamate has already been
21 shown to occur in gliomas, providing multiple benefits to tumour mass, including the increase
22 in the growth rate of tumour cells (Takano *et al.*, 2001), and an increased ability to invade
23 the surrounding tissue by increasing cellular motility (Lyons *et al.*, 2007). Glutamate release
24 also destroys neuronal cells creating a path for tumour growth (Sontheimer *et al.*, 2003).
25 Release of glutamate in exchange for cysteine is controlled by the system x_c^- cysteine
26 glutamine exchanger. The exchanger functions to generate glutathione (GSH) with
27 increased glutamate production leading to a rise in GSH concentration (De Groot *et al.*,
28 2011). In tumours, increased GSH is considered to protect against reactive oxygen species
29 further supporting tumour survival. Therefore, increased hBCAT expression, as reported
30 here, would result in neurological damage by accumulation of glutamate, increased
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3 invasiveness and free radical protection through increased glutamate production, altogether
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5 of benefit to tumour survival.
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8 Clearly, expression of hBCATc in IDH-WT tumours presents as a very attractive target for
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10 new therapies. However, this new data indicates that with increased hBCATm in IDH-WT
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12 tumours one must also consider how their expression will impact tumour growth and
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14 survival. Moreover, these studies are the first to illustrate that as the tumour progresses
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16 from grade II to grade IV the expression of both isoforms increases, supporting their role as
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18 new prognostic markers and indicators of patient survival.
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Patient number	Age at diagnosis	Diagnosis	Grade	Gender	IDH Status	MGMT status
1	43	Glioblastoma	IV	Male	IDH-	U
2	44	Glioblastoma	IV	Male	IDH-	U
3	61	Glioblastoma	IV	Male	IDH-	U
4	68	Glioblastoma	IV	Male	IDH-	M
5	64	Glioblastoma	IV	Male	IDH+	M
6	46	Glioblastoma	IV	Male	IDH+	M
7	35	Glioblastoma	IV	Male	IDH+	M
8	38	Glioblastoma	IV	Female	IDH+	M
9	26	Anaplastic astrocytoma	III	Female	IDH+	M
10	25	Anaplastic astrocytoma	III	Male	IDH+	U
11	20	Anaplastic astrocytoma	III	Male	IDH+	U
12	58	Anaplastic astrocytoma	III	Male	IDH-	M
13	67	Anaplastic astrocytoma	III	Male	IDH-	U
14	51	Anaplastic astrocytoma	III	Female	IDH-	M
15	61	Oligodendroglioma	II	Male	IDH+	M
16	44	Oligo-Astrocytoma	II	Female	IDH-	U
17	69	Anaplastic Oligodendroglioma	III	Male	IDH-	U
18	54	Anaplastic Oligodendroglioma	III	Male	IDH+	M
19	57	Anaplastic Oligodendroglioma	III	Female	IDH-	M
20	46	Anaplastic Oligodendroglioma	III	Female	IDH-	M
21	46	Anaplastic Oligodendroglioma	III	Female	IDH-	M
22	63	Glioblastoma	IV	Female	IDH-	UK
23	39	Glioblastoma	IV	Male	IDH-	U
24	28	Glioblastoma	IV	Female	IDH+	M
25	32	Oligo-Astrocytoma	II	Female	IDH+	M
26	73	Anaplastic astrocytoma	II	Male	IDH-	M
27	44	Anaplastic astrocytoma	III	Male	IDH-	U
28	54	Glioblastoma	IV	Female	IDH-	U
29	47	Glioblastoma	IV	Male	IDH-	M
30	68	Glioblastoma	IV	Male	IDH-	M
31	72	Glioblastoma	IV	Female	IDH-	M
32	56	Glioblastoma	IV	Male	IDH-	U
33	67	Anaplastic Astrocytoma	III	Male	IDH-	U
34	66	Glioblastoma	IV	Male	IDH-	M
35	51	Glioblastoma	IV	Male	IDH-	M
36	43	Glioblastoma	IV	Female	IDH-	M
37	71	Glioblastoma	IV	Female	IDH-	M
38	55	Glioblastoma	IV	Female	UK	M
39	68	Glioblastoma	IV	Male	IDH-	U
40	64	Glioblastoma	IV	Female	IDH-	M
41	66	Glioblastoma	IV	Female	IDH-	M
42	48	Glioblastoma	IV	Male	IDH-	U
43	61	Glioblastoma	IV	Male	IDH-	U
44	58	Glioblastoma	IV	Female	IDH-	U
45	57	Glioblastoma	IV	Female	IDH-	M
46	65	Glioblastoma	IV	Female	IDH-	M
47	52	Glioblastoma	IV	Male	IDH-	M
48	74	Glioblastoma	IV	Female	IDH-	U
49	53	Glioblastoma	IV	Male	IDH-	U
50	58	Glioblastoma	IV	Male	IDH-	M
51	34	Oligo-Astrocytoma	II	Male	IDH+	M
52	24	Oligo-Astrocytoma	II	Female	IDH+	M
53	29	Oligo-Astrocytoma	II	Female	IDH+	M
54	39	Diffuse Astrocytoma	II	Female	IDH+	M
55	49	Oligo-Astrocytoma	II	Female	IDH+	M
56	UK	Diffuse Astrocytoma	II	UK	IDH+	M
57	UK	Diffuse Astrocytoma	II	UK	IDH+	M
58	50	Oligo-Astrocytoma	II	Female	IDH+	M

