Prognostic and Clinical Values of Chaperone Protein P4HB in Malignant Glioma

Stella Sun
University of Hong Kong
Karrie Mei-Yee Kiang
University of Hong Kong
Gilberto Ka-Kit Leung (gilberto@hkucc.hku.hk)
The University of Hong Kong https://orcid.org/0000-0003-3147-3057

Research Article

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Abstract

Introduction. Prolyl 4-hydroxylase, beta polypeptide (P4HB) has previously been identified by our group to play important roles in association with glioma malignancy and temozolomide (TMZ) resistance through the unfolded protein response (UPR). The present study focused on the prognostic value of P4HB in glioma.

Methods. P4HB expression was assessed by immunohistochemical staining and semi-quantified by pathologist visual scoring in 73 WHO grade I-IV gliomas. Results were correlated with clinicopathological data.

Results. Our results show that P4HB expression was significantly associated several clinicopathological parameters including age (*p=0.035*), tumour grade (*p=0.002*), and the number of TMZ treatment cycles received (*p=0.043*). Using Kaplan-Meier analysis, P4HB expression was positively correlated with mortality (p=0.014) and disease progression (*p=0.026*). In patients treated with TMZ, high P4HB expression level was significantly associated with poorer overall survival (OS) (p=0.014) and progression free survival (PFS) (*p=0.027*). The association between MGMT promoter methylation and P4HB expression was also interrogated. Patients with $MGMT^{Meth}P4HB^{Low}$ tumours had the most favourable progression free survival (48 months) than patients with other combination of MGMT methylation status and P4HB expression (log rank *p=0.001*). Multivariate analysis revealed that P4HB was an independent prognostic indicator for OS (*p=0.048*).

Conclusions. P4HB could constitute an independent prognostic marker, especially for high grade glioma with the potential for informing a nuanced pathological stratification during clinical decision-making with respect to MGMT promoter methylation status and TMZ treatment.

1. Introduction

Malignant glioma is the most common subtype of primary brain tumour in adults, with an overall survival of approximately 18 months [1]. Temozolomide (TMZ), an oral DNA alkylating agent, has been the current standard of care. In the standard regimen after maximal surgical resection, patients would receive concomitant chemoradiotherapy with TMZ (75mg/m^2), followed by adjuvant TMZ (150-200mg/m^2) for 6 cycles [2]. Despite promising results in improving patient survival, drug resistance and tumour relapse is almost inevitable. The expression of DNA damage repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) in glioma cells may also protect cells from alkylating drugs including TMZ [3] whereas a methylated MGMT gene promoter may inactivate MGMT expression, leading to a greater therapeutic response to TMZ. MGMT methylation status has thus been adopted as an useful prognostic and predictive biomarker for better patient management.

The endoplasmic reticulum (ER) stress response and the associated unfolded protein response (UPR) represent one major mechanism in the development of TMZ-resistance in glioblastoma [4]. In cancer cells, adaptive ER stress response due to prolonged ER stress (e.g. caused by hypoxia and glucose

deprivation) is a common feature, and chronic ER stress imposed by long term chemotherapeutic treatments may protect against further insults by tipping the balance in favour of a pro-survival UPR response [5]. Cancer cells with constitutive activation of ER stress response and upregulation of ER chaperone have been found to better tolerate chemotherapies [4, 6]. One of the ER chaperone proteins, P4HB (prolyl 4-hydroxylase, beta polypeptide), was shown to play critical roles in TMZ-resistance and contribute to glioma recurrence driven by ER stress response. Significant upregulation of P4HB expression was found in patients with recurrent glioma as well as in TMZ-resistant xenografts [7, 8]. P4HB gene silencing also enhanced cellular apoptosis in TMZ-resistant glioma cells [4]. Furthermore, Xipell *et al*. demonstrated the adjuvant effect for TMZ treatment through targeting ER stress, where MGMT expression was reduced following a combinatorial treatment regimen [9], suggesting a dual effect in sensitizing tumours to TMZ by mediating ER stress-induced apoptosis as well as limiting the DNA damage repair by MGMT.

ER chaperones have emerged as predictive markers for treatment response. Given that P4HB is associated with glioma malignancy and TMZ-resistance, we evaluated whether the assessment of P4HB expression could provide valuable clinical information in predicting TMZ response and patient survival, irrespective of MGMT promoter methylation status. In this article, we will also discuss and highlight the potential application of P4HB as a novel biomarker to further stratify glioma patients into more clinically relevant entities.

2. Materials And Methods

2.1 Study population

This is a retrospective study on 73 patients (mean age: 47.3 ± 14.9 years) with gliomas (WHO grade I, 4.1%; WHO grade II, 17.8%; WHO grade III, 19.2% and WHO grade IV, 58.9%). Clinical data were retrieved for statistical analysis including patient's demographic data, tumour characteristics (i.e. lesion sites, pathological classification, WHO grade), treatment approaches after surgical resection (radiotherapy and/or chemotherapy), number of TMZ cycles received (≤ 6 or > 6), MGMT promoter methylation status, overall survival (OS), and progression free survival (PFS). All patients had their diagnosis confirmed by a specialist in pathology. The study was approved by the Institutional Review Board of our institution, and all tissues were collected with signed informed consent from patients.

