Distribution of *EGFR* amplification, combined chromosome 7 gain and chromosome 10 loss, and *TERT* promoter mutation in brain tumors and their potential for the reclassification of *IDH*wt astrocytoma to glioblastoma

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Abstract

EGFR amplification (*EGFR*amp), the combination of gain of chromosome 7 and loss of chromosome 10 (7+/10-), and *TERT* promoter mutation (p*TERT*mut) are alterations frequently observed in adult *IDH*-wildtype (*IDH*wt) glioblastoma (GBM). In absence of endothelial proliferation and/or necrosis, these alterations currently are considered to serve as a surrogate for upgrading *IDH*wt diffuse or anaplastic astrocytoma to GBM. Here we set out to determine the distribution of *EGFR*amp, 7+/10- and p*TERT*mut by analyzing high-resolution copy number profiles (CNP) and next generation sequencing data of primary brain tumors. In addition, we addressed the question whether combinations of partial gains on chromosome 7 and partial losses on chromosome 10 exhibited a diagnostic and prognostic value similar to that of complete 7+/10-. Several such combinations proved relevant and were combined as the 7/10 signature. Our results demonstrate that *EGFR*amp and the 7/10 signature are closely associated with *IDH*wt GBM. In contrast, p*TERT*mut is less specific for *IDH*wt GBM. We conclude that in the absence of endothelial proliferation and/or necrosis the detection of *EGFR*amp is a very strong surrogate marker for the diagnosis of GBM in *IDH*wt diffuse astrocytic tumors. The 7/10 signature is also a strong surrogate marker. However, care should be taken to exclude pleomorphic xanthoastrocytoma. p*TERT*mut is less restricted to this entity and needs companion analysis by other molecular markers in order to serve as a surrogate for diagnosing *IDH*wt GBM. A combination of any two of *EGFR*amp, the 7/10 signature and p*TERT*mut is highly specific for *IDH*wt GBM and the combination of all three alterations is frequent and exclusively seen in *IDH*wt GBM.

Keywords

EGFR amplification, chromosome 7 gain, chromosome 10 loss, 7+/10-, 7+/10q-, *TERT* promoter mutation, glioblastoma, astrocytoma, pleomorphic xanthoastrocytoma

Introduction

Isocitrate dehydrogenase *IDH*-wildtype (*IDH*wt) diffuse and anaplastic astrocytoma are considered provisional entities in the World Health Organization (WHO) classification of central nervous system tumors 2016 [20]. They comprise biologically and clinically different tumors, with the majority exhibiting molecular alterations and survival characteristics of *IDH*wt glioblastoma (GBM). A pressing question is whether *EGFR* amplification, the presence of combined gains on chromosome 7 and losses on chromosome 10, and *TERT* promoter mutation can serve as surrogate markers for upgrading *IDH*wt astrocytomas corresponding histologically to WHO grade II or III to GBM, WHO grade IV.

The combination of gains on chromosome 7 and losses on chromosome 10 are characteristic molecular alterations in *IDH*wt GBM. Recent studies have demonstrated that astrocytic tumors not fulfilling the morphological criteria for GBM but carrying typical molecular features of GBM exhibit a clinical course similar to that of morphologically unequivocal GBM [5, 11, 24, 31, 32]. Therefore, the presence of combined chromosome 7 gains and chromosome 10 losses is considered as a molecular marker for *IDH*wt GBM. However, little is known about the relevance of assessing individual arms of chromosomes 7 and 10, i.e., does gain of either arm of 7 and loss of either arm of 10 suffice or is gain of both arms of chromosome 7 (trisomy 7) and loss of both arms of chromosome 10 (monosomy 10) required. Gains of chromosome 7 and losses of chromosome 10 in GBM have been initially detected by cytogenetic analyses [25] [4]. The typical constellation found in GBM cell lines was trisomy of chromosomes 7 and monosomy of chromosome 10, most likely as a result of errors in mitotic disjunction.

EGFR amplification (*EGFR*amp) in GBM has been initially observed by molecular and cytogenetic analyses [18, 34] [3, 9]. Roughly half of all GBM exhibit a high-level amplification of this gene mostly associated with the presence of double minutes, i.e., extrachromosomal elements containing additional *EGFR* copies [28].

Telomerase reverse transcriptase (*TERT*) encodes the catalytic subunit of the telomerase complex. *TERT* promoter mutation (p*TERT*mut) initially has been detected in melanoma [12, 13]. Subsequent investigations also revealed high frequencies of p*TERT*mut in *IDH*wt GBM as well as *IDH*-mutant (*IDH*mut) and 1p/19q-codeleted oligodendroglioma. [1, 14, 15, 19, 21, 33], and demonstrated its potential use for subgrouping of gliomas [2, 7, 8, 22].

