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ORIGINAL ARTICLE

FGF2 as a potential prognostic biomarker for proneural glioma patients

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ABSTRACT

Background. The survival of high-grade glioma patients is poor and the treatment of these patients can cause severe side effects. This fosters the necessity to identify prognostic biomarkers, in order to optimize treatment and diminish unnecessary suffering of patients. The aim of this study was to identify prognostic biomarkers for high-grade glioma patients.

Methods. Eleven proteins were selected for analysis due to their suggested importance for survival of patients with other types of cancers and due to a high variation in protein levels between glioma patients (according to the Human Protein Atlas, www.proteinatlas.org). Protein expression patterns of these 11 proteins were analyzed by immunohistochemistry in tumor samples from 97 high-grade glioma patients. The prognostic values of the proteins were analyzed with univariate and multivariate Cox regression analyses for the high-grade glioma patients, including subgroup analyses of histological subtypes and immunohistochemically defined molecular subtypes.

Results. The proteins with the most significant (univariate and multivariate $p < 0.05$) correlations were analyzed further with cross-validated Kaplan-Meier analyses for the possibility of predicting survival based on the protein expression pattern of the corresponding candidate. Random Forest classification with variable subset selection was used to analyze if a protein signature consisting of any combination of the 11 proteins could predict survival for the high-grade glioma patients and the subgroup with glioblastoma patients. The proteins which correlated most significantly (univariate and multivariate $p < 0.05$) to survival in the Cox regression analyses were Myc for all high-grade gliomas and FGF2, CA9 and CD44 for the subgroup of proneural gliomas, with FGF2 having a strong negative predictive value for survival. No prognostic signature of the proteins could be found.

Conclusion. FGF2 is a potential prognostic biomarker for proneural glioma patients, and warrants further investigation.

High-grade gliomas consist of anaplastic glioma (WHO grade III) and glioblastoma (WHO grade IV) [1]. Anaplastic glioma is further subclassified according to histology as anaplastic astrocytomas, anaplastic oligodendrogliomas, anaplastic oligoastrocytomas and anaplastic ependymomas. Glioblastoma is histologically classified as astrocytoma. The most recent, clinically relevant classification of glioblastomas has

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been given by The Cancer Genome Atlas (TCGA) network based on genomic abnormalities and gene expression alterations of tumor protein p53, epidermal growth factor receptor (EGFR), neurofibromin (NF1), platelet-derived growth factor receptor alpha (PDGFRA) and isocitrate dehydrogenase 1 (IDH1), classifying it into proneural, neural, classical and mesenchymal subtypes [2]. High-grade gliomas constitute the most common and aggressive types of primary brain tumor in adults. The median survival time for anaplastic glioma is approximately 2–3 years [3] and for glioblastoma approximately 15 months [4]. The survival differs between the molecular subtypes and patients with the proneural subtype survive significantly longer than patients with the other subtypes [2].

The survival of high-grade glioma patients can be highly variable, even between patients with the same histological subtype. The standard therapy for high-grade gliomas, which consists of surgery followed by radiotherapy with the addition, since the year 2005, of chemotherapy with temozolomide for glioblastomas [5] may cause severe neurological side effects. Therefore the identification of potential prognostic biomarkers is a key task in the prediction of the patient survival. Patients with a good prognosis can avoid or receive less aggressive treatment, which may cause unwanted side effects without providing them any benefits [6]. There are currently three clinically useful prognostic markers for high-grade glioma patients; co-deletion of the chromosomes 1p and 19q, IDH1 mutation and O-6-methylguanine-DNA methyl transferase (MGMT) methylation [7,8]. 1p/19q co-deletion prognosticates longer survival for oligodendroglioma patients and can be used to identify patients which respond better to PCV (procarbazine, lomustine and vincristine) [8]. IDH1 mutation prognosticates longer survival for anaplastic gliomas and secondary glioblastomas [7]. Silencing of MGMT by DNA methylation prognosticates longer survival for glioblastoma patients [9] and it can be used to decide if elderly patients (>60 years) with glioblastoma should be treated with either hypofractionated radiotherapy or temozolomide [10].

In this study, the prognostic value of a selection of proteins, for which the expressions have previously been suggested to be important for the survival of patients with other types of cancers treated in a similar way as high-grade glioma patients, was studied for high-grade glioma patients. Information about the proteins is summarized in Table I. These 11 proteins were found to be expressed in at least a subset of high-grade gliomas according to the immunohistochemistry based data available at the Human Protein Atlas database (www.proteinatlas.org) [11]. In the present study we analyzed the expression pattern of these proteins in a well-defined clinical cohort of high-grade glioma patients.