2.2 P4HB Immunohistochemical Assay

Immunohistochemical staining procedures were performed according to the protocol described in our previous study [8]. Paraffin sections were deparaffinized in xylene and rehydrated in a descending ethanol series. Heat-induced antigen retrieval was performed and sections were quenched from endogenous tissue peroxidase by 3% hydrogen peroxide. After blocking with 5% normal goat serum (Dako, Glostrup, Denmark), sections were immunostained with rabbit monoclonal anti-P4HB (Cell Signalling Technology Inc., Danvers, MA, USA) at 4^{o}C overnight. After incubation with HRP-conjugated antibody (Invitrogen-

Zymed Laboratories, South San Francisco, CA, USA) for 1 hour, signal was detected using EnVisionTM$^+$ Kit (Dako). P4HB expression was assessed semi-quantitatively by two experienced pathologists. Ten random high-powered fields were examined under a light microscope (Olympus, Waltham, MA). The staining intensity was assigned a score of 0 (negative), 1 (weak), 2 (moderate), 3 (strong). The proportional of stained tumour cells was scored as 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51%-75%) and 4 (> 75%). The multiplication of these two variables was calculated as the final score. Samples were considered positive if the score exceeded the median value.

2.3 Statistical analysis

SPSS 18.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. The difference between two sets of data was analyzed by Chi-Square test or independent sample t-test. Kruskal-Wallis Test was used to compare differences of more than two groups of data. In the Kaplan-Meier survival analysis, patients were dichotomized into two groups and survival difference between groups was assessed by log-rank test. Risk factors for mortality after treatment were identified by univariate and multivariate analysis using Cox proportional hazards regression model. The Pearson correlation analysis was used to correlate P4HB expression with promoter DNA methylation and TMZ treatment. All statistical tests were two-sides, and a P value < 0.05 was considered statistically significant.

3. Results

3.1 Clinical correlation of P4HB level

P4HB has previously been identified by our group to be dysregulated in high grade glioma. In this study, we further analyzed its correlative relationship with other clinicopathological parameters in 73 glioma patients with complete clinical follow-up using immunohistochemical staining. Consistent with our previous findings, P4HB expression was significantly correlated with malignancy grade (*p=0.002*) (Table 1). WHO grade IV glioma showed the highest expression of P4HB among all WHO grades of glioma (Fig.1a). Chi-Square test revealed that high P4HB expression level was significantly associated with glioma patients who were greater than 55 years of age *(p=0.035)*; patients who received radiotherapy (*p=0.007*) and chemotherapy *(p=0.036*); and patients who received a greater number of TMZ treatment cycles (> 6) (*p=0.043*) (Table 1).

3.2 Prognostic value of P4HB in glioma

To explore the prognostic value of P4HB in glioma, we dichotomized all the patients into two groups, $P4HB^{Low}$ and $P4HB^{High}$, using the median of P4HB expression level obtained from immunohistochemical staining. By Kaplan-Meier analysis, patients of the two groups differed significantly in OS duration; the median survival of patients with a high level of P4HB was around 16 months, significantly shorter than patients with a lower level of P4HB (131 months, log rank p=0.015, Fig.1b). To avoid potential bias due to relatively low P4HB expression in less malignant gliomas, and to identify a meaningful subgroup for the clinical predictive value of P4HB, only WHO Grade III and IV were included for subsequent analysis

(n=57). Similar results were obtained, showing that high P4HB would predict poorer survival and vice versa ($P4HB^{Low}$ 20 months; $P4HB^{High}$ 13 months, log rank *p=0.014*, Fig.1c).

To further investigate the prognostic value of P4HB in the prediction of PFS, Kaplan-Meier survival was conducted in the same dataset of WHO III and IV gliomas. Results demonstrated that the subgroup with low P4HB expression was associated with longer time to progression whereas high P4HB demonstrated shorter time to progression ($P4HB^{Low}$:12 months; $P4HB^{High}$:6 months, log rank *p=0.026*, Fig.1d).

3.3 P4HB shows superiority in predicting survival in TMZ treated population

Whilst several chemotherapeutic agents are available for the treatment of glioma [10], TMZ is the most widely used and a FDA approved first-line chemotherapeutic [11, 12]. In our dataset, patients who received TMZ exhibited a longer overall survival (20 months) than those who did not (11 months*; p=0.001*, Fig.2a). Patients who were on TMZ treatment expressed a significant level of P4HB compared to those who did not receive TMZ (*p=0.042*, Fig.2b). We then separated the patients into those who had received TMZ treatment and those without in order to interrogate the prognostic value of P4HB with regard to TMZ treatment. Among patients who had received TMZ, a high level of P4HB expression was found to be significantly associated with shorter OS than those with a lower level of P4HB expression (*p=0.014*, Fig. 2c), whereas no such association was found in patients who received no TMZ therapy, suggesting that a potential relationship between TMZ chemotherapy response and P4HB expression (Fig.2d). The same was observed in regard to PFS: high P4HB expression level was associated with shorter PFS (8 months) than those with low P4HB expression in patients treated with TMZ (39 months; *p=0.027*) but not in those who did not receive TMZ therapy (Fig.2e & 2f). The overall findings are suggestive of a prominent predictive impact of P4HB in a subset of high-grade glioma patients who received TMZ therapy.

