

SUPPLEMENTARY MATERIAL

Title: *CA9, CYFIP2 and LGALS3BP - a novel biomarker panel to aid prognostication in glioma*

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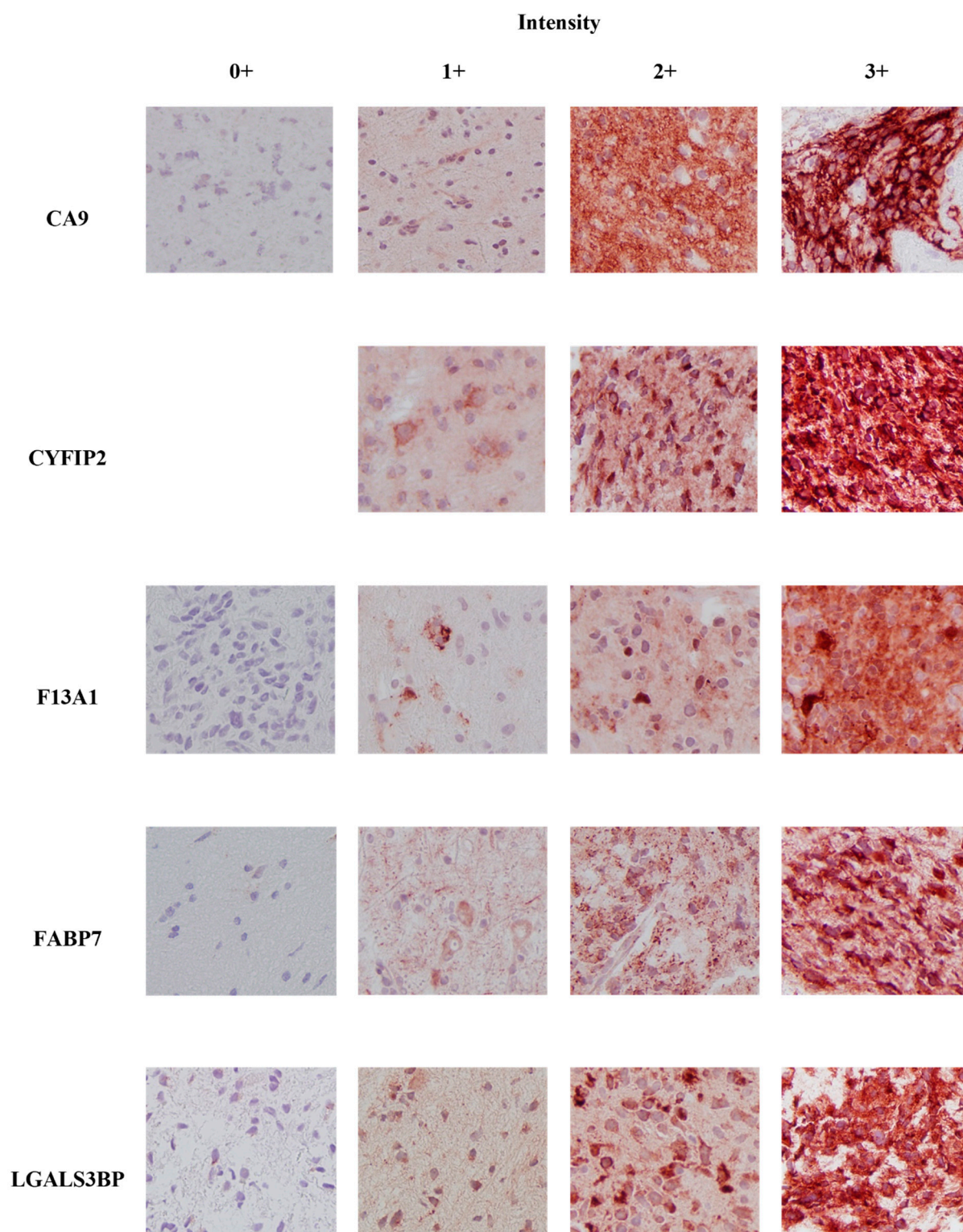
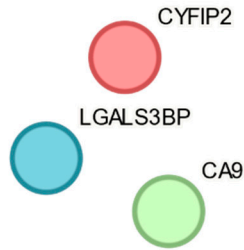


Figure S1: Representative images of the 6 candidate biomarkers in glioma tissue sections and their different scoring results. 20 × magnification.