Recent technology developments allow for generating high-resolution copy number profiles (CNP) of the human genome in tumor tissue based on next generation sequencing or microarray analyses. We set out to assess the distribution of *EGFR*amp, 7+/10- and p*TERT*mut in human brain tumors based on CNP generated from DNA methylation array data sets and from next generation sequencing data. Further we explored the prognostic association of these alterations in brain tumors diagnosed as *IDH*wt diffuse or anaplastic astrocytoma WHO grade II or III.

Material and Methods

Patient cohorts

The present analyses are based on data from three cohorts available for analysis at the Department of Neuropathology of the University Heidelberg. For each patient in the three cohorts 450K or 850K DNA methylation array data are available. All patients have received a DNA methylation based diagnosis as previously described [6].

Cohort 1 includes 2,417 brain tumor patients for whom next generation panel sequencing with a panel including the *TERT* gene and its promoter region has been performed. Cohort 1 was employed for determining the distribution of p*TERT*mut, *EGFR*amp and 7+/10- in human brain tumors. Table 1 lists the tumor diagnoses of the patients included in cohort 1 sorted by DNA methylation-based classification.

Cohort 2 includes 10,826 brain tumor patients whose tumors have been analyzed using the Illumina 450K or 850K platforms. This data set was used to assess the distribution of *EGFR*amp and 7+/10- in human brain tumors. Supplementary table 1 lists all tumor diagnoses for the patients included in cohort 2 sorted by DNA methylation-based diagnosis [6], and the respective numbers of patients. Cohort 2 encompasses all patients from cohort 1. Details on DNA methylation classes can be obtained from www.molecularneuropathology.org.

Cohort 3 comprises 939 patients from cohort 2 for whom survival data were available. In contrast to cohorts 1 and 2, the histological diagnosis according to WHO 2016 [20] including the diagnosis of *IDH*wt diffuse or anaplastic astrocytoma was the basis for survival analysis. Cohort 3 does not include patients diagnosed with GBM. There is a bias in cohort 3 because survival data have been acquired for specific tumor entities in previous studies [30]. Distribution of respective methylation groups and alterations on chromosomes 7 and 10 as well as EGFR status are given in supplementary table 2.

Generation and scoring of CNPs and mutations

DNA methylation data were generated using the Illumina 450K or 850K/EPIC platforms as previously described [6]. The copy number variation plots were generated from the same raw data using the 'conumee' R package in Bioconductor (http://www.bioconductor.org packages/release/bioc/html / conumee.html). Figure 1 shows representative CNPs. Automated assessment of copy number changes was performed using the results from conumee after additional baseline correction. EGFRamp was called amplified if the respective probes exhibited an intensity higher than 0.6 on a log2-scale.

Panel sequencing was performed as previously reported [26]. pTERTmut was scored if 10 or more reads were detected with a minimum of 10% of the reads showing either of the two TERT promoter mutations mutation.

Statistics

All patient data sets were retrospectively compiled. The size of the respective sets was determined by availability of data and not by a power calculation. OS times were analyzed by the Kaplan-Meier method. Software R version 3.4 was employed for all analyses.

Results and Discussion

Rationale and procedure

We aimed at contributing to the following three questions: (1) What is the incidence of combined 7/10 copy number alterations, *EGFR*amp and p*TERT*mut in different types of human brain tumors? (2) Are these three alterations suitable surrogate markers for diagnosing *IDH*wt GBM even in the absence of necrosis and microvascular proliferation? (3) Which patterns of partial or complete chromosome 7 gains and chromosome 10 losses might be employed for diagnostic purposes? The first two questions have been addressed by analyzing a series of 2,417 tumors (cohort 1) for which both the *TERT* promoter status and complete copy number profiles were available (table 1). Question three was addressed by analyzing an extended set of 10,826 patients (cohort 2) with complete copy number profiles available (supplementary table 1), and analyzing a subset thereof including 939 (cohort 3) patients with available overall survival data.

For all cohorts, both, a histopathological and a DNA methylation-based diagnosis were available. According to the WHO classification 2016, *IDH*wt GBM includes the H3-G34-mutant GBM, while H3-K27M-mutant diffuse midline gliomas and *IDH*mut GBM have been separated as distinct entities from *IDH*wt GBM [20]. For all questions addressing association of the markers interrogated with survival, we adhered to the current WHO definition of *IDH*wt GBM (all analyses involving cohort 3). For determination of frequencies we used the DNA methylation based diagnosis as this is highly standardized and, therefore, more suitable for this type of question.

