

**Article**

EGFR Amplification Is a Phenomenon of IDH Wildtype and TERT Mutated High-Grade Glioma: An Integrated Analysis Using Fluorescence In Situ Hybridization and DNA Methylome Profiling

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**Abstract:** Gliomas are the most common intrinsic brain tumors in adults, and in accordance with their clinical behavior and patients’ outcome, they are graded by the World Health Organization (WHO) classification of brain tumors. One very interesting candidate for targeted tumor therapy may be epidermal growth factor receptor (EGFR) amplification. Here, we performed an integrated comparative analysis of EGFR amplification in 34 glioma samples using standard fluorescence in situ hybridization (FISH) and Illumina EPIC Infinium Methylation Bead Chip and correlated results with molecular glioma hallmarks. We found that the EPIC analysis showed the same power of detecting EGFR amplification compared with FISH. EGFR amplification was detectable in high-grade gliomas (25%). Moreover, EGFR amplification was found to be present solely in IDH wildtype gliomas (26%) and TERT mutated gliomas (27%), occurring independently of MGMT promoter methylation status and being mutually exclusive with 1p/19q codeletion (LOH). In summary, EPIC Bead Chip analysis is a reliable tool for detecting EGFR amplification and is comparable with the standard method FISH. EGFR amplification is a phenomenon of IDH wildtype TERT mutated high-grade gliomas.

**Keywords:** glioma; glioblastoma; EGFR; FISH; EPIC DNA methylation analysis

1. Introduction

Gliomas are the most frequent intrinsic brain tumor of adults. According to the guidelines of the World Health Organization (WHO) classification of central nervous system (CNS) tumors, gliomas are assigned to CNS WHO Grades 1 to 4 depending on the clinical behavior and patients’ outcome [1,2]. Whilst CNS WHO Grade 1 pilocytic astrocytomas show a relatively benign course and 10-year survival of approximately 95% [3], CNS WHO Grade 4 glioblastoma show a devastating outcome with a 5-year survival rate of only 4–5% [1,4].

Since the publication of the 2021 WHO classification of tumors of the CNS, an integrated diagnosis and a layered report combining morphology and genetic findings were suggested as an integrated part of glioma classification [1]. Thus, the analysis of genomic alterations such as the isocitrate dehydrogenase 1 and 2 (IDHI, IDH2) and the histone H3 family 3A (H3F3A), HIST1H3B, and HIST1H3C genes as well as analysis of 1p and 19q status are now integrated aspects of glioma classification. Furthermore, the analysis of O(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation, mutation analysis of the telomerase reverse transcriptase (TERT) promoter, and CDKN2A/B testing are essential for evaluation of glioma [1]. Despite intensive research, survival of glioma...
patients often remains limited, and curative therapy is still lacking for the most common 
tumor entities (i.e., high-grade astrocytomas) [5–10].

A molecular target for individualized patient care is epidermal growth factor receptor
(EGFR) amplification [11]. EGFR, also termed ErbB1 and HER1, is a receptor tyrosine
kinase and is part of the ErbB receptor family [12], playing an important role in cell
proliferation, differentiation, and motility [12,13]. EGFR binds the epidermal growth factor
(EGF) and other growth factors such as growth factor-α (TGF-α), heparin-binding EGF
(HB-EGF), and amphiuregulin. Ligand binding activates the receptor couples to downstream
signaling pathways controlling cell proliferation, growth, differentiation, migration, and
inhibition of apoptosis [14,15]. Pathogenic EGFR mutations and truncations result in ligand-
independent signaling, subsequently leading to upregulation of various pro-oncogenic
processes, including chronic cell cycle proliferation [15]. EGFR gene amplification directly
correlated with protein overexpression and activated signaling [16].