3.4 P4HB predicts survival in patients with methylated MGMT

MGMT is a DNA repair enzyme that can effectively reverse DNA damages induced by TMZ, and MGMT promoter methylation status has a significant impact on glioma patient survival. High level of MGMT activity can render tumours resistant to alkylating agents and may therefore serve as both a predictive and prognostic molecular marker in high-grade glioma. As expected our data showed a significant difference in OS for patients with tumours exhibiting MGMT methylation compared to those without, regardless of treatment received in this subset of high-grade glioma patients ($MGMT^{meth}$:47 months; $MGMT^{Unmeth}$:16 months, log rank *p=0.002*, Fig.3a). There was a significant correlation between TMZ treatment and better survival in patients with methylated MGMT (Pearson correlation; *p=0.0001*, r=0.523). We then investigated the relationship between MGMT methylation status and P4HB expression. Significant difference in OS was observed between $P4HB^{Low}$ (254 months) and $P4HB^{High}$ groups (8 months) in patients with methylated MGMT tumours (*p=0.002;* Fig.3b), whereas patients with unmethylated MGMT tumours showed no significant difference in OS between P4HB subgroups (data not shown). By Cox proportional hazards model, P4HB expression could significantly distinguish between

patients with methylated MGMT tumours by OS (HR=4.261, 95% CI=1.312-13.846, *p=0.016*) but not in those with unmethylated MGMT lesions (HR=1.038, 95% CI=0.372-2.892, *p=0.944*).

MGMT is a well-established biomarker for the prediction of TMZ treatment response, and high P4HB expression has previously been identified by our group to be associated with TMZ resistance both *in vitro* and *in vivo*. Here, we further investigated the interrelationship between MGMT promoter methylation and P4HB expression by Kaplan Meier analysis on PFS. Patients were divided into four groups depending on the expression of the two markers. Among the forty-two TMZ-treated patients with high glioma, $MGMT^{Meth}P4HB^{Low}$ gave the best PFS (n=11; 48 months), followed by $MGMT^{Meth}P4HB^{High}$ (n=15; 7 months), $MGMT^{Unmeth}P4HB^{Low}$ (n= 2; 6 months), and $MGMT^{Unmeth}P4HB^{High}$ (n=14; 4 months; Fig.3c). The results suggest that P4HB may be used to assist survival prediction in patients with methylated MGMT by identifying certain patients who would nonetheless respond poorly to TMZ.

3.5 P4HB in prediction of response to TMZ in high-grade glioma

Since we found that P4HB expression was significantly associated with both OS and PFS exclusively in patients treated with TMZ, and that P4HB may play important roles in affecting glioma malignancy and TMZ resistance both *in vitro* and *in vivo,* we further investigated the clinical significance of P4HB in predicting TMZ treatment response.

Among the 57 high-grade glioma patients, 37 patients had been treated with adjuvant TMZ, and 19 patients with high P4HB expression were found to show on-treatment disease progression. The latter group exhibited a mean value of 6.74 P4HB expression, which was almost double that in those who showed favourable responses to TMZ (P4HB mean value = 3.25). High expression of P4HB was significantly associated with on-treatment disease progression (*p=0.020*) (Fig.3d).

3.6 P4HB is an independent prognostic factor for high-grade glioma

Clinicopathological factors affecting OS and PFS in patients with high-grade glioma were examined (Table 2). Univariate analysis showed that age at diagnosis (*p=0.005*), WHO grade (*p=0.000*), chemotherapy (i.e., TMZ)(*p=0.001*), MGMT methylation status (*p=0.004*), and P4HB expression level (*p=0.018*) were significantly associated with OS. Multivariate analysis using Cox regression model demonstrated that not only WHO grade *(p=0.002)* and chemotherapy (TMZ) (*p=0.001*), but also P4HB expression level were independent prognostic markers by OS (*p=0.048*). Patients with high P4HB expression also had less favorable PFS outcomes compared to those with low P4HB expression. On univariate analysis, WHO grade (*p=0.007*), MGMT (*p=0.010*), the number of TMZ cycles (*p=0.020*) and P4HB expression (*p=0.035*) were significant prognostic factors by PFS. Multivariate analysis showed that among all prognostic factors, whilst P4HB expression level did not reach statistical significance, it was the only factor showing marginal association with PFS, suggestive of its potential as an independent prognostic factor in terms of PFS (Table 2).

