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Epigenetic profiling reveals a strong association between lack of 5-ALA fluorescence and *EGFR* amplification in *IDH*-wildtype glioblastoma

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Abstract

Background: 5-aminolevulinic acid (5-ALA) fluorescence-guided resection increases the percentage of complete CNS tumor resections and improves the progression-free survival of *IDH*-wildtype glioblastoma patients. A small subset of *IDH*-wildtype glioblastoma shows no 5-ALA fluorescence. An explanation for these cases is missing. In this study, we used DNA methylation profiling to further characterize non-fluorescent glioblastomas.

Methods: Patients with newly diagnosed and recurrent *IDH*-wildtype glioblastoma that underwent surgery were analyzed. The intensity of intraoperative 5-ALA fluorescence was categorized as non-visible or visible. DNA was extracted from tumors and genome-wide DNA methylation patterns were analyzed using Illumina EPIC (850k) arrays. Furthermore, 5-ALA intensity was measured by flow cytometry on human gliomasphere lines (BT112 and BT145).

Results: Of 74 included patients, 12 (16.2%) patients had a non-fluorescent glioblastoma, which were compared to 62 glioblastomas with 5-ALA fluorescence. Clinical characteristics were equally distributed between both groups. We did not find significant differences between DNA methylation subclasses and 5-ALA fluorescence ($P = .24$). The distribution of cells of the tumor microenvironment was not significantly different between the non-fluorescent and fluorescent tumors. Copy number variations in *EGFR and* simultaneous EGFRvIII expression were strongly associated with 5-ALA fluorescence since all non-fluorescent glioblastomas were *EGFR*-amplified ($P < .01$). This finding was also demonstrated in recurrent tumors. Similarly, *EGFR*-amplified glioblastoma cell lines showed no 5-ALA fluorescence after 24 h of incubation.

Conclusions: Our study demonstrates an association between non-fluorescent *IDH*-wildtype glioblastomas and *EGFR* gene amplification which should be taken into consideration for recurrent surgery and future studies investigating *EGFR*-amplified gliomas.

Keywords: 5-ALA; 5-aminolevulinic acid; EGFR; glioma; subclass.