Nodes:

Network nodes represent proteins

splice isoforms or post-translational modifications are collapsed, i.e. each node represents all the proteins produced by a single, protein-coding gene locus.

Node Color



*colored nodes:
query proteins and first shell of interactors*



*white nodes:
second shell of interactors*

Node Content



*empty nodes:
proteins of unknown 3D structure*



*filled nodes:
a 3D structure is known or predicted*

Edges:

Edges represent protein-protein associations

associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding to each other.

Known Interactions



from curated databases



experimentally determined

Predicted Interactions



gene neighborhood



gene fusions



gene co-occurrence

Others



textmining



co-expression



protein homology

Your Input:

● CYFIP2

Cytoplasmic FMR1-interacting protein 2; Involved in T-cell adhesion and p53/TP53-dependent induction of apoptosis. Does not bind RNA. As component of the WAVE1 complex, required for BDNF-NTRK2 endocytic trafficking and signaling from early endosomes (By similarity). (1278 aa)

● CA9

Carbonic anhydrase 9; Reversible hydration of carbon dioxide. Participates in pH regulation. May be involved in the control of cell proliferation and transformation. Appears to be a novel specific biomarker for a cervical neoplasia. (459 aa)

● LGALS3BP

Galectin-3-binding protein; Promotes integrin-mediated cell adhesion. May stimulate host defense against viruses and tumor cells. (585 aa)

Your Current Organism:

Homo sapiens

NCBI taxonomy Id: [9606](#)

Other names: H. sapiens, human, man

SI Figure 2: Interaction schematic of the CA9, CYFIP2 and LGALS3BP using STRING (accessed 5th December 2023) confirming no published interaction have been identified [1].

Table S1: Clinical characteristics of discovery cohort.

| | Number of patients (%) |
|---|------------------------|
| Gender | |
| Female | 4 (66.7) |
| Male | 2 (33.3) |
| Age at diagnosis (years) | |
| Median | 38 |
| Range | 30-46 |
| IDH | |
| <i>IDH1 R132H</i> | 5 (83.3) |
| <i>IDH1 R132G</i> | 1 (16.7) |
| Primary tumour grade | |
| II | 4 (66.6) |
| III | 2 (33.3) |
| Recurrent tumour grade | |
| III | 1 (16.6) |
| IV | 5 (83.3) |
| Treatment (in between specimens) | |
| Radiotherapy | 3 (50) |
| None | 3 (50) |
| Time to recurrence (Months)* | |
| Median | 46 |
| Range | 14-99 |
| Outcome# | |
| Alive | 4 (66.7) |
| Dead | 2 (33.3) |

*calculated as the months between the primary and recurrent surgery; #censor date, 29th Feb 2020.

Table S2: Immunohistochemistry antibodies.

| Specificity | Host species/Isotype | Concentration (µg/ml) | Company | Catalogue number |
|--|-----------------------------|------------------------------|-------------------|-------------------------|
| CA9 (carbonic anhydrase 9) | Rabbit IgG | 0.5 | Abcam | Ab15086 |
| CYFIP2 (cytoplasmic FMR1-interacting protein 2) | Rabbit IgG | 2.5 | Life Technologies | PA568449 |
| F13A1 (Coagulation factor XIII A chain) | Rabbit IgG | 0.25 | Sigma-Aldrich | HPA001804 |
| FABP7 (Fatty acid-binding protein, brain) | Rabbit IgG | 0.25 | Sigma-Aldrich | HPA028825 |
| LGALS3BP (galectin-3-binding Protein) | Rabbit IgG | 0.25 | Sigma-Aldrich | HPA000554 |
| PRKAR1A (cAMP-dependent protein kinase type I-alpha regulatory subunit) | Rabbit IgG | 1 | Sigma-Aldrich | HPA049979 |

