*Supplementary material for* Sooman L, et al. FGF2 as a potential prognostic biomarker for proneural glioma patients. Acta Oncol 2015;54:385–94.

## Supplementary methods

*Cross-validated Kaplan-Meier analyses*

The analyses were done as follows for each variable:

1. Patients were divided randomly into five similarly sized groups: P1, P2, P3, P4 and P5.
2. One of the groups was assigned test group and the remaining groups were assigned training group.
3. A threshold value was determined for the variable that divided the patients in the training group in two parts resulting in the lowest possible two-sided Mann-Whitney p-value.
4. The patients in the test group were designated as either having a low or a high value of the variable according to if the value of the variable was above or below the determined threshold.
5. Paragraphs 2–4 were repeated, changing the group designated test group each round, until all groups had been test groups once and all patients were designated either as having a low or a high value of the variable.
6. The cross-validated Kaplan-Meier plot was plotted and a Mann-Whitney U test statistic was calculated.
7. A p-value was calculated by comparing the Mann-Whitney U test statistic with the permutation distribution of Mann-Whitney U test statistics. The permutation distribution was obtained by permutating the survival time and subsequently repeating paragraph 1–6 500 times.

*Random Forest classification with variable subset selection*

1. Annotation data was randomly divided into training and test data sets. 80% of the data constituted training data and 20% test data.
2. For each variable, a two-sided Mann-Whitney U test was performed on the training data comparing the variable values for the two classes, resulting in one p-value per variable.
3. For each value of $k = 1, 2, \ldots, 5$, k variables with lowest p-values were selected. Using an inner 5-fold cross validation the performance of each Random Forest model was assessed based on these k variables. As a performance measure error rate was used, i.e. the fraction of incorrect classifications.
4. The k that gave the best performance was selected and a model using Random Forest was built based on the k best variables.
5. The model was used to predict the class for each of the patients in the test data set and error rate was computed.

1–5 was repeated 100 times, resulting in 100 error rates, which were summarized in an average error rate.

Permutations were adopted to estimate the significance of such an average error rate, i.e. the survival times were randomly permuted so that the measurements were randomly connected to a survival time. The permutation was repeated 500 times and for each permutation the variable subset selection, Random Forest model and evaluation procedure was performed.

## References

[1] Muramatsu T, Miyauchi T. Basigin (CD147): A multifunctional transmembrane protein involved in reproduction, neural function, inflammation and tumor invasion. Histol Histopathol 2003;18:981–7.

[2] Ju XZ, Yang JM, Zhou XY, Li ZT, Wu XH. EMMPRIN expression as a prognostic factor in radiotherapy of cervical cancer. Clin Cancer Res 2008;14:494–501.

[3] Tsai WC, Chen Y, Huang LC, Lee HS, Ma HI, Huang SM, et al. EMMPRIN expression positively correlates with WHO grades of astrocytomas and meningiomas. J Neurooncol 2013;114:281–90.

[4] Tian L, Zhang Y, Chen Y, Cai M, Dong H, Xiong L. EMMPRIN is an independent negative prognostic factor for patients with astrocytic glioma. PloS One 2013;8:e58069.

[5] Sameshima T, Nabeshima K, Toole BP, Inoue T, Yokogami K, Nakano S, et al. Correlation of emmprin expression in vascular endothelial cells with blood-brain-barrier function: a study using magnetic resonance imaging enhanced by Gd-DTPA and immunohistochemistry in brain tumors. Virchows Archiv 2003;442:577–84.

[6] McDonald PC, Winum JY, Supuran CT, Dedhar S. Recent developments in targeting carbonic anhydrase IX for cancer therapeutics. Oncotarget 2012;3:84–97.

[7] Schrijvers ML, van der Laan BF, de Bock GH, Pattje WJ, Mastik MF, Menkema L, et al. Overexpression of intrinsic hypoxia markers HIF1alpha and CA-IX predict for local recurrence in stage T1-T2 glottic laryngeal carcinoma treated with radiotherapy. Int J Radiat Oncol Biol Phys 2008;72:161–9.