Methods

Patient cohort

One hundred and fifty patients were diagnosed with high-grade glioma and treated with radiotherapy at the Department of Oncology, Uppsala University Hospital, from 1990 to 1998. The diagnosis of the patients in the cohort was reassessed [12] and consisted thereafter of 97 high-grade glioma patients who were at least 18 years of age at diagnosis and for whom paraffin-embedded tumor samples with sufficient material for reassessment and tissue microarray construction were available (Table II). The study was approved by Regional Ethical Review Board in Uppsala (application number 2006/084).

Diagnostic reassessment and tissue microarray construction

Tissue microarrays (TMAs) were constructed from paraffin-embedded tumor samples from each patient according to [13]. The diagnostic reassessment of histology and grade and the classification of molecular subtypes have previously been described for the patients in our cohort [12]. Popova et al. [12] developed a method to classify patients into molecular subtypes based on immunohistochemical analyses of EGFR, CD44 molecule, c-mer proto-oncogene tyrosine kinase, p53 and oligodendrocyte lineage transcription factor 2. This method was applied to the patients in our cohort.

Protein selection

Since this is a retrospective study and we do not have information about the response to therapy for the patients in our cohort we focused on finding prognostic biomarkers which potentially may have predictive value for high-grade glioma patients. Proteins were selected for which the expressions have been suggested to be prognostic for patients with other types of cancers who received similar treatment as the patients in our cohort (surgery + radiotherapy $\pm$ chemotherapy with alkylating agents and/or tubulin inhibitors) or predictive for the response to radiotherapy for patients with other types of cancers. A biomarker that is predictive for radiotherapy may have prognostic value in our patient cohort since all patients were treated with radiotherapy. The selection was done by going through all search results in PubMed after searching for: (radiation OR radiotherapy) AND (predictive OR prognostic) AND

Table I. Summary of the proteins selected for analysis of their prognostic value.

Protein	Importance for cancer cells	Cancer types for which the protein expression has prognostic or predictive value	Importance of protein expression for high-grade glioma patients
BSG	Immunosuppression and invasion [1]	Cervical cancer [2]	BSG is overexpressed in high-grade gliomas [3], its expression is a negative prognostic factor for astrocytic gliomas (including both low- and high-grade) [4] and it is associated with an intact BBB function [5]
CA9	Cell growth, survival and invasiveness [6]	Glottic laryngeal carcinoma [7], NSCLC [8] and breast [9], cervical [10] and head and neck cancers [11]	The combined expression of HIF1A, CA9, and SPP1 is a negative prognostic factor for high-grade glioma patients [12] and CA9 expression is a negative prognostic factor for grade II and III oligodendrogliomas [13] and grade II-IV astrocytomas [14]
CD44	Cell migration, adhesion and proliferation [15]	Larynx cancer [16]	CD44 is overexpressed in high-grade gliomas [17]
EIF4EBP1	Cell growth and proliferation [18]	Cervical carcinoma [19]	N/A
FGF2	Cell proliferation, migration, survival angiogenesis and inhibition of differentiation [20]	NSCLC [21], head and neck [22] and breast cancers [23]	FGF2 is overexpressed in high-grade gliomas [24]
MCM2	DNA replication [25]	Oral squamous cell carcinoma [26]	MCM2 is overexpressed in high-grade oligodendroglioma [27]
MIF	Inflammation, immune response, proliferation and angiogenesis [28]	Head and neck cancer [29]	Elevated MIF expression correlates with tumor recurrence and poor prognosis of patients with gliomas (including both low- and high-grade) [30]
Myc	Cell growth, proliferation, survival and malignant transformation [31]	Cervical carcinoma [32]	Myc is overexpressed in malignant astrocytomas [33] and Myc expression is a positive prognostic factor for glioblastoma patients [34]
SOD2	Cell survival [35]	Buccal mucosal squamous cell [36] and cervical carcinomas [37]	SOD2 expression is downregulated in high-grade astrocytomas [38]
p53	Cell cycle arrest, apoptosis, senescence and DNA repair [39]	NSCLC [40], rectal [41], breast [42], ovarian [43] esophageal [44], head and neck [45], prostate [46] cancers etc.	p53 expression is a negative prognostic factor for oligodendroglioma patients [47] a positive prognostic factor for anaplastic astrocytoma patients [48] and can either be a positive [49] or a negative [50] prognostic factor for glioblastoma patients
TP73	Proliferation, differentiation and cell death [51]	Cervical [52] and colorectal [53] cancers	N/A