Epigenomic DNA-methylation profiling is an emerging approach in tumor classifi-
cation [1,17,18]. DNA-methylation acts as control of gene transcription enabling on/off
switching of transcription by demethylation/methylation of CpG dinucleotide sequences
in the gene promoter [17,18]. The Illumina Infinium EPIC (850 k) Bead Chip is an advanced
tool for profiling approximately 850,000 CpGs in parallel throughout the human genome
with a highly streamlined protocol [17,18]. This information is subsequently analyzed in
bioinformatical pipelines enabling the deduction of copy number variation profiles and
similarity calculations, e.g., random forest trees and principal component analysis [17–20].

Here, we analyzed 34 glioma specimens of WHO Grades I to IV with regard to EGFR
amplification status using fluorescence in situ hybridization (FISH) and Illumina Infinium
EPIC Bead Chip Arrays. Furthermore, we performed an integrated analysis of EGFR
amplification in the context of other established molecular hallmarks.

2. Materials and Methods

2.1. Tissue Collection

In this study, we analyzed 34 anonymized tissue samples, entailing 2 pilocytic astro-
cytomas CNS WHO Grade 1, 2 oligodendrogliomas CNS WHO Grade 2, 2 astrocytomas
CNS WHO Grade 2, 3 astrocytomas CNS WHO Grade 4, and 23 glioblastomas CNS WHO
Grade 4, and 2 diffuse midline gliomas H3 K27 altered WHO Grade 4.

The gliomas were assigned to CNS WHO Grades 1 to 4, and integrated molecular
profiling was performed according to the 2021 WHO classification of CNS tumors [1]. All
tumor samples were provided by the University Institute of Pathology of the University
Hospital Salzburg. The samples used in this study were formalin-fixed and paraffin
embedded (FFPE). Prior to study inclusion, samples were anonymized according to the
ethics guidelines. Details about glioma samples are listed in Table 1.

Routine immunohistochemical (IHC) analysis was performed on a Ventana BenchMark
Ultra device (Roche) using Ventana ready-to-use antibodies against GFAP (760-4345), Ki67
(790-4286), PHH3 (760-4591), and EGFR (3C6) according to the manufacturer’s protocols.
EGFR protein expression levels were scored according to Avilés-Salas et al. within a scale
of 0 to 3 [21].

Table 1. Details on glioma samples. Indicated are details on analyzed samples including age, sex,
EGFR analysis, and molecular genetic hallmarks. n.a.: not available, wt: wildtype, u unmethylated,
m: methylated, d: deleted, n: not deleted.

| ID | Diagnosis | Grade | Age (y) | Sex | FISH | EPIC | IHC | IDH1 | IDH2 | 1p/19q | TERT | H3F3A | MGMT | CDKN2A/B |
|----|-----------|-------|---------|-----|------|------|-----|------|------|--------|------|-------|------|----------|
| T01| Pilocytic astrocytoma| 1 | 38 | f | 1.00 | 0.00 | 0 | wt | wt | n.a. | wt | n.a. | n | n |
| T02| Oligod. IDH mut. 1p/19q codel. | 1 | 16 | m | 1.00 | 0.00 | 0 | wt | wt | n.a. | wt | n.a. | n | n |
| T03| Oligod. IDH mut. 1p/19q codel. | 2 | 27 | m | 1.00 | 0.00 | 1 | R132H | wt | 1p/19q | C228T | n.a. | n | n |
| T04| Oligod. IDH mut. 1p/19q codel. | 2 | 63 | f | 1.00 | 0.00 | n.a. | R132H | wt | 1p/19q | C250T | n.a. | n | n |
### Table 1. Cont.