Table S3: Cut off points for defining low and high protein expression by IHC.

| Protein (Cellular localisation) | Expression level | Concordance score (Tumour cells staining (%) × intensity) |
|--|-------------------------|--|
| CA9 (membranous/ cytoplasmic) | None | 0 |
| | Any (Low- high) | > 0 |
| F13A1 (cytoplasmic) | | |
| CYFIP2 (cytoplasmic) | Low | ≤ 200 |
| FABP7 (cytoplasmic) | High | >200 |
| LGALS3BP (cytoplasmic) | Low | ≤ 95 |
| | High | >95 |
| PRKAR1A (cytoplasmic) | Low | ≤ 40 |
| | High | > 40 |

Table S4: Differentially expressed proteins identified in discovery cohort. *Samples were further stratified into patients that received no treatment (A) and those that received radiotherapy (B) in between their surgeries. P-value <0.05 indicates significant results.*

| Direction of change* | Comparison | Uniprot ID | Protein Name | Protein abbreviation | Log 2 FC | P - value |
|----------------------|------------|------------|---|----------------------|----------|-----------|
| UP | A | Q16527 | Cysteine and glycine-rich protein 2 | CSRP2 | 2.09 | 0.027 |
| | A | Q08380 | Galectin-3-binding protein | LGALS3BP | 1.52 | 0.024 |
| | A | P04004 | Vitronectin | VTN | 1.43 | 0.034 |
| | A | Q6IAA8 | Ragulator complex protein | LAMTOR1 | 1.10 | 0.035 |
| | A | P01042 | Kininogen-1 | KNG1 | 1.02 | 0.016 |
| | B | P00488 | Coagulation factor XIII A chain | F13A1 | 1.39 | 0.044 |
| DOWN | A | Q9NZJ7 | Mitochondrial carrier homolog 1 | MTCH1 | - 1.07 | 0.013 |
| | A | O00429 | Dynamin-1-like protein | DNM1L | - 1.08 | 0.032 |
| | A | P31946 | 14-3-3 protein beta/alpha | YWHAB | - 1.15 | 0.012 |
| | A | P08195 | 4F2 cell-surface antigen heavy chain | SLC3A2 | - 1.19 | 0.013 |
| | A | P07954 | Fumarate hydratase | FH | 1.21 | 0.014 |
| | A | P21796 | Voltage-dependent anion-selective channel protein 1 | VDAC1 | - 1.23 | 0.007 |
| | A | Q9P2R7 | Succinate-CoA ligase | SUCLA2 | - 1.24 | 0.015 |
| | A | Q9H936 | Mitochondrial glutamate carrier 1 | SLC25A22 | - 1.28 | 0.040 |
| | A | O43301 | Heat shock 70 kDa protein 12A | HSPA12A | - 1.31 | 0.045 |
| | A | Q99798 | Aconitate hydratase | ACO2 | - 1.31 | 0.033 |
| | A | O75306 | NADH dehydrogenase | NDUFS2 | - 1.36 | 0.005 |
| | A | O15540 | Fatty acid-binding protein | FABP7 | - 1.38 | 0.008 |