Discussion

TMZ has been widely used for treating primary and recurrent high-grade gliomas. However, the efficacy of TMZ is often limited by the development of chemo-resistance. Intertumoral heterogeneity between glioma patients, such as epigenetic silencing of MGMT is the most studied mechanism and is a promising predictive marker for TMZ response [13]. Whilst the prognosis of high-grade malignant glioma patients is generally poor (with median OS <16 months in our cohort), we found that it is possible to further identify subgroups of patients with differing prognoses according to P4HB expression, on top of MGMT promoter methylation status. As a key member of the protein disulfide isomerases (PDI) family, P4HB acts as a chaperone mediator in the UPR and modulates ER stress response similar to GRP78, which is a master regulator of UPR [14]. Activation of UPR via inhibition of chaperone proteins has previously been identified to be associated with a reduction in DNA repair capacity [15], and may create vulnerabilities that sensitize aggressive tumours to cytotoxic drugs and prevent cancer progression and/or recurrence [16, 17].

P4HB was previously identified by our group to possess oncogenic (pro-survival) properties in malignant glioma [7]. PDI has recently been reported as promising target for survival prediction and tumour progression in glioma [18]. However, the reported findings were limited to bioinformatics modeling of gene expression using datasets from TCGA and CGGA database; no clinical specimens were involved in the study. Here, glioma cohort with 73 patients were included in the current study to evaluate the prognostic significance of P4HB. Our findings were consistent with the literature that showed upregulation of P4HB in high-grade gliomas when compared to low grade lesions. Moreover, overexpression of P4HB was significantly associated with several clinical parameters including older age and the prolonged use of TMZ, which may be indicative of a more aggressive tumour that required extended treatment.

We found that P4HB expression can be used to predict OS and PFS in patients with malignant glioma treated with TMZ. The predictive value of P4HB was especially prominent in subgroups of TMZ-treated malignant glioma population than those who did not receive TMZ. This could be explained by the poor pre-existing neurological or general condition of the patients who were not fit for chemotherapy, as the median OS in both $P4HB^{low}$ and $P4HB^{high}$ patients in the non-TMZ-treated group were only 10 and 12 months, respectively. It is important to note that, the median OS in $P4HB^{low}$ patients were significantly longer (254 months) than in $P4HB^{high}$ patients (14 months), suggesting that tumours with low P4HB may be more responsive to TMZ treatment compared to tumours with P4HB upregulation. In other words, overexpression of P4HB may confer resistance to chemotherapies secondary to increased ER stress and UPR. Indeed, aberrant expression of P4HB was found to be associated with TMZ-resistant D54 and U87 glioma cells lines [7]. Furthermore, inhibition of P4HB could attenuate TMZ resistance [4] whereas overexpression could promote malignancy via MAPK signaling [8]. Chronic TMZ treatment may also exacerbate drug resistance by further upregulating P4HB expression [4].

Strikingly, P4HB expression level enabled the stratification of treatment outcome towards TMZ within the methylated MGMT population. Whilst MGMT promoter methylation status retained significance in predicting treatment response, P4HB expression was found to have additional value, when used alone or

in combination with MGMT methylation status, for identifying patients at risk of adverse health outcome [19]. Notably, the use of $P4HB^{Low}$ in conjunction with $MGMT^{Meth}$ gave the strongest predictive power for PFS in TMZ-treated malignant glioma patients whereas $P4HB^{High}$ $MGMT^{Unmeth}$ tumours are more likely to show suboptimal response to TMZ. It is noteworthy that MGMT promoter methylation status may be altered upon tumour recurrence. Kohsaka *et al*. demonstrated upregulation of MGMT protein expression during the acquisition of TMZ resistance in U87 glioma cells [20]. Whilst it was unclear whether this was the result of MGMT promoter unmethylation, the increase in MGMT expression during the course of TMZ treatment appeared to further reduce TMZ responsiveness.

It is a common observation that initially TMZ-sensitive malignant glioma may eventually become resistant, partly due to the restoration of MGMT activity [21]. Our findings suggest that P4HB expression could be used to identify tumours likely to benefit from prolonged TMZ treatment even before recurrence. For recurrent diseases, rechallenge with TMZ is a commonly adopted strategy but no reliable predictive factors have been identified so far [22]. We surmise that P4HB expression could be used to identify recurrent tumours that might still respond favourably to TMZ rechallenge. And our findings have the potential for informing future treatment paradigm. While the stratification of patient subgroups by P4HB were primarily based on MGMT methylation status, the clinical significance of P4HB maybe limited by the small cohort size and missing clinical parameters such as extent of resection and other prognostic markers including IDH mutation status. Future studies may also include molecular investigations to determine drug response upon P4HB inhibition in glioma.

Conclusion

P4HB is a component of UPR that plays important roles in mediating glioma survival, therapeutic resistance, and tumour progression. In this context, P4HB expression has been shown to be significantly associated with PFS and OS in malignant glioma patients and can be used as an independent prognostic marker. P4HB can be used singly or in combination with MGMT to stratify patients who are good responders to glioma therapeutics. Furthermore, P4HB expression may also inform a more nuanced approach to the use of extended TMZ regimen as well as TMZ rechallenge. Future research may be conducted in a larger and standardized cohort, and evaluate the association between P4HB expression and other important biomarkers, that eventually give rise to fruitful clinical translations.