| ID  | Diagnosis                        | Grade | Age (y) | Sex  | FISH | EPIC | IDH1    | IDH2    | 1p/19q | TERT | H3F3A | MGMT | CDKN2A/B |
|-----|----------------------------------|-------|---------|------|------|------|---------|---------|--------|------|-------|------|----------|
| T05 | Astrocytoma IDH mutant           | 2     | 47      | m    | 1.00 | 0.00 | 0       | R132S   | wt     | wt   | wt    | n.a. | n        |
| T06 | Astrocytoma IDH mutant           | 2     | 22      | m    | 1.00 | 0.00 | n.a.    | R132C   | wt     | wt   | wt    | n.a. | u        |
| T07 | Glioblastoma IDH wildtype        | 4     | 47      | m    | 9.20 | 0.98 | 3       | wt      | wt     | n.a. | n.a.  | u    | n        |
| T08 | Astrocytoma IDH mutant           | 4     | 37      | f    | 1.00 | 0.32 | 1       | R132H   | wt     | wt   | wt    | n.a. | m        |
| T09 | Glioblastoma IDH wildtype        | 4     | 70      | f    | 1.00 | 0.00 | n.a.    | wt      | wt     | n.a. | C250T | n.a. | m        |
| T10 | Glioblastoma IDH wildtype        | 4     | 66      | m    | 7.74 | 1.07 | 3       | wt      | wt     | n.a. | C228T | n.a. | m        |
| T11 | Glioblastoma IDH wildtype        | 4     | 62      | m    | 1.13 | 0.00 | n.a.    | wt      | wt     | n.a. | C250T | n.a. | m        |
| T12 | Astrocytoma IDH mutant           | 4     | 45      | f    | 1.00 | 0.00 | 1       | R132H   | wt     | wt   | wt    | n.a. | m        |
| T13 | Glioblastoma IDH wildtype        | 4     | 77      | m    | 1.00 | 0.00 | 0       | wt      | wt     | n.a. | C228T | n.a. | u        |
| T14 | Glioblastoma IDH wildtype        | 4     | 74      | m    | 8.32 | 0.94 | 3       | wt      | wt     | n.a. | C250T | n.a. | m        |
| T15 | Glioblastoma IDH wildtype        | 4     | 43      | f    | 1.08 | 0.24 | n.a.    | wt      | wt     | n.a. | C250T | n.a. | u        |
| T16 | Astrocytoma IDH mutant           | 4     | 38      | m    | 1.00 | 0.00 | n.a.    | R132H   | wt     | wt   | wt    | n.a. | m        |
| T17 | Glioblastoma IDH wildtype        | 4     | 26      | m    | 1.20 | 0.00 | 0       | wt      | wt     | n.a. | n.a.  | u    | n        |
| T18 | Glioblastoma IDH wildtype        | 4     | 69      | m    | 9.25 | 1.04 | 3       | wt      | wt     | n.a. | C228T | n.a. | m        |
| T19 | Glioblastoma IDH wildtype        | 4     | 63      | f    | 1.00 | 0.00 | 0       | wt      | wt     | n.a. | C250T | n.a. | m        |
| T20 | Glioblastoma IDH wildtype        | 4     | 32      | m    | 1.15 | 1.00 | 0       | wt      | wt     | 1p   | C228T | n.a. | u        |
| T21 | Glioblastoma IDH wildtype        | 4     | 72      | m    | 1.15 | 0.00 | 0       | wt      | wt     | wt   | C228T | n.a. | m        |
| T22 | Glioblastoma IDH wildtype        | 4     | 79      | f    | 9.4  | 1.12 | n.a.    | wt      | wt     | wt   | C228T | n.a. | u        |
| T23 | Glioblastoma IDH wildtype        | 4     | 75      | f    | 1.00 | 0.00 | n.a.    | wt      | wt     | 1p   | C250T | n.a. | u        |
| T24 | Glioblastoma IDH wildtype        | 4     | 45      | f    | 1.05 | 0.00 | 0       | wt      | wt     | n.a. | C228T | wt    | m        |
| T25 | Glioblastoma IDH wildtype        | 4     | 77      | f    | 7.90 | 0.98 | 3       | wt      | wt     | n.a. | C228T | n.a. | u        |
| T26 | Glioblastoma IDH wildtype        | 4     | 49      | m    | 8.35 | 1.17 | n.a.    | wt      | wt     | n.a. | C250T | n.a. | m        |
| T27 | Glioblastoma IDH wildtype        | 4     | 25      | m    | 1.00 | 0.00 | 0       | wt      | wt     | wt   | wt    | n.a. | u        |
| T28 | Glioblastoma IDH wildtype        | 4     | 51      | m    | 1.00 | 0.00 | 0       | wt      | wt     | n.a. | C250T | n.a. | m        |
| T29 | Glioblastoma IDH wildtype        | 4     | 63      | f    | 1.00 | 0.36 | 1       | wt      | wt     | n.a. | C250T | n.a. | m        |
| T30 | Glioblastoma IDH wildtype        | 4     | 65      | f    | 1.00 | 0.00 | 1       | wt      | wt     | n.a. | C250T | n.a. | m        |
| T31 | Glioblastoma IDH wildtype        | 4     | 76      | f    | 1.05 | 0.00 | 0       | wt      | wt     | n.a. | n.a.  | n.a. | m        |
| T32 | Glioblastoma IDH wildtype        | 4     | 72      | f    | 1.00 | 0.12 | n.a.    | wt      | wt     | n.a. | C250T | n.a. | u        |
| T33 | Dif. midline glioma H3 K27 alt. | 4     | 38      | m    | 1.00 | 0.00 | 0       | wt      | wt     | n.a. | C228T | K27M | u        |
| T34 | Dif. midline glioma H3 K27 alt. | 4     | 33      | f    | 1.00 | 0.00 | 0       | wt      | wt     | n.a. | wt    | n.a. | K27M    |