BSG, basigin (Ok blood group); CA9, carbonic anhydrase 9; CD44, CD44 molecule; EIF4EBP1, eukaryotic translation initiation factor 4E binding protein 1; FGF2, fibroblast growth factor 2; HIF1A, hypoxia inducible factor 1, alpha subunit; MCM2, minichromosome maintenance complex component 2; MIF, macrophage migration inhibitory factor; Myc, v-Myc myelocytomatosis viral oncogene homolog; N/A, not available; NSCLC, non-small cell lung cancer; SOD2, superoxide dismutase 2, mitochondrial; SPP1, secreted phosphoprotein 1; p53, tumor protein p53; TP73, tumor protein p73.

expression). Proteins which were already reported to be predictive for the response to radiotherapy for high-grade glioma patients or prognostic for high-grade glioma patients who received similar treatment as the patients in our cohort in studies with a similarly sized or larger patient cohort as ours were excluded from the selection, such as EGFR [14] and cyclin-dependent kinase inhibitor 2A (CDKN2A) [15]. Also proteins for which most papers in the literature report negative findings regarding their prognostic or predictive value were excluded. Since a prognostic factor needs to discriminate patients based on, e.g. differences in relative levels of protein expression, the 11 proteins which demonstrated the largest variations of protein levels between patients, according to their immunohistochemical staining in glioma patient samples in the Human Protein Atlas, were selected to be analyzed for their prognostic value (Table III). The variation in protein levels was defined as the standard deviation of the fraction of patients at each antibody staining level (mean fraction for all antibodies used).

Table II. The characteristics and treatment of the 97 high-grade glioma patients. For parameters where information was missing in the patients' journals the number of patients for which information was available is given in parenthesis.

	Number of patients
WHO grade	
III	21
IV	76
Histological subtype	
Anaplastic astrocytoma	10
Anaplastic oligodendroglioma	11
Glioblastoma	76
Molecular subtype	
Classical	33 (85)
Proneural	26 (85)
Mesenchymal	23 (85)
Other	3 (85)
Treatment	
Surgery	86 (96)
Radiotherapy	97
Adjuvant proton radiation	19 (28)
Second line radiotherapy	4 (90)
Adjuvant PCV	1 (93)
Second line temozolomide	1 (90)
Second line temozolomide + PCV	1 (90)
Second line PCV	17 (90)
Second line PCV + other chemotherapeutic drugs	3 (90)
Second line other chemotherapeutic drugs	9 (90)
Median OS [months]	
Anaplastic astrocytoma	13.8
Anaplastic oligodendroglioma	9.3
Glioblastoma	10.4
Age	
Range	22.5–80.5
Median	59.5
Gender	
Male	55
Female	42
Performance status	
Poor	14 (87)
Good	73 (87)

Immunohistochemistry

Immunohistochemistry was done as previously described [13]. Primary antibodies used were anti-BSG 11989-1-AP (Proteintech, IL, USA) diluted 1:200, anti-CA9 2330 (Strategic Diagnostics Inc., DE, USA) diluted 1:2000, anti-CD44 M7082 diluted 1:100, anti-p53 M7001 diluted 1:1000 (both from Dako Sweden AB, Stockholm, Sweden), anti-EIF4EBP1 1557-1 (Epitomics, CA, USA) diluted 1:75, anti-FGF2 sc-79 diluted 1:400 (Santa Cruz Biotechnology, CA, USA), anti-MCM2 HPA031496 (Atlas Antibodies AB, Stockholm, Sweden) diluted 1:1100, anti-SOD2 06-984 diluted 1:600 and anti-p73 AB7824 diluted 1:100 (both from Merck Millipore, MA, USA). Immunohistochemical staining of MIF [16] and Myc [17] have previously been done for the patients in this cohort. Due to the absence of tumor cells in some cores on the TMA sections, up to 10 patients per protein were excluded from further evaluation. IHC stainings were annotated by two pathologists in the designated localizations (Table III) in regard to intensity of immunoreactivity in tumor cells (0 = negative, 1 = weak, 2 = moderate and 3 = strong) and fraction of immunoreactive tumor cells ($0 \leq 2\%$, 1 = 2–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75% and $5 \geq 75\%$). An IHC score, ranging from 0 to 15, was calculated by multiplying the intensity level with the quantity level.