Defining the 7/10 status

An open question is whether 7+/10- is prognostically relevant in *IDH*wt astrocytic gliomas only if both arms of each chromosome are affected or also aberrations of only one arm of either chromosome are detectable. Furthermore, how much of each chromosome arm needs to be affected by copy number changes to suffice for prognostic relevance. This question is of particular interest in light of many of the available data to date being based on focused FISH analysis and, therefore, not providing representative information on the extent of copy number imbalances on each chromosome. Our approach is based on an array platform thereby covering the entire chromosome arms with ten thousands of probes each. We selected two different thresholds with one being 50% and the other 80% of chromosomal representation on each arm being gained or lost for calling the respective alteration in cohort 1. Supplementary table 1 provides an overview of 7+ and 10- combinations using both thresholds. In the predominant GBM subgroup characterized by gain of entire 7 and loss of entire 10, 1,185 patients (75% of all GBM) scored positive with the 80% and 1,265 patients (81% of all GBM) with the 50% threshold (supplementary table 1). We therefore went on using the 50% threshold for all subsequent analyses. Of 9 possible combinations exhibiting both, gain of at least one arm on 7 and loss of at least one arm on 10, 7+/10- represents the most frequent (1,265/1,598; 79%) constellation followed by 7+/10q- (87/1,598; 5%), by 7p+/10- (74/1,598; 5%) and by 7q+/10- (70/1,598; 4%) (supplementary table 1).

Next we analyzed which variants of gains on chromosome 7 and losses on chromosome 10 were associated with unfavorable clinical outcome in *IDH*wt astrocytic glioma patients. To this end we analyzed the respective combinations in patients from cohort 3.

Of the nine possible combinations of chromosome 7 gains and chromosome 10 losses we encountered 7+/10- (n=97), 7q+/10- (n=12), 7+/10q- (n=9), 7q+/10q- (n=7), 7p+/10- (n=3), both 7+/10p- and 7q+/10p- (n=1), and both 7p+/10p and 7p+/10q- (n=0) in patients from cohort 3.

Survival analysis was performed for patients whose tumors carried the combinations 7+/10- (n=97), 7q+/10- (n=12), 7+/10q- (n=9), 7q+/10q- (n=7) and 7p+/10- (n=3). Three combinations, 7+/10-, 7q+/10- and 7+/10q- were associated with poor survival similar to that of patients with GBM (figure 2a). We therefore defined all patients with 7+/10-, 7+/10q- and 7q+/10- as carrying the prognostic 7/10 signature. However, additional studies should be encouraged for evaluation of the prognostic power of the rare combinations of chromosome 7 gains and chromosome 10 losses. Noteworthy, DNA methylation-based classification identified 52 patients in cohort 3 without a 7/10 signature but with typical survival characteristics of GBM (figure 2b, orange graph), thus demonstrating the power of this method. This set exhibited p*TERT*mut in 52% (n =25) comparable to 67% and *EGFR*amp in 25% (n = 52) lower than 36% seen in all GBM included in cohort 1. Only one of these patients was allotted to the methylation class GBM, *IDH*wt, H3.3 G34-mutant (supplementary table 3).

Distribution of *EGFR*amp, 7/10 signature and p*TERT*mut in human brain tumors

Comparison of the distribution of all three parameters was performed using cohort 2, although for the distribution of *EGFR*amp and 7/10 signature a higher resolution could be obtained from cohort 1.

p*TERT*mut was observed in 363 of 544 (67%) GBM, *IDH*wt, in 95 of 120 (79%) oligodendrogliomas, *IDH*mut and 1p/19q-codeleted, in 12 of 17 (71%) melanomas, in 19 of 42 (45%) medulloblastomas of DNA methylation subclass SHH A, and in 7 of 34 (21%) pleomorphic xanthoastrocytomas (PXA). Fractions of p*TERT*mut tumors in entities with low mutation frequencies or entities with only few tumors analyzed are given in the table 1. Of the three parameters, p*TERT*mut exhibited highest sensitivity (67%) but lowest specificity (89%) for identification of *IDH*wt GBM (table 2).

The 7/10 signature was more specific for GBM being detected in 323 of 544 (59%) GBM, *IDH*wt, in 9 of 140 (6%) *IDH*mut anaplastic astrocytomas and GBM, and in 5 of 54 (9%) medulloblastoma of DNA methylation subgroup 4 (table 1). Sensitivity was 59% and specificity was 98% for *IDH*wt GBM (table 2).