### 2.2. Molecular Genetic Characterization of Gliomas

Molecular genetic analysis of glioma samples was performed as previously described [22]. Representative tumor tissues with at least 90% of viable tumor cells were microscopically identified. DNA extraction was conducted applying the Maxwell system (Promega) according to the manufacturer’s instructions. Mutational analysis of *IDH1* and *IDH2* genes were performed with the AmpliSeq for Illumina Cancer Hotspot Panel v2 (Illumina) or the AmpliSeq for Illumina Focus Panel (Illumina), respectively, on an Illumina MiniSeq next-generation sequencing device following the manufacturer’s protocols. Identification of mutations within the hot spot loci of *TERT* promoter, *H3F3A*, *H3F1H3B*, and *HIST1H3C* genes were analyzed by Sanger sequencing as described previously [22–24]. Homozygous losses of cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B) were assessed by EPIC.
copy number variation (CNV) analysis according to Capper et al. [18] and in concordance with the guidelines of the 2021 WHO classification [1].

ZytoLight 1p/1q and 19q/19p probe sets (ZytoVision) were applied according to the manufacturer’s protocols to evaluate the 1p/19q codeletion status of IDH mutant gliomas. Deletions of 1p and 19q were double-checked applying EPIC CNV profiles to avoid false positive results that may be due to partial 1p and 19q losses. According to the guidelines of the 2021 WHO classification, 1p/19q status was assessed for all IDH mutated gliomas, as loss of 1p and 19q is only occurring in IDH mutant gliomas [1].

2.3. Fluorescence In Situ Hybridization (FISH) Analysis

To evaluate the EGFR amplification status, we applied the ZytoLight SPEC EGFR/CEN 7 Dual Color Probe set (ZytoVision). Slides were reviewed using fluorescence microscopy, and FISH signals for individual probes were recorded. To assess EGFR amplification, we calculated the ratio of green (EGFR) and red (CEN7) signals. In accordance with French et al., we set the cut-off value for amplification to equal or greater than 2 [25]. Tumors with polysomy for chromosome 7 but without focal amplification of the EGFR gene were considered to be EGFR non-amplified.

2.4. Infinium Methylation EPIC Array Analysis

Methylation analysis of glioma samples was performed using the Infinium Methylation EPIC Bead Chip (Illumina) according to manufacturer’s protocol. Raw data (idat-files) were analyzed using the molecularneuropathology.org bioinformatics pipeline of the German Cancer Research Center (DKFZ) and the current brain tumor classifier [17]. Copy-number variation (CNV) analysis is an integrated part of the molecularneuropathology.org bioinformatics pipeline. EGFR amplifications were assessed using the generated CNV plots and ImageJ. EGFR status was interpreted in accordance to Stichel et al. as being considered amplified if the respective probes showed an intensity of more than 0.6 on the log2-scale from the CNV after baseline correction (relative probe intensity) [26].