Statistics

All calculations were done in R [18].

Single protein analyses. To analyze correlations between overall survival and the intensity, fraction and score annotations of the IHC stainings for each protein, univariate Cox regression was used. To rule out the influence of tumor grade, age, sex, performance status, extent of tumor resection and second-line chemotherapy (Table II) on the correlation, multivariate Cox regression analysis was used. All regressions were done separately for the whole patient cohort, for patients with the histological subtypes anaplastic astrocytoma ($n = 10$), anaplastic oligodendroglioma ($n = 11$) and glioblastoma ($n = 76$) and for patients with the molecular subtypes (classified in [12]) classical ($n = 33$), proneural ($n = 26$), and mesenchymal ($n = 23$). The neural subtype was not included because it was not possible to classify this subtype with immunohistochemistry [12]. Overall survival was defined as time from surgery to death. For patients who had progressed from a lower grade tumor the diagnosis and time of surgery for the tumor sample with highest grade was used.

The most significant IHC staining intensities, fractions or scores (= variables) from univariate Cox regression (with both univariate and multivariate $p < 0.05$) were analyzed further by five-fold cross-validated Mann-Whitney tests and illustrated by cross-validated Kaplan-Meier plots (according to [19] and Supplementary Methods, available online at: http://informahealthcare.com/doi/abs/10.3109/0284186X.2014.951492). P-values were calculated by permutation analysis.

Protein signature analyses. To analyze the possibility of predicting survival time from protein signatures generated from combinations of the intensity and fraction annotations (the scores were not included as they are a product of intensity and score and will be included anyway in the built non-linear model) of the IHC stains of the 11 proteins, Random Forest classification with variable subset selection was adopted (as described in Supplementary Methods

Table III. Summary of annotations of IHC stainings for the selected proteins.

Protein symbol	Protein name	Analyzed localization	Number of analyzed patients	Percentage of patients with detectable protein levels	IHC staining range	
					Intensity	Fraction of positive tumor cells
BSG	Basigin	Cytoplasm	90	82	weak to moderate	11–75%
		Membrane		90	weak to moderate	2–75%
CA9	Carbonic anhydrase IX	Membrane	93	16	weak to strong	2–25%
CD44	CD44 molecule	Cytoplasm	94	13	weak to moderate	11–50%
EIF4EBP1	Eukaryotic translation initiation factor 4E binding protein 1	Membrane		34	moderate to strong	2->75%
		Cytoplasm	90	66	weak to moderate	2->75%
FGF2	Fibroblast growth factor 2	Cytoplasm	90	80	moderate to strong	2->75%
MCM2	Minichromosome maintenance complex component 2	Nucleus	90	88	moderate to strong	2->75%
MIF	Macrophage migration inhibitory factor	Cytoplasm	92	70	weak to strong	2->75%
		Nucleus		2	moderate to strong	11–25%
Myc	v-Myc myelocytomatosis viral oncogene homolog	Cytoplasm	86	90	weak to strong	2->75%
		Nucleus	86	49	weak to strong	2–75%
p53	Tumor protein p53	Nucleus	92	43	weak to strong	2->75%
SOD2	Superoxide dismutase 2, mitochondrial	Nucleus	92	100	weak to strong	2->75%
TP73	Tumor protein p73	Cytoplasm	56	95	weak to strong	26->75%
		Nucleus	57	96	weak to strong	2->75%

available online at: http://informahealthcare.com/doi/abs/10.3109/0284186X.2014.951492). The analysis was done for all high-grade glioma patients (n = 97) and the subgroup with glioblastoma patients (n = 76) separately. The patients were divided into two classes based on their survival time, those living longer than one year (35 of all high-grade glioma patients and 25 of the glioblastoma patients) and those living shorter (62 of all high-grade glioma patients and 51 of the glioblastoma patients). The threshold one-year overall survival was used instead of the median as the median will vary slightly between different training sets, and we find one year to be a reasonable split point as it is close to the median.

Multiple test correction. It is difficult to determine the exact number of independent tests that have been done in this study since the patient groups are partly overlapping and the protein levels may to some extent be dependent on each other. Hence, p-values were not corrected for multiple testing. They are instead used for ranking the significance of the results in this study rather than specifying the probability that the results are true positives.