[8] Giatromanolaki A, Koukourakis MI, Sivridis E, Pastorek J, Wykoff CC, Gatter KC, et al. Expression of hypoxia-inducible carbonic anhydrase-9 relates to angiogenic pathways and independently to poor outcome in non-small cell lung cancer. Cancer Res 2001;61:7992–8.

[9] Brennan DJ, Jirstrom K, Kronblad A, Millikan RC, Landberg G, Duffy MJ, et al. CA IX is an independent prognostic marker in premenopausal breast cancer patients with one to three positive lymph nodes and a putative marker of radiation resistance. Clin Cancer Res 2006;12:6421–31.

[10] Liao SY, Darcy KM, Randall LM, Tian C, Monk BJ, Burger RA, et al. Prognostic relevance of carbonic anhydrase-IX in high-risk, early-stage cervical cancer: A Gynecologic Oncology Group study. Gynecol Oncol 2010;116: 452–8.
[11] Koukourakis MI, Bentzen SM, Giatromanolaki A, Wilson GD, Daley FM, Saunders MI, et al. Endogenous markers of two separate hypoxia response pathways (hypoxia inducible factor 2 alpha and carbonic anhydrase 9) are associated with radiotherapy failure in head and neck cancer patients recruited in the CHART randomized trial. J Clin Oncol 2006;24:727–35.
[12] Erpolat OP, Gocun PU, Akmansu M, Ozgun G, Akyol G. Hypoxia-related molecules HIF-1alpha, CA9, and osteopontin: Predictors of survival in patients with high-grade glioma. Strahlenther Onkol 2013;189:147–54.
[13] Jarvela S, Parkkila S, Bragge H, Kahkonen M, Parkkila AK, Soini Y, et al. Carbonic anhydrase IX in oligodendroglial brain tumors. BMC Cancer 2008;8:1.
[14] Haapasalo JA, Nordfors KM, Hilvo M, Rantala IJ, Soini Y, Parkkila AK, et al. Expression of carbonic anhydrase IX in astrocytic tumors predicts poor prognosis. Clin Cancer Res 2006;12:473–7.
[15] Naor D, Sionov RV, Ish-Shalom D CD44: Structure, function, and association with the malignant process. Advance Cancer Res 1997;71:241–319.
[16] de Jong MC, Pramana J, van der Wal JE, Lacko M, Peutz-Kootstra CJ, de Jong JM, et al. CD44 expression predicts local recurrence after radiotherapy in larynx cancer. Clin Cancer Res 2010;16:5329–38.
[17] Yoshida T, Matsuda Y, Naito Z, Ishiwata T. CD44 in human glioma correlates with histopathological grade and cell migration. Pathol Int 2012;62:463–70.
[18] Martineau Y, Azar R, Bousquet C, Pyronnet S. Anti-oncogenic potential of the eIF4E-binding proteins. Oncogene 2013;32:671–7.
[19] Benavente S, Verges R, Hermosilla E, Fumanal V, Casanova N, Garcia A, et al. Overexpression of phosphorylated 4E-BP1 predicts for tumor recurrence and reduced survival in cervical carcinoma treated with postoperative radiotherapy. Int J Radiat Oncol Biol Phys 2009;75:1316–22.
[20] Brooks AN, Kilgour E, Smith PD. Molecular pathways: Fibroblast growth factor signaling: A new therapeutic opportunity in cancer. Clin Cancer Res 2012;18:1855–62.
[21] Rades D, Setter C, Dahl O, Schild SE, Noack F. Fibroblast growth factor 2 – a predictor of outcome for patients irradiated for stage II-III non-small-cell lung cancer. Int J Radiat Oncol Biol Phys 2012;82:442–7.
[22] Rades D, Seibold ND, Gebhard MP, Noack F, Schild SE. Fibroblast growth factor 2 is of prognostic value for patients with locally advanced squamous cell carcinoma of the head and neck. Strahlenther Onkol 2014;190:68–74.
[23] Yiangou C, Gomm JJ, Coope RC, Law M, Luqmani YA, Shousha S, et al. Fibroblast growth factor 2 in breast cancer: Occurrence and prognostic significance. Br J Cancer 1997; 75:28–33.
[24] Takahashi JA, Fukumoto M, Igarashi K, Oda Y, Kikuchi H, Hatanaka M. Correlation of basic fibroblast growth factor expression levels with the degree of malignancy and vascularity in human gliomas. J Neurosurgery 1992; 76:792–8.