2.5. Computational Data Analysis

Statistical analysis was performed using Prism 9 (GraphPad) software suite and Microsoft Excel applying Student’s t-test. Statistical significance was assumed for p-values < 0.05. Regression analysis was performed using Prism 9 and Microsoft Excel.

3. Results

3.1. Fluorescence In Situ Hybridization (FISH) and Infinium EPIC Methylation Bead Chip Analysis Are Equally Valid Methods in Detection of EGFR Amplifications

In this study, we investigated the validity of EGFR detection using conventional FISH analysis and epigenome-wide methylation analysis using the Illumina EPIC Methylation Bead Chip with integrated CNV profiling. DNA methylome analysis by EPIC arrays is a reliable approach in molecular glioma classification [17]. Thus, we performed both FISH and EPIC analysis on all 34 histologically well-characterized gliomas. We found that both FISH and EPIC analysis enabled discrimination of EGFR non-amplification and amplification status and that the results are consistent with EGFR protein levels assessed by immunohistochemistry (Figure 1a–h). Using FISH, we detected seven gliomas with EGFR amplification (Figure 2a). This is in perfect concordance with EPIC analysis that revealed the same seven gliomas being EGFR amplified (Figure 2b). Results also correlate with protein expression detected by immunohistochemistry (Figure 2c). Cut-off values were defined as suggested by French et al. (FISH) [25] and Stichel et al. (EPIC) [26]. Protein expression was scored according to Avilés-Salas et al. [21]. Regression analysis showed perfect match of FISH and EPIC analysis (R² = 0.9411, p < 0.0001) as well as of FISH and IHC (R² = 0.8618, p < 0.0001) and EPIC and IHC (R² = 0.9019, p < 0.0001) (Figure 2d).
Figure 1. Detection of EGFR copy numbers in gliomas. Analysis of 34 glioma samples showed that it is possible to distinguish between EGFR non-amplification (e.g., sample T34) (a–d) and EGFR amplification (e.g., sample T25) (e–h) using FISH (b,f) and EPIC analysis (c,g). These findings also correlate with protein expression levels using IHC (d,h). HE: Hematoxylin–Eosin; FISH: fluorescence in situ hybridization; EPIC: Illumina Infinium EPIC Bead Chip; IHC: immunohistochemistry. Scale bars: 50 µm (a,d,e,h), 10 µm (b,f).
Figure 2. Comparison of two different methodologies for EGFR amplification detection. Using both FISH (a) and EPIC analysis (b), we found seven samples with EGFR amplification. Cut-off values for EGFR amplification are indicated by a green line and were set according to French et al. to equal or greater than 2 for FISH [25] and according to Stichel et al. to a relative probe intensity of more than 0.6 for EPIC analysis [26]. The results are well in line with protein expression levels detected by immunohistochemistry (c) scored according to Avilés-Salas et al. [21]. Regression analysis showed good correlation of EGFR amplification using FISH and EPIC analysis as well as FISH and IHC and EPIC and IHC (d). Analysis of WHO Grade and EGFR status showed that EGFR amplification is a hallmark of high-grade CNS WHO Grade 4 gliomas using both FISH (e) and EPIC analysis (f) as well as IHC (g). All gliomas with EGFR amplification were of CNS WHO Grade 4 (h). #: not performed due to tissue limitation.
3.2. EGFR Amplifications Predominantly Occur in High-Grade Glioma

Detailed analysis showed that EGFR amplification is unevenly distributed across gliomas. Of all 34 gliomas, we found 7 gliomas (21%) with EGFR amplification: While we did not find EGFR amplification in low-grade gliomas (0%)—i.e., CNS WHO Grade 1 (0%) and CNS WHO Grade 2 (0%)—25% of high-grade astrocytomas and glioblastomas CNS WHO Grade 4 gliomas showed EGFR amplification (Figure 2a–c). Mean EGFR gene amplification was 3 in CNS WHO Grade 4 gliomas using FISH (Figure 2e). These results are in concordance with EPIC analysis: We did not find EGFR amplification in WHO Grade 1 and 2 gliomas but found mean EGFR relative probe intensities of 0.3 in CNS WHO Grade 4 gliomas (Figure 2f). Immunohistochemistry showed mean EGFR protein expression of 1 in CNS WHO Grade 4 gliomas (Figure 2g). All identified gliomas with EGFR amplification were of CNS WHO Grade 4 (seven cases) (Figure 2h).