Table 1: Patient details, IDH/MGMT of sample Glioblastomas

FIGURE LEGENDS

Figure. 1. Increased hBCAT expression in IDH-WT gliomas relative to IDH1 tumours. Western blot analysis and immunohistochemistry was used to evaluate the expression of BCKDC, hBCATm and hBCATc in IDH-WT and IDH1 mutation gliomas. **Panel A:** Patient details and Western blot analysis of glioma homogenates. The density of each band was measured using ImageJTM software and analysed for significance using a one-way ANOVA test in MinitabTM (n=55). **Panel B:** Panels show interquartile range (box) sample variability (whiskers) and the median (horizontal line within the interquartile range) of Western blot analysis for BCKDC, hBCATm and hBCATc relative to α -tubulin. Statistical analysis reveals a significant increase in hBCATm ($p=0.036$) and hBCATc ($p=0.007$) protein expression in IDH-WT gliomas compared to IDH mutation gliomas. **Panel C:** BCKDC, hBCATm, hBCATc staining of glioma sections, where increased staining was observed for hBCATm and hBCATc for IDH-WT relative to IDH mutation gliomas (n=51). Magnification for all sections, X20. Abbreviations: F – female; M – male; Me – methylated; MGMT – methylguanine-DNA methyltransferase methylation; U – unmethylated.

Figure. 2. Kaplan Meier analysis for IDH status and hBCATm expression. Statistics were calculated from Log rank Mantel-Cox analysis. **Panel A:** Cumulative survival of IDH status demonstrates significantly increased rate of survival in IDH mutation gliomas compared to IDH-WT ($p=0.005$) (n=47). **Panel B:** Cumulative survival of hBCATm expression in IDH-WT patients (including grades 2, 3 and 4): demonstrates significantly increased rate of survival in low (median split) hBCATm expressing gliomas ($p=0.003$) (n=35). **Panel C:** Cumulative survival of hBCATm expression in IDH-WT patients (grade 4 only) demonstrates significantly increased rate of survival in low (median split) hBCATm expressing gliomas ($p=0.043$) (n=24).

Figure. 3. hBCAT expression increases with tumour grade. Western blot analysis of BCKDC, hBCATm and hBCATc in glioma samples, grade 2-4. There is a significant increase in hBCATm ($p=0.010$) with increasing grade, where a trend for increased hBCATc is also observed (n=55). Abbreviations: F – female; M – male; Me – methylated; MGMT – methylguanine-DNA methyltransferase methylation; O1 – Oligodendroglioma; O2 – Oligo-Astrocytoma; U – unmethylated.

Figure. 4. Multiple banding for hBCATm in Oligodendric gliomas and Anaplastic oligodendrogliomas. Western blot analysis of BCKDC, hBCATm and hBCATc in IDH-WT and IDH mutation glioma samples. Abbreviations: F – female; M – male; Me – methylated; MGMT – methylguanine-DNA methyltransferase methylation; O1 – Oligodendroglioma; O2 – Oligo-Astrocytoma; U – unmethylated.