*EGFR*amp was observed in 196/544 (36%) GBM, *IDH*wt, showing lowest sensitivity (36%) but highest specificity (100%) for this entity (table 2).

Overall, p*TERT*mut (562/2,417) is more frequent than *EGFR*amp (199/2,417) and 7/10 (361/2,417). The combinations of p*TERT*mut – *EGFR*amp (28 cases), p*TERT*mut – 7/10 (146 cases) and *EGFR*amp – 7/10 (30 cases) were strongly associated with GBM, *IDH*wt, and the triple combination of p*TERT*mut – 7+/10- - *EGFR*amp (124 cases) was exclusively seen in this entity (table 1).The sensitivity of any combination of double or triple positives was 58% and the specificity was 99% (table 2).

Distribution of *EGFR*amp, 7/10 signature and p*TERT*mut across DNA methylation classes of *IDH*wt GBM

Subdivision of *IDH*wt GBM by DNA methylation-based classification results in 7 subgroups. These subgroups exhibit striking differences in the frequencies of the three molecular parameters interrogated. Tumors of the DNA methylation class GBM, *IDH*wt, H3.3 G34-mutant (n=17) did not exhibit p*TERT*mut or *EGFR*amp. The 7/10 signature was observed only in four H3.3 G34-mutant GBM. This finding is quite similar to that in H3.3 K27-mutant diffuse midline gliomas and argues for separating the H3.3 G34-mutant GBM from the other *IDH*wt GBM. Tumors of the DNA methylation class glioblastoma, *IDH*wt, subclass *MYCN* (n=22) exhibited p*TERT*mut and *EGFR*amp in less than a quarter of all cases and the 7/10 signature only in a single tumor. DNA methylation class glioblastoma, *IDH*wt, subclass RTK I (n=71) presented with p*TERT*mut in 55 cases (77%). A total of 18 (25%) tumors carried *EGFR*amp and 46 (65%) tumors carried the 7/10 signature. DNA methylation class glioblastoma, *IDH*wt, subclass RTK II (n=203) constituted the most frequent GBM subgroup and presented with p*TERT*mut in 166 cases (83%), *EGFR*amp in 128 cases (63%), and the 7/10 signature in 160 cases (79%). The DNA methylation class glioblastoma, *IDH*wt, subclass RTK III (n=23) was predominantly encountered in young patients and exhibited p*TERT*mut in 11 (48%), *EGFR*amp in 8 (35%) and 7/10 in 3 (13%) instances. The DNA methylation class glioblastoma, *IDH*wt, subclass mesenchymal (n=157) was frequent and included 123 tumors with p*TERT*mut (78%), 37 tumors with *EGFR*amp (24%), and 109 tumors with the 7/10 signature (69%). Finally, tumors falling into the DNA methylation class glioblastoma, *IDH*wt, subclass midline (n=51), which is an as yet poorly characterized group of tumors with morphology and survival characteristics comparable to that of other types of *IDH*wt GBM [24], exhibited p*TERT*mut in only 4 tumors (8%), while *EGFR*amp and the 7/10 signature was absent in this group. As *IDH*wt GBM comprises all these distinct DNA methylation subgroups, the sensitivity of *EGFR*amp, 7/10 signature and p*TERT*mut based grading is compromised by the low prevalence or absence in some of the molecular subgroups.

Diagnostic use of the three parameters *EGFR*amp, 7/10 signature and p*TERT*mut

A single molecular marker for diagnosing *IDH*wt GBM in absence of microvascular proliferation and/or necrosis would be a major contribution to daily routine diagnostics. While *EGFR*amp, p*TERT*mut or 7/10 signature are very good candidates, single use is not warranted for each of these parameters. p*TERT*mut obviously needs accompanying analysis for *IDH1* or *IDH2* mutation in order to exclude *IDH*mut and 1p/19q-codeleted oligodendroglioma and *IDH*mut astrocytoma. Also rare cases of medulloblastoma in adults [23] and anaplastic meningioma [10, 27], as well as solitary fibrous tumor/hemangiopericytoma frequently carry p*TERT*mut and need to be distinguished by their distinct histologies and appropriate additional molecular tests. The presence of p*TERT*mut in PXA [16] should be addressed by testing for the *BRAF*V600E mutation, typical for the latter [29]. However, an overlap with the rare epithelioid GBM cannot be ruled out [17]. The 7/10 signature also needs additional testing for *IDH* mutation to exclude *IDH*-mutant diffuse and anaplastic gliomas and for *BRAF* mutation as it might occasionally occur in PXA (table 1). *EGFR*amp, however, has the highest specificity (<99%) of all three parameters for GBM and *IDH* mutation testing can separate the very rare occurrences in *IDH*-mutant glioma. Assuming a threshold for specificity of 99% a reasonable

compromise, our data support the single use of *EGFR*amp detection or any double positive combination of the three parameters *EGFR*amp, p*TERT*mut or 7/10 signature for upgrading *IDH*wt astrocytoma to *IDH*wt GBM. For the low sensitivity for *EGFR*amp we suggest the assessment of all three parameters.

