Integrated characterization of EGFR amplification architecture and EGFRvIII status in patient-derived glioblastoma stem cells

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Glioblastoma (GB) is the most common malignant brain tumor in adults and is frequently characterized by EGFR amplification, often occurring on extrachromosomal DNA (ecDNA). Glioma stem cells (GSCs), which contribute to tumor progression, recurrence and therapeutic resistance, are particularly enriched for EGFR amplification and expression of the mutant EGFRvIII variant. We investigated the genomic architecture of EGFR alterations and EGFRvIII status and evaluated their association with response to Osimertinib in patient-derived GSCs.

Array comparative genomic hybridization (aCGH) identified EGFR amplifications in 4 of 11 analyzed GSC cultures. Karyotype analysis revealed the presence of ecDNA as double minutes (DMs) in two of the four EGFR amplified cell lines. Dual-color Fluorescence In Situ Hybridization (FISH), using probes for the HER1/EGFR locus and chromosome 7 centromere, mapped EGFR to extrachromosomal DMs in two cell lines and to intrachromosomal homogeneously staining regions (HSRs) in one cell line.

EGFR amplification was consistently detected by FISH, targeted NGS (Oncomine Precision Assay) and whole-genome sequencing, the latter further analyzed with Amplicon Classifier to identify ecDNA elements. Molecular profiling identified two representative GSC lines with distinct EGFR alteration patterns: GSC7, harboring EGFR amplification on ecDNA and lacking EGFRvIII, and GSC23, carrying EGFRvIII with EGFR amplification on HSRs. RNA-seq analysis revealed a sixfold higher EGFR expression in GSC7 than in GSC23.

Osimertinib cytotoxicity evaluated in both GSC lines showed GSC23 being more sensitive to treatment. Extreme Limiting Dilution Assay (ELDA) showed reduced neurospheres formation after drug exposure, while wound healing assays demonstrated decreased migratory capacity in both lines after treatment.

These findings highlight the genomic heterogeneity of EGFR alterations and suggest that EGFR amplification architecture and EGFRvIII status are associated with distinct biological features and differential response to Osimertinib. Comprehensive molecular profiling may support the development of targeted therapeutic approaches.