Figure 5. IDH-WT and IDH1 mutation metabolism of citrate, linked with BCAA metabolism. **A.** IDH-WT catalyses the oxidative carboxylation of isocitrate to α -ketoglutarate as part of the TCA cycle. **B.** In glioblastomas with IDH mutation the product of catalysis is 2-hydroxyglutarate, accumulation of which causes methylation of histones within DNA, ultimately impairing differentiation. **C.** In IDH-WT tumours increased hBCAT expression contributes to tumour survival and neurotoxicity of neighbouring healthy brain cells.

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2
3 **Table 1. Glioma cases used in immunohistochemical and Western blot analysis**
4 **of BCKD, hBCATc and hBCATm.** All cases were from the South West Brain
5 Tumour Bank (Southmead Hospital) and had no significant comorbidities (such as
6 Vascular dementia or Parkinson's disease). Abbreviations: M – methylated; MGMT –
7 methylguanine-DNA methyltransferase methylation; U – unmethylated; UK -
8 unknown.
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For Peer Review

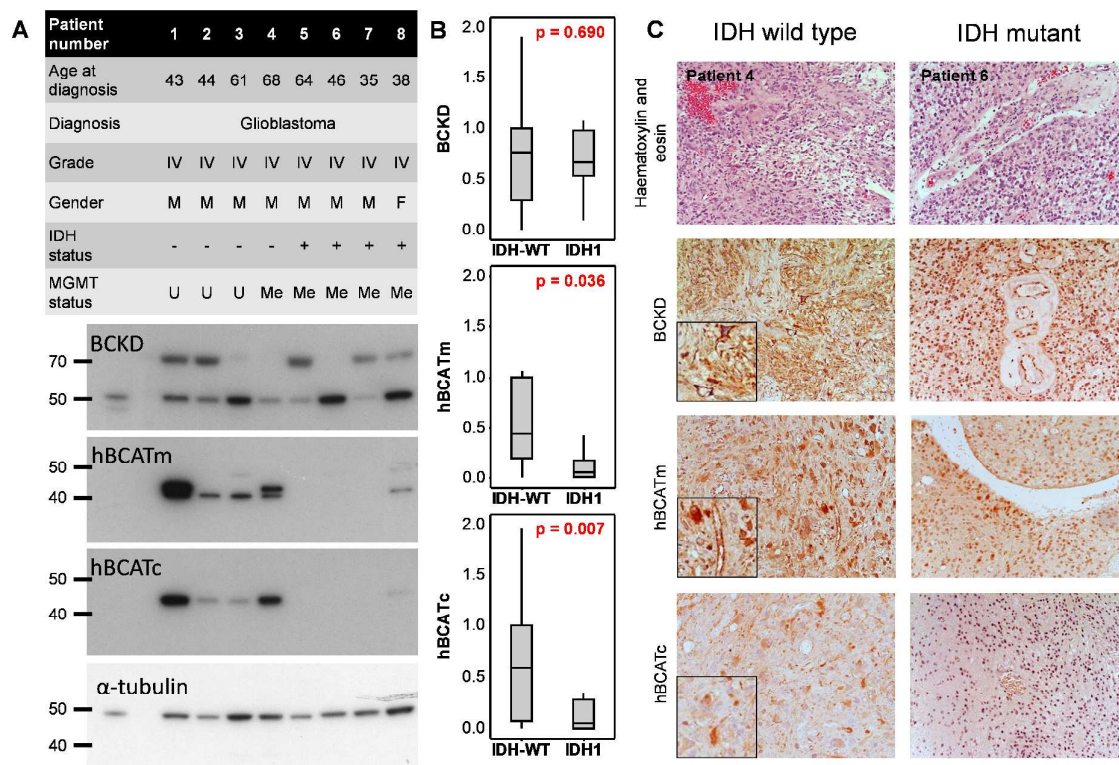


Figure 1

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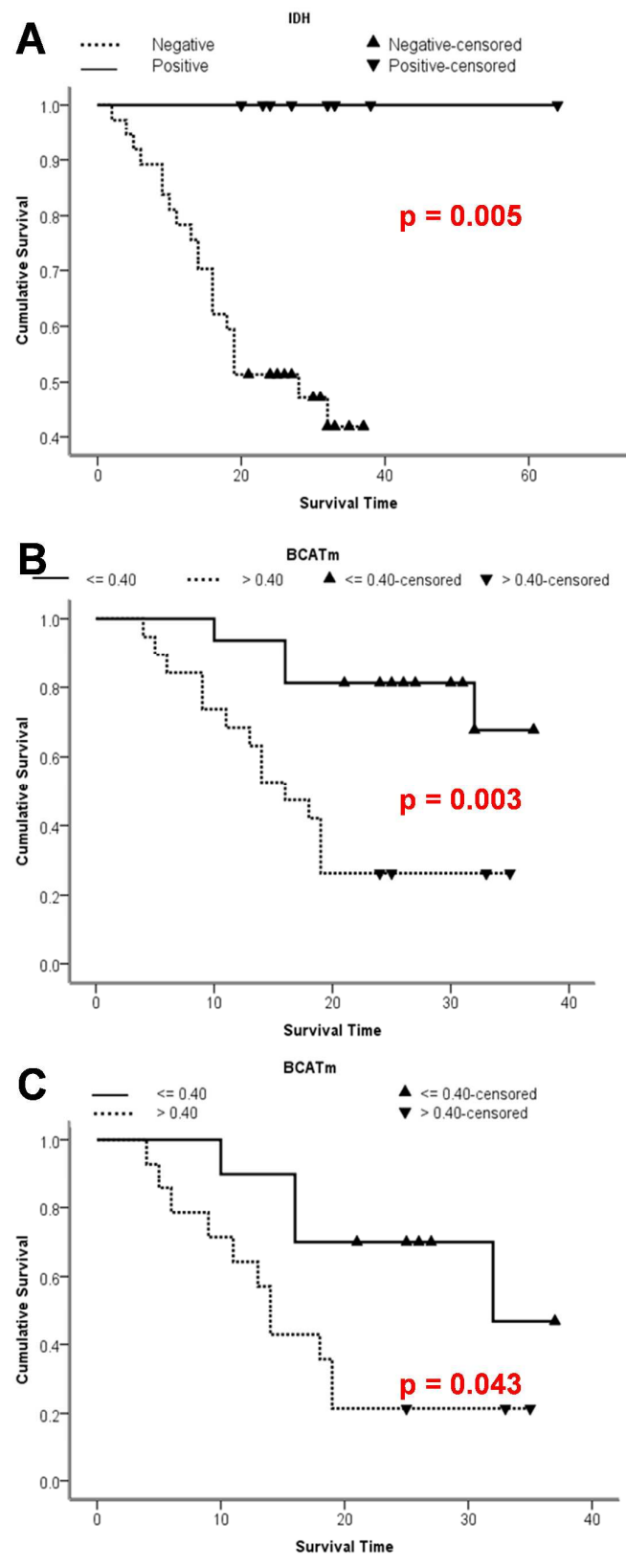