Conclusions

Our data supports the use of the molecular alterations *EGFR*amp, p*TERT*mut or the 7/10 signature as diagnostic biomarkers for the upgrading of *IDH*wt diffuse astrocytoma WHO grade II or anaplastic astrocytoma WHO grade III to *IDH*wt GBM WHO grade IV, pending additional molecular tests. *EGFR*amp and the combination of a positive finding for any two of the three markers are highly specific for *IDH*wt GBM, while the combination of all three markers is exclusively seen in *IDH*wt GBM.

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Table 1 Classifier prediction, *EGFR*amp, 7/10 and p*TERT*mut status in patient cohort 1

Methylation class abbreviation	n total	n *TERT*	n *EGFR*	n 7/10	triple wt	single 7/10	single EGFR	single *TERT*	double 7/10 + EGFR	double 7/10 + *TERT*	double EGFR + *TERT*	triple 7/10 + EGFR + *TERT*
PXA	34	7	0	2	26	1	0	6	0	1	0	0
EFT_CIC	3	0	0	0	3	0	0	0	0	0	0	0
HGNET_BCOR	9	0	0	0	9	0	0	0	0	0	0	0
HGNET_MN1	6	0	0	0	6	0	0	0	0	0	0	0
CNS_NB_FOXR2	1	0	0	0	1	0	0	0	0	0	0	0
EWS	5	1	0	0	4	0	0	1	0	0	0	0
O_IDH	120	95	0	1	25	0	0	94	0	1	0	0
A_IDH	154	5	0	1	148	1	0	5	0	0	0	0
A_IDH_HG	140	11	1	9	121	7	1	9	0	2	0	0
ANA_PA	75	2	0	1	72	1	0	2	0	0	0	0
ATRT_MYC	4	0	0	0	4	0	0	0	0	0	0	0
ATRT_SHH	7	0	0	0	7	0	0	0	0	0	0	0
ATRT_TYR	6	0	0	0	6	0	0	0	0	0	0	0
CN	18	0	0	0	18	0	0	0	0	0	0	0
LIPN	2	0	0	0	2	0	0	0	0	0	0	0
CHGL	1	0	0	0	1	0	0	0	0	0	0	0
CHORDM	4	1	1	2	2	0	0	0	1	1	0	0
CPH_ADM	2	0	0	0	2	0	0	0	0	0	0	0
CPH_PAP	4	0	0	0	4	0	0	0	0	0	0	0
DLGNT	9	0	0	0	9	0	0	0	0	0	0	0
DMG_K27	63	0	0	0	63	0	0	0	0	0	0	0
ETMR	25	0	0	0	25	0	0	0	0	0	0	0
EPN_RELA	14	0	0	0	14	0	0	0	0	0	0	0
EPN_YAP	1	0	0	0	1	0	0	0	0	0	0	0
EPN_MPE	15	0	0	1	14	1	0	0	0	0	0	0
EPN_PF_A	34	0	0	2	32	2	0	0	0	0	0	0
EPN_PF_B	6	1	0	0	5	0	0	1	0	0	0	0
EPN_SPINE	4	0	0	0	4	0	0	0	0	0	0	0
ENB_A	3	0	0	1	2	1	0	0	0	0	0	0
ENB_B	2	0	0	0	2	0	0	0	0	0	0	0
GBM_G34	17	0	0	4	13	4	0	0	0	0	0	0
GBM_MYCN	22	4	5	1	14	0	4	2	0	1	1	0
GBM_RTK_I	71	55	18	46	4	8	2	13	2	28	6	8
GBM_RTK_II	203	166	128	160	6	7	4	21	20	41	12	92
GBM_RTK_III	23	11	8	3	7	0	4	6	1	2	3	0
GBM_MES	157	123	37	109	12	15	1	29	6	64	6	24
GBM_MID	51	4	0	0	47	0	0	4	0	0	0	0
HMB	4	0	0	0	4	0	0	0	0	0	0	0
IHG	7	1	0	0	6	0	0	1	0	0	0	0
LGG_MYB	14	0	0	0	14	0	0	0	0	0	0	0
LGG_DIG_DIA	3	0	0	0	3	0	0	0	0	0	0	0
LGG_DNT	19	0	0	0	19	0	0	0	0	0	0	0
LGG_GG	12	3	0	2	9	0	0	1	0	2	0	0
LGG_RGNT	8	0	0	0	8	0	0	0	0	0	0	0
LGG_PA_GG_ST	30	1	0	1	29	0	0	0	0	1	0	0
LGG_PA_MID	34	0	0	0	34	0	0	0	0	0	0	0
LGG_PA_PF	78	0	0	0	78	0	0	0	0	0	0	0
LGG_SEGA	13	0	0	0	13	0	0	0	0	0	0	0
LYMPHO	13	0	0	0	13	0	0	0	0	0	0	0
MB_WNT	34	2	0	0	32	0	0	2	0	0	0	0
MB_SHH_CHL_AD	42	19	0	0	23	0	0	19	0	0	0	0
MB_SHH_INF	47	1	0	0	46	0	0	1	0	0	0	0
MB_G3	33	0	0	1	32	1	0	0	0	0	0	0
MB_G4	54	4	0	5	45	5	0	4	0	0	0	0
MELCYT	17	0	0	0	17	0	0	0	0	0	0	0
MELAN	17	12	0	1	5	0	0	11	0	1	0	0
SCHW_MEL	8	1	0	1	6	1	0	1	0	0	0	0
MNG	476	22	0	2	453	1	0	21	0	1	0	0
PTPR_A	4	0	0	0	4	0	0	0	0	0	0	0
PTPR_B	14	0	0	0	14	0	0	0	0	0	0	0
PGG_nC	8	0	0	0	8	0	0	0	0	0	0	0
PIN_T_PPT	12	0	0	0	12	0	0	0	0	0	0	0
PIN_T_PB_A	3	0	0	1	2	1	0	0	0	0	0	0
PIN_T_PB_B	7	0	0	1	6	1	0	0	0	0	0	0
PITUI	5	2	0	0	3	0	0	2	0	0	0	0
PITAD_STH_DNS_B	1	0	0	0	1	0	0	0	0	0	0	0
PLEX_AD	5	0	0	1	4	1	0	0	0	0	0	0
PLEX_PED_A	6	0	0	2	4	2	0	0	0	0	0	0
PLEX_PED_B	28	3	1	0	24	0	1	3	0	0	0	0
SCHW	18	0	0	0	18	0	0	0	0	0	0	0
SFT_HMPC	16	4	0	0	12	0	0	4	0	0	0	0
SUBEPN_PF	2	1	0	0	1	0	0	1	0	0	0	0
SUBEPN_SPINE	4	0	0	0	4	0	0	0	0	0	0	0
SUBEPN_ST	6	0	0	0	6	0	0	0	0	0	0	0
sum	**2,417**	**562**	**199**	**361**	**1747**	**61**	**17**	**264**	**30**	**146**	**28**	**124**