Declarations

Competing interest statement: The authors declare no competing financial interests.

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Conflicts of interest/Competing interests: None.

Availability of data and material: Associated data will not be deposited.

Code availability: Not applicable.

Authors' contributions: SS collected the data, performed statistical analysis and wrote the paper. KK participated in the study design and wrote the paper. GL conceived the study and contributed to the critical revision.

Ethics approval: Study protocol was approved by the Institution Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster.

Consent to participate: Human tissue specimens were obtained with informed consent from the patient.

Consent for publication: The manuscript was seen and approved by all authors.

References

1. Di Carlo DT, Cagnazzo F, Benedetto N, Morganti R, Perrini P: Multiple high-grade gliomas: epidemiology, management, and outcome. A systematic review and meta-analysis. *Neurosurg Rev* 2019, 42(2):263-275.

2. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U *et al*: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005, 352(10):987-996.

3. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L *et al*: MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005, 352(10):997-1003.

4. Sun S, Lee D, Ho AS, Pu JK, Zhang XQ, Lee NP, Day PJ, Lui WM, Fung CF, Leung GK: Inhibition of prolyl 4-hydroxylase, beta polypeptide (P4HB) attenuates temozolomide resistance in malignant glioma via the endoplasmic reticulum stress response (ERSR) pathways. *Neuro Oncol* 2013, 15(5):562-577.

5. Malhotra JD, Kaufman RJ: The endoplasmic reticulum and the unfolded protein response. *Semin Cell Dev Biol* 2007, 18(6):716-731.

6. Luo B, Lee AS: The critical roles of endoplasmic reticulum chaperones and unfolded protein response in tumorigenesis and anticancer therapies. *Oncogene* 2013, 32(7):805-818.

7. Sun S, Wong TS, Zhang XQ, Pu JK, Lee NP, Day PJ, Ng GK, Lui WM, Leung GK: Protein alterations associated with temozolomide resistance in subclones of human glioblastoma cell lines. *J Neurooncol* 2012, 107(1):89-100.

8. Sun S, Kiang KMY, Ho ASW, Lee D, Poon MW, Xu FF, Pu JKS, Kan ANC, Lee NPY, Liu XB *et al*: Endoplasmic reticulum chaperone prolyl 4-hydroxylase, beta polypeptide (P4HB) promotes malignant phenotypes in glioma via MAPK signaling. *Oncotarget* 2017, 8(42):71911-71923.

9. Xipell E, Aragon T, Martinez-Velez N, Vera B, Idoate MA, Martinez-Irujo JJ, Garzon AG, Gonzalez-Huarriz M, Acanda AM, Jones C *et al*: Endoplasmic reticulum stress-inducing drugs sensitize glioma cells to temozolomide through downregulation of MGMT, MPG, and Rad51. *Neuro Oncol* 2016, 18(8):1109-1119.

10. Nanegrungsunk D, Onchan W, Chattipakorn N, Chattipakorn SC: Current evidence of temozolomide and bevacizumab in treatment of gliomas. *Neurol Res* 2015, 37(2):167-183.

11. Messaoudi K, Clavreul A, Lagarce F: Toward an effective strategy in glioblastoma treatment. Part I: resistance mechanisms and strategies to overcome resistance of glioblastoma to temozolomide. *Drug Discov Today* 2015, 20(7):899-905.

12. Wait SD, Prabhu RS, Burri SH, Atkins TG, Asher AL: Polymeric drug delivery for the treatment of glioblastoma. *Neuro Oncol* 2015, 17 Suppl 2:ii9-ii23.

13. Tan AC, Ashley DM, Lopez GY, Malinzak M, Friedman HS, Khasraw M: Management of glioblastoma: State of the art and future directions. *CA Cancer J Clin* 2020, 70(4):299-312.

14. Liu K, Tsung K, Attenello FJ: Characterizing Cell Stress and GRP78 in Glioma to Enhance Tumor Treatment. *Front Oncol* 2020, 10:608911.

15. Liu Y, Ji W, Shergalis A, Xu J, Delaney AM, Calcaterra A, Pal A, Ljungman M, Neamati N, Rehemtulla A: Activation of the Unfolded Protein Response via Inhibition of Protein Disulfide Isomerase Decreases the Capacity for DNA Repair to Sensitize Glioblastoma to Radiotherapy. *Cancer Res* 2019, 79(11):2923-2932.

16. Chen X, Cubillos-Ruiz JR: Endoplasmic reticulum stress signals in the tumour and its microenvironment. *Nat Rev Cancer* 2021, 21(2):71-88.

17. Penaranda Fajardo NM, Meijer C, Kruyt FA: The endoplasmic reticulum stress/unfolded protein response in gliomagenesis, tumor progression and as a therapeutic target in glioblastoma. *Biochem Pharmacol* 2016, 118:1-8.