Figure 2

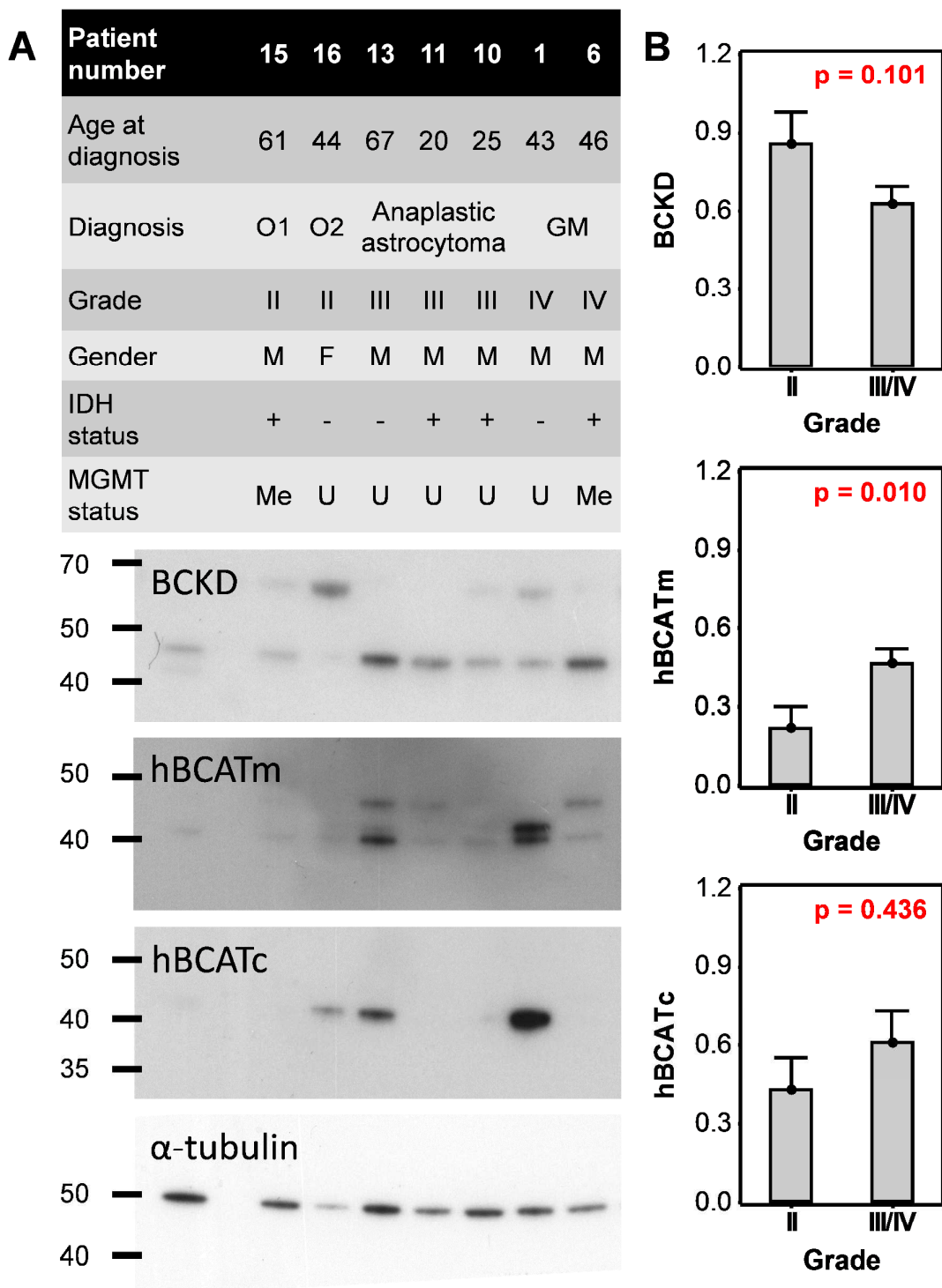


Figure 3

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Patient number	15	16	17	18	19	20	21
Age at diagnosis	61	44	69	54	57	46	46
Diagnosis	O1	O2	Anaplastic oligodendroglioma				
Grade	II	II	III	III	III	III	III
Gender	M	F	M	M	F	F	F
IDH status	+	-	-	+	-	-	-
MGMT status	Me	U	U	Me	Me	Me	Me