Full text for abbreviated methylation classes is provided in supplementary table 1. A characterization of methylation classes by Classifier prediction can be obtained from www.molecularneuropathology.org.

Table 2 Sensitivity and specificity of p*TERT*mut, *EGFR*amp and the 7/10 signature as single parameters and in combination for 544 *IDH*wt GBM in a series of 2,417 brain tumors

n total	n *TERT*	n *EGFR*	n 7/10	double 7/10 + *EGFR*	double 7/10 + *TERT*	double *EGFR* + *TERT*	triple 7/10 + *EGFR* + *TERT*	any double or triple
true positive	363	196	323	29	136	28	124	317
true negative	1674	1870	1835	1872	1863	1873	1873	1862
false positive	199	3	38	1	10	0	0	11
false negative	181	348	221	515	408	516	420	227
sensitivity	**66.7%**	**36.0%**	**59.4%**	**5.3%**	**25.0%**	**5.1%**	**22.8%**	**58.3%**
specificity	**89.4%**	**99.8%**	**98.0%**	**99.9%**	**99.5%**	**100.0%**	**100.0%**	**99.4%**

Supplementary table 1 Distribution of *EGFR*amp and status of chromosomes 7 and 10 in 10,826 tumors (cohort 2). Chromosome 7 and 10 status is given for two different thresholds requiring loss >50% or >80% of the respective arms. Combinations not qualifying for any form of a combined 7 gain and 10 loss are indicated by print in grey

Supplementary table 2 Distribution of methylation groups and alterations on chromosomes 7 and 10 as well as EGFR status in 939 patients from cohort 2 for whom survival data were available