| | | | | | | |
|--|---|--------|---|---------|-----------|-------|
| | A | Q96F07 | Cytoplasmic FMR1-interacting protein 2 | CYFIP2 | - 1.43 | 0.024 |
| | A | P46459 | Vesicle-fusing ATPase | NSF | - 1.65 | 0.045 |
| | A | P68366 | Tubulin alpha-4A chain | TUBA4A | - 1.69 | 0.046 |
| | A | Q9BRX8 | Redox-regulatory protein FAM213A | PRXL2A | - 1.81 | 0.018 |
| | A | Q99719 | Septin-5 | SEPTIN5 | - 1.85 | 0.034 |
| | A | P80723 | Brain acid soluble protein 1 | BASP1 | - 1.87 | 0.048 |
| | B | P13637 | Sodium/potassium-transporting ATPase subunit alpha-3 | ATP1A3 | - 1.02 | 0.004 |
| | B | P62873 | Guanine nucleotide-binding protein | GNB1 | - 1.06 | 0.044 |
| | B | P37840 | Alpha-synuclein | SNCA | - 1.07 | 0.006 |
| | B | P10644 | cAMP-dependent protein kinase type I-alpha regulatory subunit | PRKAR1A | - 1.09 | 0.013 |
| | B | P60880 | Synaptosomal-associated protein 25 | SNAP25 | - 1.13 | 0.011 |
| | B | P08247 | Synaptophysin | SYP | - 1.14 | 0.007 |
| | B | P17600 | Synapsin-1 | SYN1 | - 1.41 | 0.002 |
| | B | P63027 | Vesicle-associated membrane protein 2 | VAMP2 | - 1.50 | 0.006 |

**in recurrent samples.*

Table S5: Cancer related functions and association with prognosis of candidate biomarkers.

| Protein name (Symbol, Uniprot ID) | Function | Associations with cancer | References |
|--|--|---|------------------|
| Carbonic anhydrase 9 (CA9, Q16790) | pH regulation; hypoxia inducible | Glioma - silencing of CA9 reduced invasion, enhanced susceptibility to temozolomide (TMZ) and radiotherapy <i>in vitro</i> ; inhibition of CA9 enhances pro-apoptotic effects of TMZ; overexpression is associated with poorer overall survival. | [12, 13, 28, 43] |
| Cytoplasmic FMR1-interacting protein 2 (CYFIP2, Q96F07) | Pro-apoptotic protein and a direct target of TP53. Involved in T-cell adhesion. | Repression of CYFIP2 promoted colon cancer cell proliferation, growth and survival. Knockdown promoted proliferation, chemoresistance and inhibited apoptosis in gastric cancer cells. | [34, 44] |
| Coagulation factor XIII A chain (F13A1, P00488) | Key component in the coagulation cascade. F13A1 cross-links fibrin chains and protects them from fibrinolysis to strengthen the fibrin clot. | High expression associated with progression in lung squamous carcinomas. Downregulated in tumour-educated platelets in non-small cell lung cancer. Plasma levels in ovarian cancer patients were significantly higher compared to controls. Serum levels of the activation peptide of F13A1 were significantly reduced in colorectal patients. | [45-49] |
| Fatty acid-binding protein, brain (FABP7, O15540) | Important in the formation of the radial glial fibre in the developing brain. | Glioma - highly expressed in grade IV gliomasU251 glioma cells Overexpression or knockdown in glioma cell line U251 promoted or reduced proliferation, respectively. In glioma neurospheres, expression increased after radiation and resulted in enhanced migration and proliferation. Melanoma, breast cancer, renal cell carcinoma - overexpressed and associated with proliferation, higher | [50-57] |

| | | | |
|--|---|--|---------|
| | | grade of malignancy and shorter overall survival. | |
| Galectin-3-binding protein (LGALS3BP, Q08380) | Essential role in centrosome structure and function; modulates cell-cell and cell-matrix interactions. | Overexpressed in prostate, colorectal and epithelial ovarian cancers. Overexpression inhibits neutrophil-mediated tumour cell killing in colorectal cells. Breast cancer – over expression promotes angiogenesis and metastasis; associated with poor survival. | [58-62] |
| cAMP-dependent protein kinase type I-alpha regulatory subunit (PRKAR1A, P10644) | Regulatory subunit of the cyclic adenosine monophosphate (cAMP)- dependent protein kinases involved in cAMP signaling in cells. | Potential tumour suppressor. Lung adenocarcinoma - low expression correlated with poor overall survival. Inactivating mutations causes Carney Complex (patients develop multiple benign tumours). | [63-67] |