Results

Protein selection

PubMed searches for proteins for which the protein levels have been suggested to be important for survival for cancer patients yielded 143 possible proteins to analyze. Ten of these proteins were excluded due to that they had already been reported to be either prognostic or predictive for radiotherapy in a similar cohort of high-grade glioma patients as in our study. Three proteins were excluded due to that the majority of the publications in the literature analyzing their prognostic or predictive value reported negative findings. Eleven proteins were excluded due to that information regarding the protein expression in glioma tissue was not available in the Human Protein Atlas. Of the remaining 119 proteins, the 11 proteins with the highest variation in protein levels between glioma patients, according to the Human Protein Atlas, were selected for further analyses.

Single protein analyses

Protein expression patterns of the selected 11 proteins were measured with immunohistochemistry in 97 high-grade glioma patients. The clinical characteristics of the patients are summarized in Table II and the annotations of the IHC analyses are summarized in Table III. The prognostic value of each protein was analyzed, by univariate and multivariate Cox regression analyses, for all high-grade glioma patients, subgroups of patients with the histological subtypes anaplastic astrocytoma (n = 10), anaplastic oligodendroglioma (n = 11) and glioblastoma (n = 76) and subgroups of patients with the molecular subtypes classical (n = 33), proneural (n = 26), and mesenchymal (n = 23), in accordance

with immunohistochemistry-based classification [12]. The results from the Cox regression analyses are summarized in Supplementary Table I (available online at: http://informahealthcare.com/doi/abs/10.3109/0284186X.2014.951492).

The most significant correlations (univariate and multivariate $p < 0.05$) from Cox regression analyses between protein levels and survival time were for the score of nuclear Myc in all high-grade gliomas (univariate $p = 0.041$ and multivariate $p = 0.003$) and the subgroup of glioblastomas (univariate $p = 0.015$ and multivariate $p = 0.044$) and the fraction of tumor cells positive for cytoplasmic FGF2 (univariate $p = 0.002$ and multivariate $p = 0.001$), the intensity of membranous CA9 (univariate $p = 0.009$ and multivariate $p = 0.037$) and the fraction of tumor cells positive for membranous CD44 (univariate $p = 0.030$ and multivariate $p = 0.020$) in proneural gliomas (Figure 1). High levels of all these proteins correlated to shorter survival for the patients. Since we have not corrected the p-values for multiple testing there is a probability that some of these results are false positives. However, we used the p-values to select the best correlations for further analysis.

To illustrate the possibility of predicting patient survival depending on whether the patients had a high or a low level of these proteins which correlated most significantly to survival, cross-validated Kaplan-Meier plots were generated and corresponding permutation p-values for cross-validated Mann-Whitney tests were computed (see Supplementary Methods available online at: http://informahealthcare.com/doi/abs/10.3109/0284186X.2014.951492, for more details). The protein levels which could best predict survival, with regard to whether the patient had a low (including patients with no protein expression) or a high level of the protein, were the fraction of tumor cells positive for cytoplasmic FGF2 ($p = 0.002$) and intensity of membranous CA9 ($p = 0.026$) for proneural gliomas (Figure 2). For Myc ($p = 0.126$ for high-grade gliomas and $p = 0.584$ for glioblastomas) and CD44 ($p = 0.056$) the survival time could very poorly be predicted by determining which patients have low and which have high protein levels. Since the p-value for FGF2 was relatively low (the observation would be significant at the 0.05 level if the maximum number of independent hypothesis tests in this study was as high as 25) we believe that the fraction of tumor cells positive for cytoplasmic FGF2 may be a prognostic biomarker for proneural glioma patients. The intensity of the IHC staining of cytoplasmic FGF2 may be important for survival since also the score of IHC staining was predictive of survival ($p = 0.002$). Protein levels of FGF2 were variable in the proneural gliomas and FGF2 was present in 72% of the samples from proneural tumors which were available for analysis. The protein levels ranged from moderate in 2–10% of the tumor cells to strong in 51–75% of the tumor cells in the samples (Table III and Figure 3). For CA9 however, there were only a few patients that had detectable protein levels and the p-value was relatively high considering the multiple hypothesis tests that have been done in this study. Thereby, further studies in a larger patient cohort are needed to assess the potential of CA9 as a prognostic biomarker for proneural gliomas.