3.3. Integrated Analysis of EGFR Amplification and Molecular Glioma Hallmarks

Next, we performed an integrated analysis of EGFR amplification and molecular glioma hallmarks: IDH1/2 mutations, TERT promoter mutations, MGMT promoter methylation, and LOH 1p/19q.

An analysis of EGFR amplification and IDH mutation status revealed that IDH mutated glioma showed fewer copies of EGFR using FISH (Figure 3a) and EPIC (Figure 3b) without this being statistically significant ($p > 0.05$, Student’s $t$-test). Of all analyzed IDH wildtype gliomas, 26% showed EGFR amplification (Figure 3c), and among IDH mutated gliomas, none showed an EGFR amplification (Figure 3d).

Analysis of TERT promoter mutation status and EGFR amplification showed that TERT mutated glioma show increased copies of EGFR using FISH (Figure 3e) and EPIC (Figure 3f) without statistical significance ($p > 0.05$, Student’s $t$-test). Of all analyzed TERT wildtype gliomas, none show an EGFR amplification (Figure 3g). Of all TERT mutated gliomas, 27% showed EGFR amplifications (Figure 3h).

An analysis of EGFR amplification and MGMT promoter methylation revealed that there is no association between EGFR copy numbers and MGMT methylation status using FISH (Figure 3i) and EPIC (Figure 3j) ($p > 0.05$, Student’s $t$-test). Of all analyzed MGMT methylated gliomas, 19% showed EGFR amplification (Figure 3k), and 22% of MGMT unmethylated gliomas showed an EGFR amplification (Figure 3l).

Analysis of LOH 1p/19q status and EGFR amplification showed increased copies of EGFR in gliomas without LOH 1p/19q using FISH (Figure 3m) and EPIC (Figure 3n) without being statistically significant ($p > 0.05$, Student’s $t$-test). Of all gliomas without LOH 1p/19q, 11% showed EGFR amplification (Figure 3o). Of all gliomas with LOH 1p/19q, none showed an EGFR amplification (Figure 3p).
Figure 3. Integrated analysis of EGFR amplification and molecular genetic hallmarks of glioma. Correlation of EGFR amplification and IDH status showed EGFR amplification in IDH wildtype gliomas using both FISH (a) and EPIC analysis (b) \( (p > 0.05, \text{Student's } t\text{-test}) \), with 26% of IDH wildtype gliomas being EGFR amplified (c) and no EGFR amplified case in IDH mutated gliomas (d). In the case of TERT mutation status, we found that EGFR amplification occurs in TERT mutated cases using FISH (e) and EPIC analysis (f) \( (p > 0.05, \text{Student's } t\text{-test}) \), with no EGFR amplified case in TERT wildtype gliomas (g) and 27% of EGFR amplified cases in TERT mutated gliomas (h). Analysis of EGFR amplification and MGMT methylation status showed no differences in EGFR amplification in MGMT methylated and unmethylated gliomas using FISH (i) and EPIC analysis (j) \( (p > 0.05, \text{Student's } t\text{-test}) \), 19% of MGMT methylated cases (k), and 22% of MGMT unmethylated cases showing EGFR amplification (l). In terms of LOH 1p/19q EGFR amplification was found in gliomas without LOH 1p/19q using FISH (m) and EPIC analysis (n) \( (p > 0.05, \text{Student's } t\text{-test}) \), with 11% of cases without LOH 1p/19q (o) and no case with LOH 1p/19q being EGFR amplified (p). wt: wildtype; m: methylated; u: unmethylated; LOH: loss of heterozygosity.
4. Discussion

Glioblastomas are the most frequent and most aggressive brain tumors in adults, with a 5-year overall relative survival of only 6.8% [1,27]. One hallmark in glioblastoma therapy was the identification of MGMT promoter methylation that is associated with good therapy response using the alkylating agent temozolomide [8,28,29] and with better outcome [7].