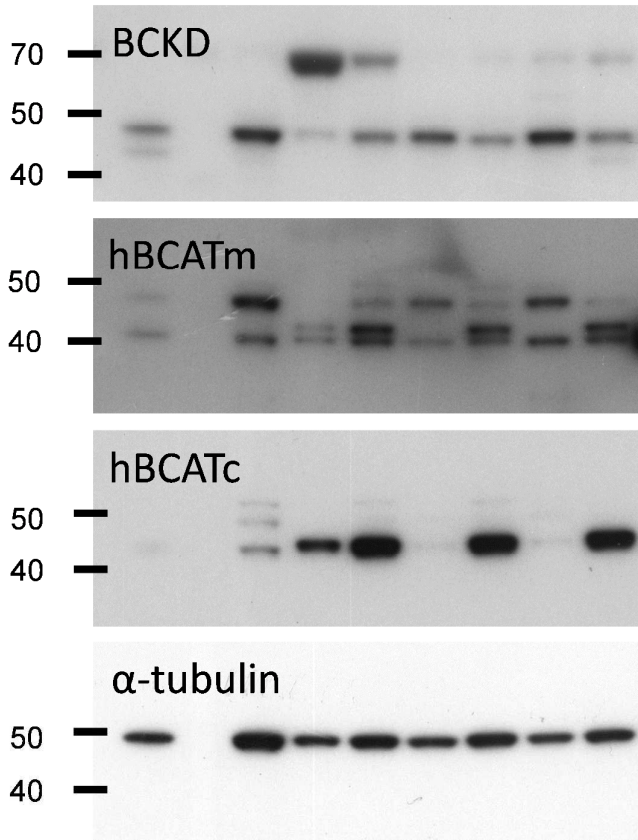


Figure 4

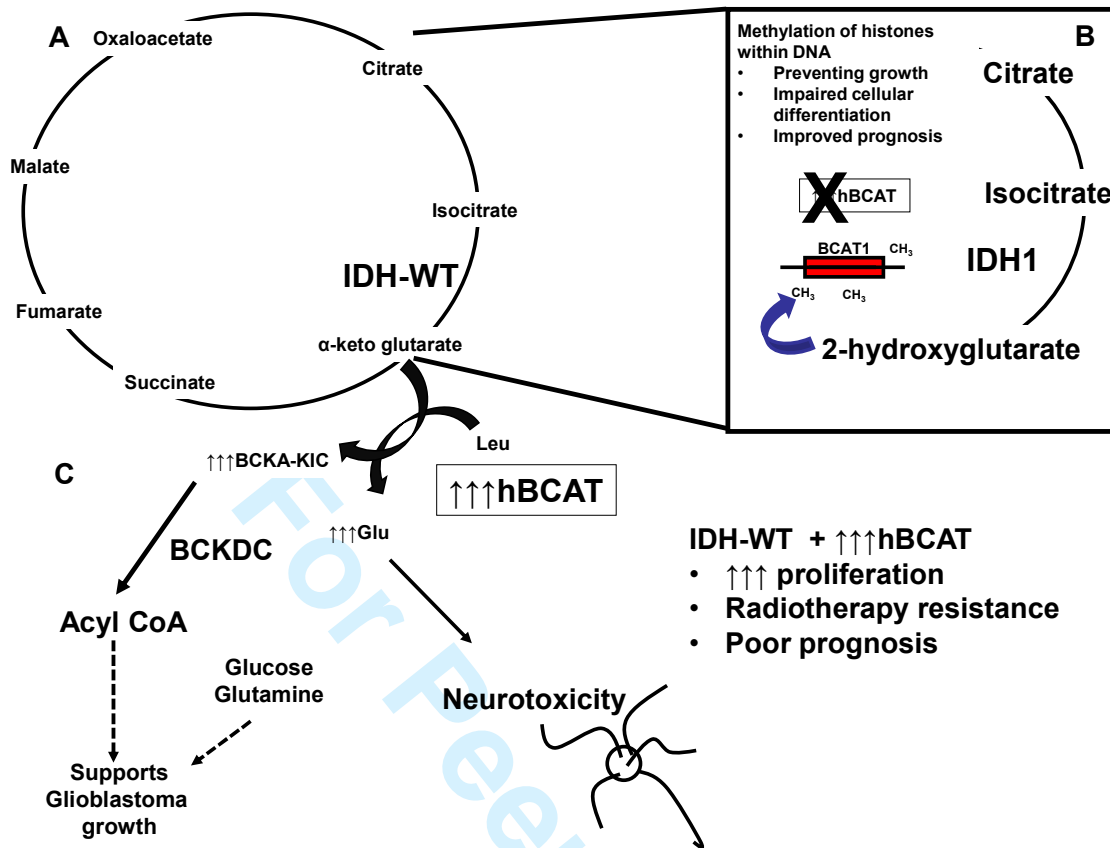


Figure 5

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