[25] Costa A, Ilves I, Tamberg N, Petojevic T, Nogales E, Botchan MR, et al. The structural basis for MCM2-7 helicase activation by GINS and Cdc45. Nat Struct Mol Biol 2011;18: 471–7.
[26] Szelachowska J, Dziegiel P, Jelen-Krzeszewska J, Jelen M, Matkowski R, Pomiecko A, et al. Mcm-2 protein expression predicts prognosis better than Ki-67 antigen in oral cavity squamocellular carcinoma. Anticancer Res 2006;26:2473–8.
[27] Wharton SB, Chan KK, Anderson JR, Stoeber K, Williams GH. Replicative Mcm2 protein as a novel proliferation marker in oligodendrogliomas and its relationship to Ki67 labelling index, histological grade and prognosis. Neuropathol App Neurobiol 2001;27:305–13.
[28] Babu SN, Chetal G, Kumar S. Macrophage migration inhibitory factor: A potential marker for cancer diagnosis and therapy. Asian Pacific J Cancer Prevent 2012;13:1737–44.
[29] Suzuki F, Nakamaru Y, Oridate N, Homma A, Nagahashi T, Yamaguchi S, et al. Prognostic significance of cytoplasmic macrophage migration inhibitory factor expression in patients with squamous cell carcinoma of the head and neck treated with concurrent chemoradiotherapy. Oncol Rep 2005;13: 59–64.
[30] Wang XB, Tian XY, Li Y, Li B, Li Z. Elevated expression of macrophage migration inhibitory factor correlates with tumor recurrence and poor prognosis of patients with gliomas. J Neurooncol 2012;106:43–51.
[31] Dang CV. MYC on the path to cancer. Cell 2012;149: 22–35.
[32] Vijayalakshmi N, Selvaluxmi G, Mahji U, Rajkumar T. C-myc oncoprotein expression and prognosis in patients with carcinoma of the cervix: an immunohistochemical study. Eur J Gynaecol Oncol 2002;23:135–8.
[33] Hayashi S, Yamamoto M, Ueno Y, Ikeda K, Ohshima K, Soma G, et al. Expression of nuclear factor-kappa B, tumor necrosis factor receptor type 1, and c-Myc in human astrocytomas. Neurol Med Chir 2001;41:187–95.
[34] Cenci T, Martini M, Montano N, D'Alessandris QG, Falchetti ML, Annibali D, et al. Prognostic relevance of c-Myc and BMI1 expression in patients with glioblastoma. Am J Clin Pathol 2012;138:390–6.
[35] Dhar SK, St Clair DK. Manganese superoxide dismutase regulation and cancer. Free Rad Biol Med 2012;52: 2209–22.
[36] Fu TY, Hou YY, Chu ST, Liu CF, Huang CH, Chen HC, et al. Manganese superoxide dismutase and glutathione peroxidase as prognostic markers in patients with buccal mucosal squamous cell carcinomas. Head Neck 2011;33:1606–15.
[37] Nakano T, Oka K, Taniguchi N. Manganese superoxide dismutase expression correlates with p53 status and local recurrence of cervical carcinoma treated with radiation therapy. Cancer Res 1996;56:2771–5.
[38] Pu PY, Lan J, Shan SB, Huang EQ, Bai Y, Guo Y, et al. Study of the antioxidant enzymes in human brain tumors. J Neurooncol 1996;29:121–8.
[39] Hussain SP, Harris CC. p53 biological network: At the crossroads of the cellular-stress response pathway and molecular carcinogenesis. J Nippon Med School 2006;73:54–64.
[40] Langendijk JA, Thunnissen FB, Lamers RJ, de Jong JM, ten Velde GP, Wouters EF. The prognostic significance of accumulation of p53 protein in stage III non-small cell lung cancer treated by radiotherapy. Radiother Oncol 1995; 36:218–24.
[41] Chen MB, Wu XY, Yu R, Li C, Wang LQ, Shen W, et al. P53 status as a predictive biomarker for patients receiving neoadjuvant radiation-based treatment: A meta-analysis in rectal cancer. PloS One 2012;7:e45388.
[42] Kim K, Chie EK, Han W, Noh DY, Park IA, Oh DY, et al. Prognostic value of p53 and bcl-2 expression in patients treated with breast conservative therapy. J Korean Med Sci 2010;25:235–9.
[43] Skirnisdottir I, Sorbe B, Karlsson M, Seidal T. Prognostic importance of DNA ploidy and p53 in early stages of epithelial ovarian carcinoma. Int J Oncol 2001;19:1295–302.