18. Peng Z, Chen Y, Cao H, Zou H, Wan X, Zeng W, Liu Y, Hu J, Zhang N, Xia Z *et al*: Protein disulfide isomerases are promising targets for predicting the survival and tumor progression in glioma patients. *Aging (Albany NY)* 2020, 12(3):2347-2372.

19. Gruenewald TL, Seeman TE, Ryff CD, Karlamangla AS, Singer BH: Combinations of biomarkers predictive of later life mortality. *Proc Natl Acad Sci U S A* 2006, 103(38):14158-14163.

20. Kohsaka S, Wang L, Yachi K, Mahabir R, Narita T, Itoh T, Tanino M, Kimura T, Nishihara H, Tanaka S: STAT3 inhibition overcomes temozolomide resistance in glioblastoma by downregulating MGMT expression. *Mol Cancer Ther* 2012, 11(6):1289-1299.

21. Huang H, Xiang Y, Su B, Xiong W, Zhang X: Potential roles for Gfi1 in the pathogenesis and proliferation of glioma. *Med Hypotheses* 2013, 80(5):629-632.

22. Franceschi E, Lamberti G, Visani M, Paccapelo A, Mura A, Tallini G, Pession A, De Biase D, Minichillo S, Tosoni A *et al*: Temozolomide rechallenge in recurrent glioblastoma: when is it useful? *Future Oncol* 2018, 14(11):1063-1069.

Tables

Due to technical limitations, table 1-2 is only available as a download in the Supplemental Files section.