Supplementary table 3 Methylation-based classification of 52 patients in cohort 3 without a 7/10 signature but with typical survival characteristics of GBM

Figure legends

Fig. 1 Representative CNP-plots: a) *IDH*wt GBM with 7+/10- and *EGFR*amp; b) *IDH*wt GBM with 7+/10q- and *EGFR*amp; c) PXA with 7+/10-

Fig. 2 a) OS in 939 patients (cohort 3) who have been diagnosed with *IDH*wt glioma, excluding GBM, stratified for different combinations of alterations of chromosomes 7 and 10. Of all possible combinations with losses on chromosomes 7 and 10, only 7+/10-, 7+/10q-, 7p+10-, 7q+/10q- and 7q+/10- were represented more than 3 times. Survival of glioma patients with 7+/10-, 7+/10q- and 7q+/10- was significantly worse than that of patients without these alterations. b) OS in167 patients from cohort 3 who in addition have received the classifier diagnosis GBM, *IDH*wt. DNA methylation based classification identifies 52 additional patients without the 7/10 signature. The black graph is a

reference series of additional 261 patients diagnosed with *IDH*wt GBM by both, histology and DNA methylation based classification

figure 1

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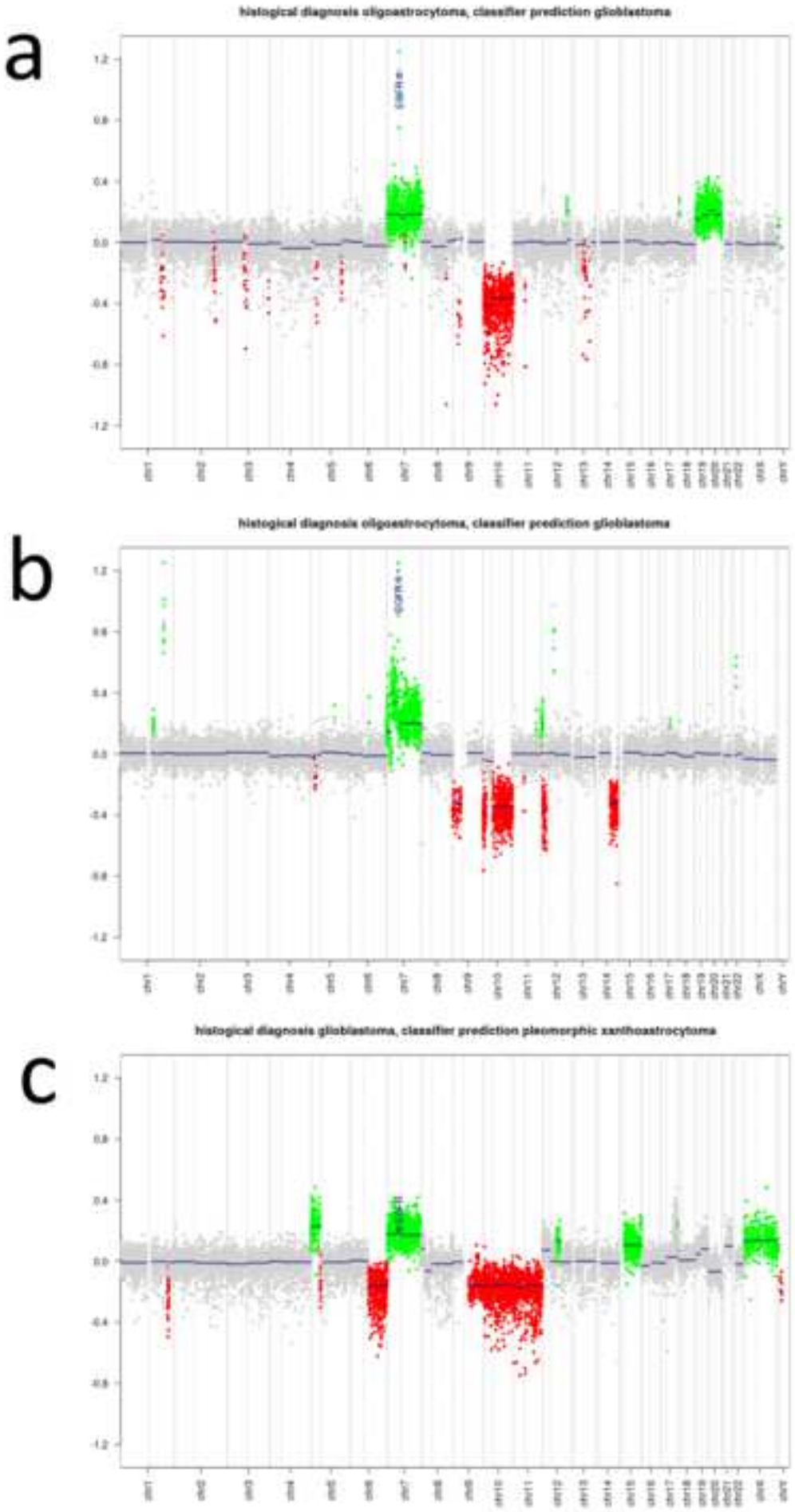