Protein signature analyses

To analyze if a protein signature consisting of the intensity and fraction annotation of the IHC stainings of any combination of the 11 proteins included in the study could predict survival for the high-grade glioma patients and the subgroup with glioblastoma patients, Random Forest classification with variable subset selection was used. The average error rate (over the 100 repeated holdouts) for the classification model was 0.4 for both the whole cohort and the subgroup with glioblastoma patients, with or without variable subset selection. This was on the same level as the measured error rate for the permuted case, suggesting that the classification model could not be used to predict longer or shorter survival. In addition, Random Forest regression with variable subset selection was done but did not generate any models that could predict survival time (data not shown). Hence, no prognostic signature predicting survival could be found.

Discussion

In this study, we have analyzed the prognostic significance of the protein levels of 11 proteins in a cohort of 97 high-grade glioma patients. The median overall survival of the patients in our cohort (Table II) was slightly shorter than survival times reported in the literature [1]. This may be due to that the patients in our cohort have been treated during the 1990s, and since then adjuvant, concomitant and second-line chemotherapy is more commonly used for these patients. The introduction of temozolomide in the standard treatment of glioblastoma patients increased the median survival with 2.5 months [4] and studies have shown that oligodendroglioma patients, especially those with 1p/19q co-deletion, benefit from adjuvant PCV [20]. Consequently, since there is a smaller variation in survival between the patients in our cohort than what is common the possibility of predicting survival according to differences in protein levels between patients may be more