Figures

Figure 1

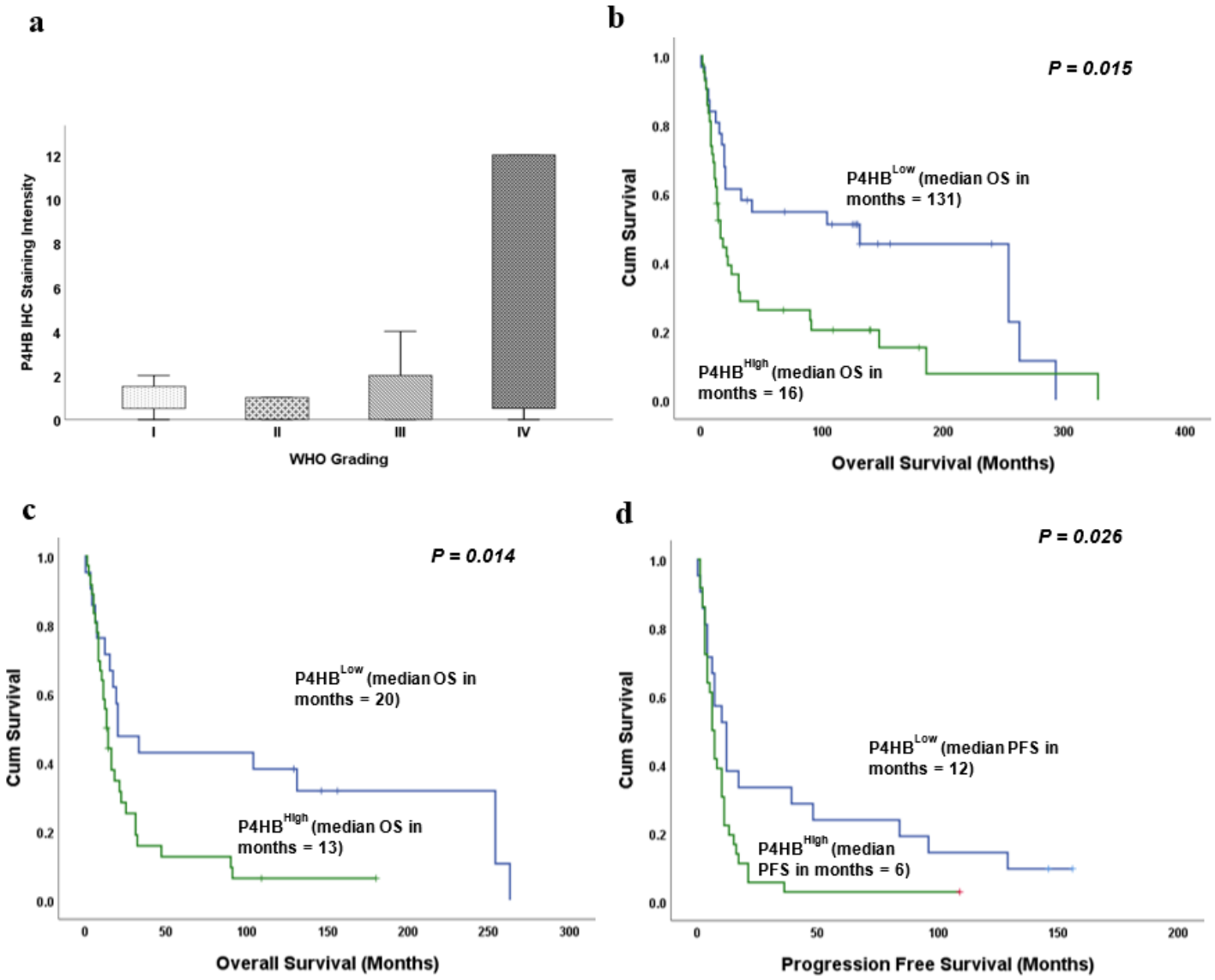

Figure 1

High P4HB expression is associated with poor glioma patient outcome in clinical cohort a) Immunohistochemical staining of P4HB shows that P4HB expression was significantly upregulated with the ascending tumour grades (n=73; p= 0.0001). Kaplan-Meier survival analysis after classifying patients into two groups P4HBLow and P4HBHigh based on immunohistochemical staining scores. Overall survival prediction in b) All WHO grades gliomas (n=73), c) WHO grade III and IV gliomas (n=57). Progression-free survival prediction in d) WHO grade III and IV gliomas (n=57). Log rank test was performed to determine the p-value indicated on the graphs.

Figure 2

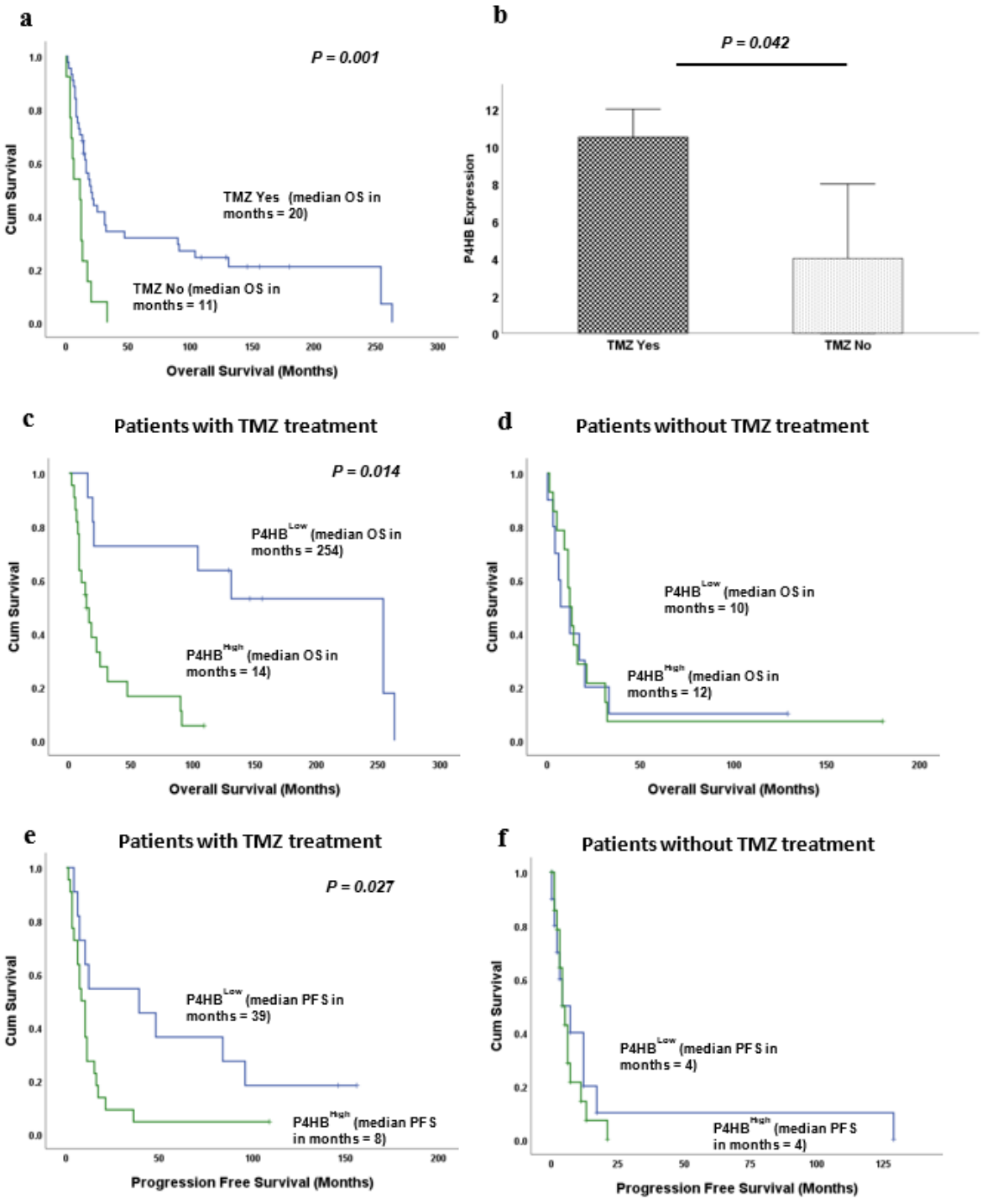

Figure 2

P4HB predicts survival in TMZ treated population a) Kaplan-Meier survival curves showed that patients who received TMZ chemotherapy revealed better overall survival than patients who did not receive TMZ (p=0.001). b) The use of TMZ chemotherapy was significantly associated with higher P4HB expression (p=0.042). c) Better overall survival outcome was revealed in TMZ treated population who have a low P4HB expression (P4HBLow) than those with a higher P4HB expression level (P4HBHigh). d) Patients without TMZ treatment, no significant difference was observed in OS. Progression free survival by Kaplan-Meier analysis shows that e) Patients who received TMZ treatment demonstrated better survival in P4HBLow group than P4HBHigh group (p=0.027) whereas f) No significant difference was demonstrated between P4HBLow and P4HBHigh groups in non-TMZ treated population.