[44] Pomp J, Blom J, Zwinderman AH, Van Krimpen C. P53 and radiotherapy for oesophageal carcinoma: A comparison between 4 different antibodies. Oncol Rep 2000;7:1075–8.
[45] Nagy B, Tiszlavicz L, Eller J, Molnar J, Thurzo L. Ki-67, cyclin D1, p53 and bcl-2 expression in advanced head and neck cancer. In vivo 2003;17:93–6.
[46] Scherr DS, Vaughan ED, Jr., Wei J, Chung M, Felsen D, Allbright R, et al. BCL-2 and p53 expression in clinically localized prostate cancer predicts response to external beam radiotherapy. J Urol 1999;162:12–6; discussion 16–7.
[47] Kros JM, Godschalk JJ, Krishnadath KK, Van Eden CG. Expression of p53 in oligodendrogliomas. J Pathol 1993;171: 285–90.
[48] Kyritsis AP, Bondy ML, Hess KR, Cunningham JE, Zhu D, Amos CJ, et al. Prognostic significance of p53 immunoreactivity in patients with glioma. Clin Cancer Res 1995;1:1617–22.
[49] Malkoun N, Chargari C, Forest F, Fotso MJ, Cartier L, Auberdiac P, et al. Prolonged temozolomide for treatment of glioblastoma: Preliminary clinical results and prognostic value of p53 overexpression. J Neurooncol 2012;106: 127–33.
[50] Soini Y, Niemela A, Kamel D, Herva R, Bloigu R, Paakko P, et al. p53 immunohistochemical positivity as a prognostic marker in intracranial tumours. Acta Patholog Microbiol Immunol Scand 1994;102:786–92.
[51] Collavin L, Lunardi A, Del Sal G. p53-family proteins and their regulators: Hubs and spokes in tumor suppression. Cell Death Different 2010;17:901–11.
[52] Liu SS, Chan KY, Cheung AN, Liao XY, Leung TW, Ngan HY. Expression of deltaNp73 and TAp73alpha independently associated with radiosensitivities and prognoses in cervical squamous cell carcinoma. Clin Cancer Res 2006;12:3922–7.
[53] Huang MY, Wang JY, Chang HJ, Kuo CW, Tok TS, Lin SR. CDC25A, VAV1, TP73, BRCA1 and ZAP70 gene overexpression correlates with radiation response in colorectal cancer. Oncol Rep 2011;25:1297–306.

Supplementary Table I. P-values for the univariate and multivariate Cox regression analyses. NA=P-value is missing because there were too few samples to do Cox regression analysis.