figure 2

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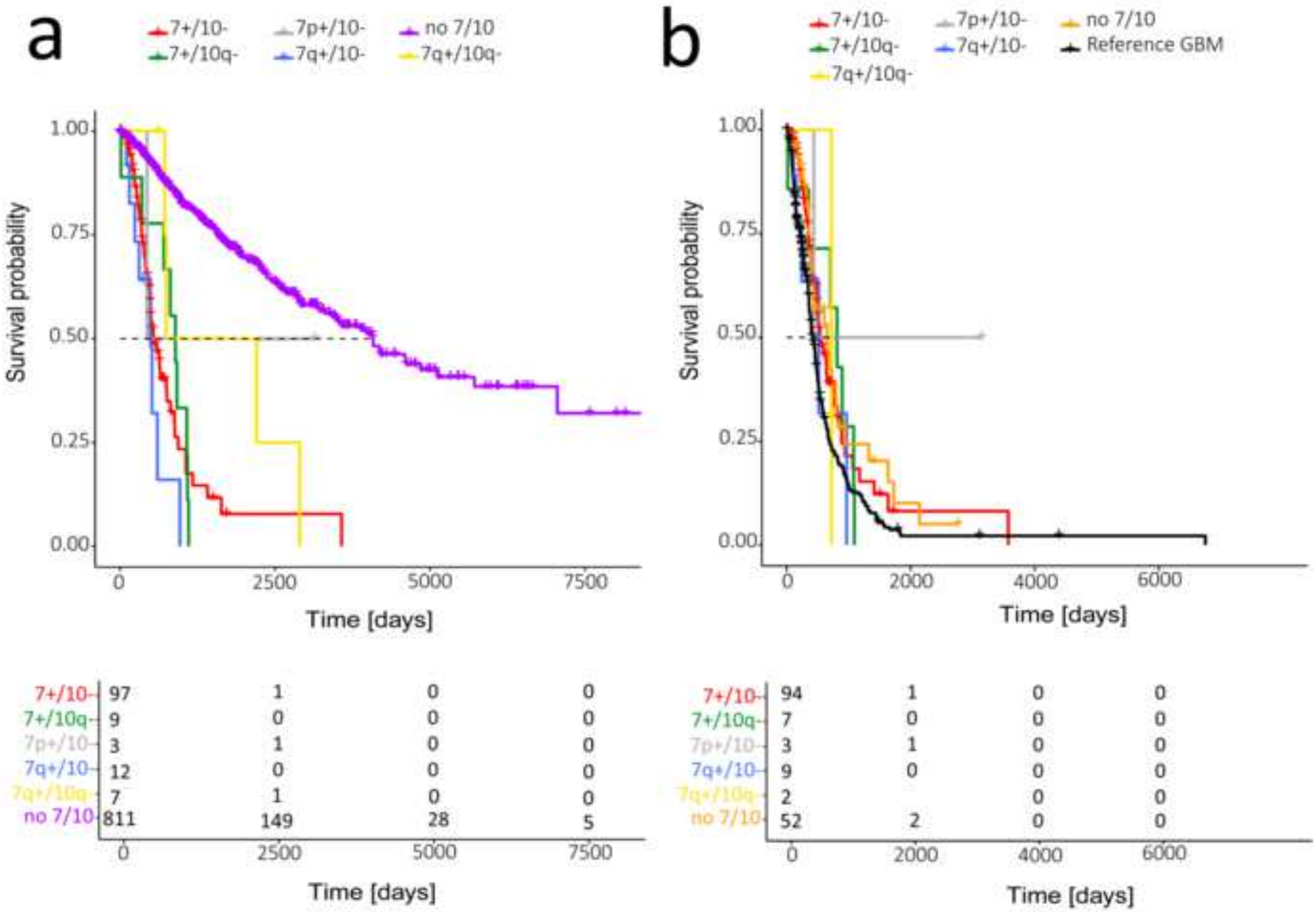

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