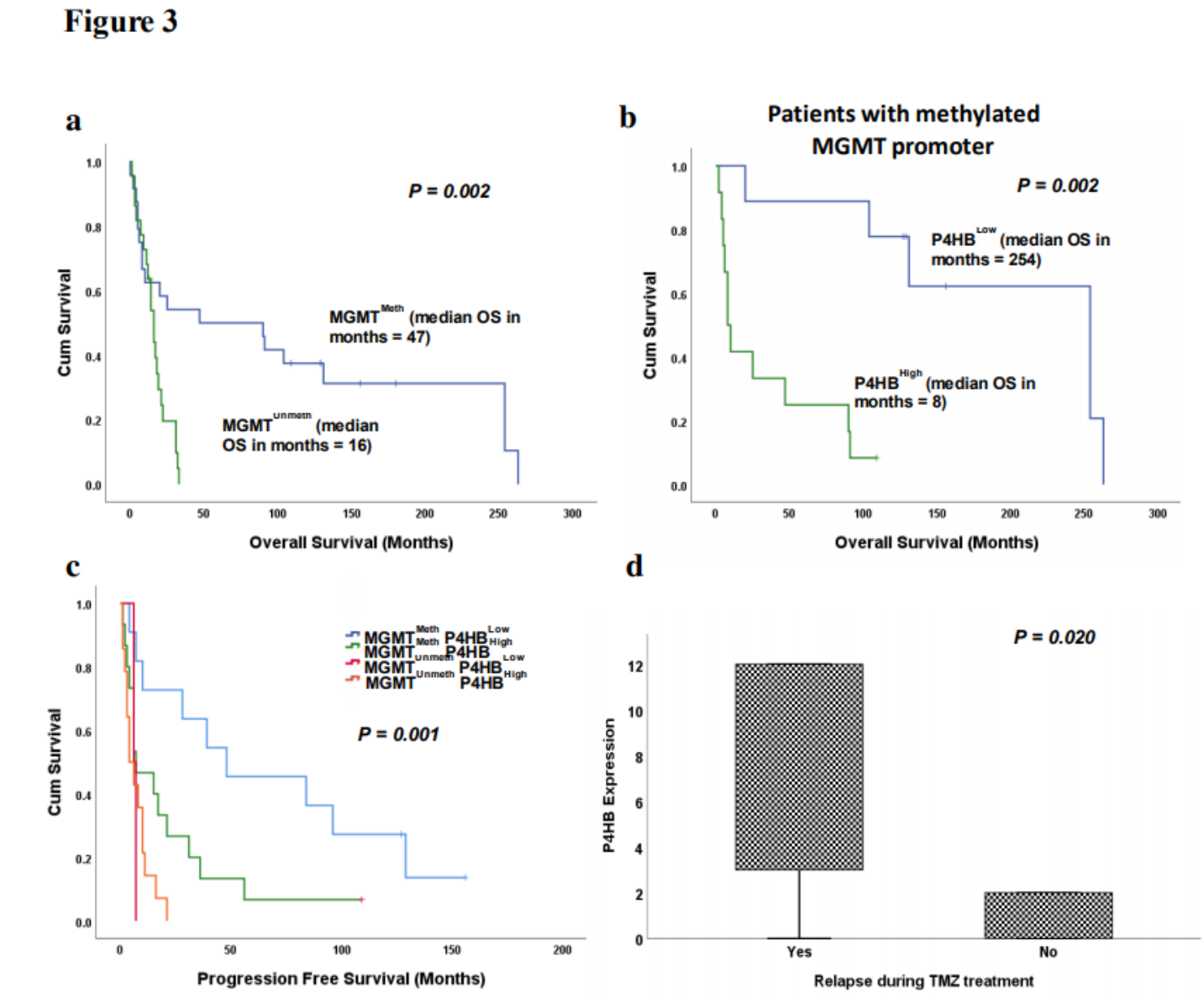

Figure 3

P4HB predicts TMZ treatment response in patients with methylated MGMT a) Kaplan-Meier survival curves showed that patients who exhibited methylated MGMT in their tumours revealed better overall survival outcome than patients who had unmethylated MGMT in the glioma lesions (p=0.002). b) P4HBLow predicts better survival outcome in patients with methylated MGMT compared to P4HBHigh group (p=0.002). c) Progression free survival shows MGMTMeth P4HBLow exhibited the best survival outcome among the different combination of MGMT methylation status and P4HB expression (p=0.001). d) High P4HB expression was significantly associated with increased number of patients relapsed during TMZ treatment (p=0.020).

Supplementary Files

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