A promising target in glioblastoma therapy may be EGFR overexpression. EGFR inhibition by monoclonal antibodies or small-molecule tyrosine kinase inhibitors (TKIs) has been approved for the treatment of tumor entities such as RAS wildtype colorectal cancers, squamous cell carcinoma of the head and neck (HNSCC), and EGFR mutated non-small-cell lung cancer (NSCLC) [11].

Here, we assessed EGFR gene amplification using FISH and Infinium Methylation EPIC Bead Chip analysis—a technique that is routinely used for molecular brain tumor classifications [17]. We were able to demonstrate, that both FISH and EPIC Bead Chip analysis are equally valid in identifying EGFR amplifications: Regression analysis of FISH and EPIC Array revealed very high concordance of both methods for the analysis of EGFR amplification (Figure 2d).

Detailed workup showed that EGFR amplification is a phenomenon of high-grade CNS WHO Grade 4 gliomas (Figure 2e–g). Integrated analysis of molecular key hallmarks in glioma (IDH, TERT, MGMT methylation, and LOH 1p/19q) and EGFR amplification showed that EGFR amplification is a phenomenon that can be predominantly found in IDH wildtype (Figure 3a–d) and TERT mutated (Figure 3e–h) gliomas, as well in gliomas without LOH 1p/19q (Figure 3m–p).

Since the importance of EGFR amplification has already been established as a precision medicine target in other cancers, such as colorectal cancers, HNSCC, and NSCLC [11], our findings may also open new therapeutic approaches in future brain tumor therapy [30,31]. Thereby, our results are well in line with published data: Bale et al. found that IDH wildtype gliomas have a higher prevalence of EGFR gene amplification and overexpression than IDH mutated gliomas [32]. In terms of EGFR amplification and TERT promoter mutation, our findings confirm published results: Jaunmuktane et al. found that 82.88% of IDH and TERT wildtype gliomas were EGFR non-amplified, while only 17.12% were EGFR amplified. Of IDH wildtype and TERT mutant gliomas, 58.13% were EGFR non-amplified and 41.87% were EGFR amplified [33]. In terms of MGMT promoter methylation, Bale et al. found that EGFR amplification occurred independently of MGMT promoter methylation status [32]. Our data also support the finding that EGFR amplification occurs independently of MGMT promoter methylation. Furthermore, Bale et al. stated that EGFR amplification was mutually exclusive of codeletion of chromosomes 1p and 19q (LOH) [32]. Our data also support the finding that EGFR amplification is mutually exclusive of codeletions of 1p and 19q.

The detection of combined loss of 1p and 19q in IDH mutated astrocytoma is an essential aspect in integrated diagnosis according to the 2021 WHO classification [1]. Thereby, it is important to detect whole arm losses of 1p and 19q [1]. Since FISH probes cover only distinct genomic regions, the use of FISH as the only method is a limitation that may lead to false-positive results [1]. Thus, we additionally double-checked 1p/19q losses detected by FISH in CNV profiles of the EPIC results.

In summary, our findings demonstrate that Infinium EPIC Bead Chip analysis that is routinely applied in molecular brain tumor classification [17] is a reliable technique for detecting EGFR amplifications compared with standard FISH analysis. We found that EGFR amplification is a phenomenon that predominantly occurs in high-grade glioma.

5. Conclusions

In conclusion, we demonstrated that EPIC Bead Chip analysis is a reliable tool in detecting EGFR amplification that is comparable with the standard FISH method. We found that EGFR amplification is a phenomenon of IDH wildtype TERT mutated high-grade gliomas.
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