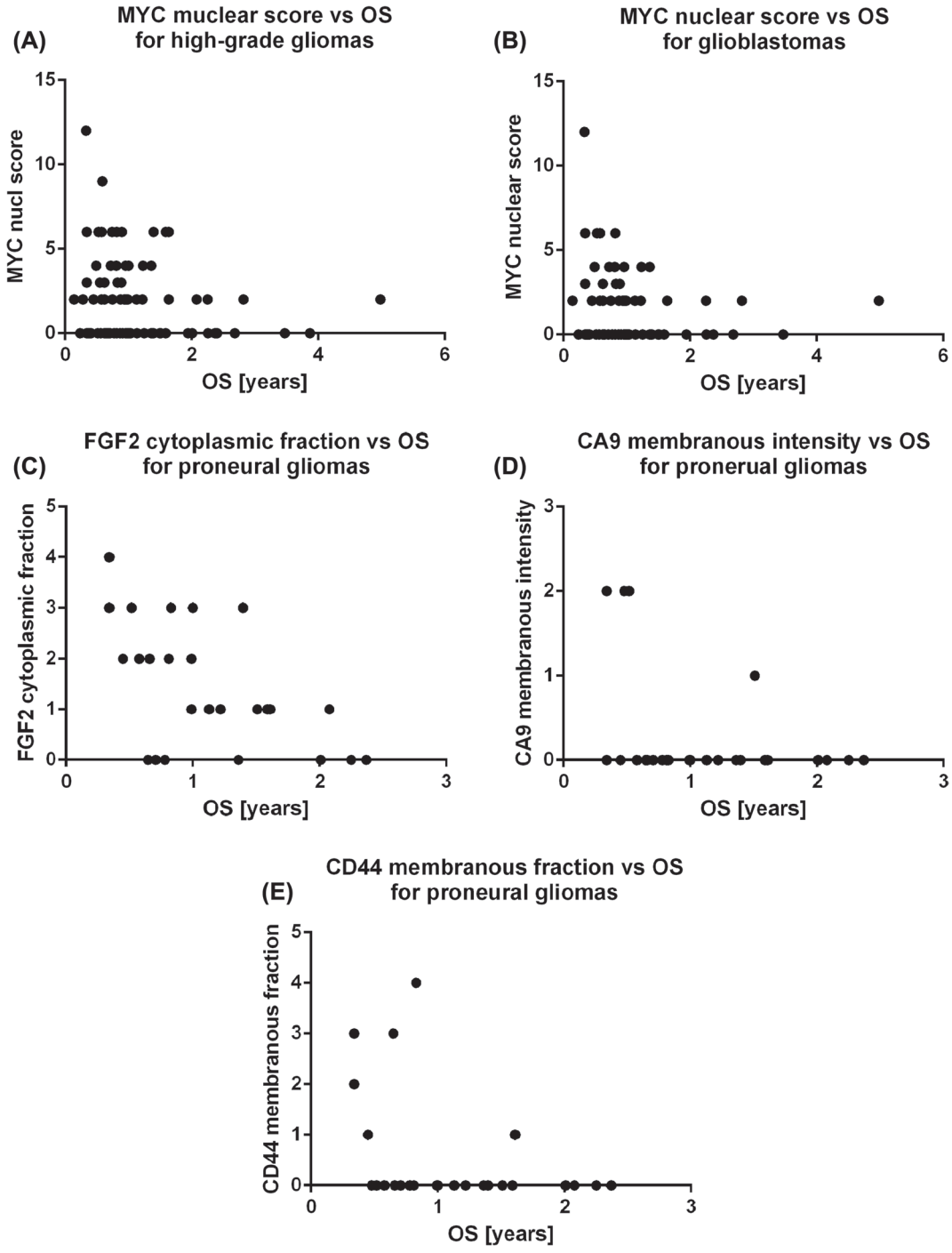

Figure 1. The most significant correlations between protein levels and overall survival (OS) according to Cox regression analyses. The graphs present the survival time plotted against the annotations of the score of nuclear Myc in high-grade gliomas (A) and in glioblastomas (B) and the fraction of tumor cells positive for cytoplasmic FGF2 (C), the intensity of membranous CA9 (D) and the fraction of tumor cells positive for membranous CD44 (E) in proneural gliomas.

difficult in our cohort and the prognostic significance of the proteins included in this study may be underestimated.

The protein which could best predict survival based on whether the patients have a low or high protein level was FGF2 for proneural glioma patients, where a high fraction of tumor cells positive for cytoplasmic FGF2 predicted shorter survival. Proneural glioma is a molecular subtype of gliomas which is characterized by alterations of *PDGFRA* and mutation of *IDH1* [2]. Patients with proneural gliomas are significantly younger and have longer survival than patients with the other subtypes. However, these patients do not have any survival benefit

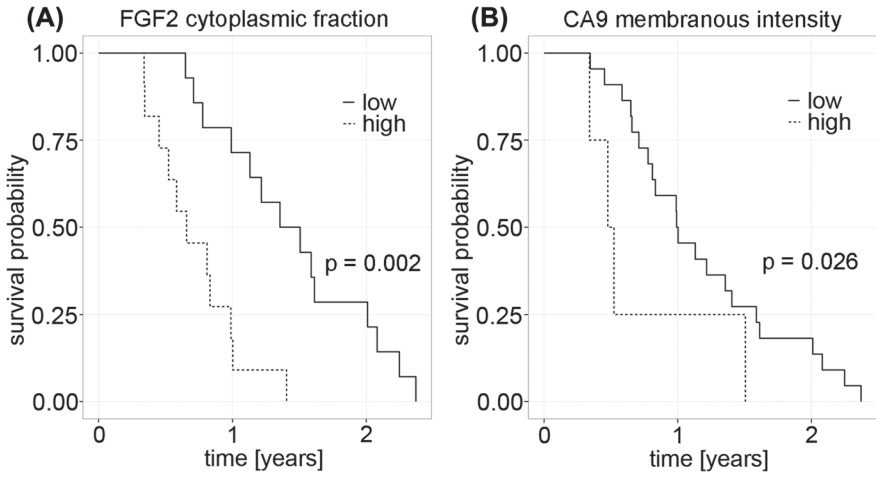

Figure 2. The proteins which could best predict survival time according to cross-validated Kaplan-Meier analyses. The graphs represent the overall survival of patients with high or low levels of the fraction of tumor cells positive for cytoplasmic FGF2 (A) and the intensity of membranous CA9 (B).

from aggressive treatment, defined as concurrent chemo- and radiotherapy or more than three subsequent cycles of chemotherapy [2]. FGF2 is a growth factor involved in tumor formation and malignancy through its promotion of cell proliferation, migration, survival, angiogenesis and its inhibition of differentiation [21]. Its expression in tumor tissue is prognostic for head and neck cancer patients [22] and both prognostic and predictive for the response to therapy for lung cancer [23] and breast cancer patients [24]. FGF2 is often overexpressed in high-grade glioma [25] and constitutively active fusions of its receptor, the fibroblast growth factor receptor (FGFR), with transforming acidic coiled-coil containing protein (TACC) 1 or TACC2 are present in a fraction of high-grade gliomas [26]. Hence, FGF2 may be involved in regulating survival of high-grade glioma patients and according to our study it may be a valuable prognostic biomarker for proneural glioma patients. Prospective clinical trials are needed to find out whether the long-term survivors of the proneural glioma patients may suffice with less aggressive treatment than standard treatment. In that case FGF2 may be used to spare these patients from unnecessary suffering. In addition, with prospective studies the potential of FGF2 as a predictive biomarker for the response to therapy for high-grade glioma patients could be evaluated.

Since high expression of FGF2 correlated to worse survival for the high-grade proneural glioma patients, FGF2 and its receptor may also constitute potential therapeutic targets for these patients. Inhibiting FGF2 or FGFR in glioblastoma inhibits cell proliferation in vitro and tumor growth in vivo [27] and targeting FGFR fusions in mice with glioblastoma prolongs their survival [28]. There are several inhibitors for FGFs and FGFRs which have performed well in early clinical trials for various types of cancers [29,30]. However, none are used so far in routine cancer therapy and none have been tested in glioma patients. Hence, it would be interesting to test the effect of these inhibitors in proneural high-grade glioma patients with high FGF2 expression.

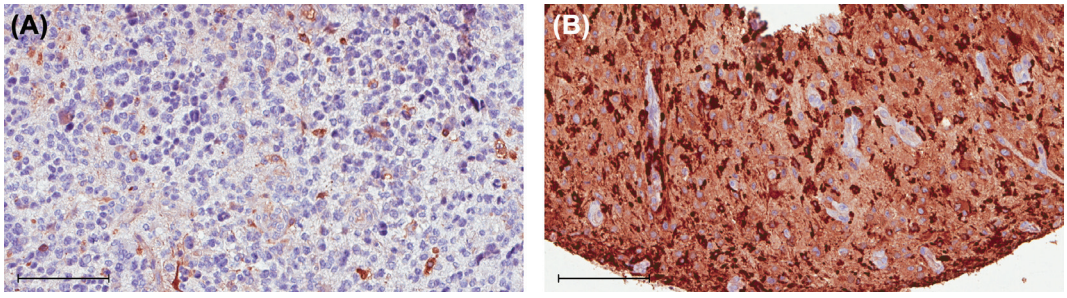

Figure 3. Protein levels of FGF2 in tumor samples from proneural gliomas detected by immunohistochemistry. The protein levels of cytoplasmic FGF2 ranged from moderate in 2–10% of the tumor cells (A) to strong in 51–75% of the tumor cells (B) in the tumor samples. Scale bars represent 0.1 mm.

There was a trend that the four proneural glioma patients which had detectable protein levels of CA9 had shorter survival than patients with no detectable protein levels of CA9. CA9 is an enzyme that catalyzes the reversible hydration of carbon dioxide and is thereby vital to many biological and physical functions. In tumors its expression is often upregulated by hypoxia and its expression promotes cell growth, survival and invasiveness [31]. CA9 expression is correlated to poor prognosis for patients with various cancers [32,33]. The combined expression of HIF1A, CA9, and SPP1 is a negative prognostic factor for high-grade glioma patients [34] and CA9 expression is a negative prognostic factor for grade II and III oligodendrogliomas [35] and grade II–IV astrocytomas [36,37]. Hence, there are many indications that CA9 may be involved in causing shorter survival for high-grade glioma patients. However, further studies in a larger patient cohort are needed to assess its role as a prognostic biomarker for proneural glioma patients.

Since all the proteins which correlated most significantly to shorter survival in the Cox regression analysis, Myc, FGF2, CA9 and CD44, are involved in signaling during hypoxia [38–40] and since tumor hypoxia causes resistance to cancer therapy and increased metastasis [41], it is possible that upregulation of these proteins in the high-grade glioma tumors reflects a higher degree of hypoxia and that this is the reason for the shorter survival for the patients. Various hypoxic markers are correlated to worse survival for patients with different types of cancers [42].

No prognostic signature including the proteins analyzed in this study could be found for all high-grade glioma patients or the subgroup with glioblastoma patients. This indicates that no combination of the 11 proteins collaboratively affect the survival of these patients.

In conclusion, we propose FGF2 to be a useful prognostic biomarker for proneural glioma patients and encourage further prospective clinical trials to validate this finding.

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Supplementary material available online

Supplementary Methods. Methodology of the cross-validated Kaplan-Meier analyses and the Random Forest with subset variable selection analyses and Supplementary Table 1 available online at: http://informahealthcare.com/doi/abs/10.3109/0284186X.2014.951492.