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Gauging heterogeneity in primary versus recurrent glioblastoma

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See the article by van den Bent et al., on pages 935–941.

Genetic heterogeneity of malignant tumors is high on the research agenda. Glioblastoma is a prime example of a heterogeneous tumor, in terms of both heterogeneity among patients and within a given tumor. Heterogeneity has been highlighted in large-scale studies comparing gene expression profiles, copy number alterations, gene mutations, and DNA methylation patterns among large cohorts of patients.[1–5] Recently several studies have provided evidence for genetically heterogeneous clones within an individual tumor, at the level of copy number aberration and gene expression,[6] DNA ploidy,[7] gene amplification and expression of receptor tyrosine kinases.[8,9] Amplification of the epidermal growth factor receptor (EGFR) gene is found in 50%–60% of glioblastomas, and about half of these display concomitant expression of the EGFR deletion mutant lacking exons 2–7, commonly termed EGFR variant (v)III. EGFRvIII displays low-level constitutive pathway activity and represents a promising therapeutic target because of its tumor specificity. However, it has long been known that EGFRvIII is detected in only a fraction of cells in EGFR amplified tumors,[10] challenging the effectiveness of EGFRvIII targeting agents. Of note, expression studies are largely based on primary tumor material, while the distribution of EGFRvIII is hardly known in recurrent glioblastoma.

EGFRvIII in Recurrent Glioblastoma

The study by van den Bent and colleagues (this issue of *Neuro-Oncology*) addresses exactly this simple but clinically relevant question about the evolution of EGFR and EGFRvIII expression in recurrences.[11] They investigated EGFR gene amplification and EGFRvIII expression status in 55 paired primary and recurrent tumors. EGFR amplification was determined by DNA-based PCR and EGFR/EGFRvIII expression by quantitative reverse transcription PCR. All patients had undergone standard radiotherapy and temozolomide treatment, thus representing a homogeneous study group. The cohort presented a relatively high proportion of EGFR amplified tumors (73%) compared with other studies, suggesting a tendency for increased reoperation in EGFR amplified tumors in their center, although the reasons for this are unclear.

As expected, EGFR expression was strongly correlated with EGFR gene amplification, and EGFRvIII expression was detected only in EGFR amplified tumors. EGFR amplification status was found to be largely constant between primary and recurrent tumors, meaning that amplified and non-amplified tumors retained their original status (84%). Among the amplified tumors, some changes were observed in the level of amplification, but these were rather modest. The situation was different for EGFRvIII. Although the overall status (presence or absence of EGFRvIII) was maintained in 79% of cases, about half of EGFRvIII-positive tumors had lost the expression in the recurrent setting, while the remaining often displayed reduced expression (Fig. 1).

Clinical Relevance

Although the mechanism leading to this change in receptor distribution is currently not known, the clinical consequences are considerable in view of various glioblastoma resistance mechanisms and adaptation to EGFR targeting therapies. The finding is of particular concern for clinical trials targeting EGFR/EGFRvIII in the recurrent setting; however, it should also be considered when targeting the primary tumor. It was previously reported that EGFR amplification status remained unchanged after treatment with EGFR tyrosine kinase inhibitors.[12] In this context, the loss of extrachromosomal mutant EGFR has recently been proposed as one of the resistance mechanisms to EGFR therapies.[13] The present study suggests that this mechanism may already be at play after standard of care, a phenomenon that may be exacerbated by tyrosine kinase inhibitor treatment.

The study also has important consequences for the interpretation of immunotherapy studies with rindopepimut, a promising peptide vaccine targeting EGFRvIII. An ongoing phase III trial (ACT IV) investigates the effect of the vaccine in newly diagnosed tumors, while a phase II trial (ReACT) targets recurrent glioblastoma.[14] Since recurrent glioblastomas are rarely reoperated, the presence of the target molecule will be largely unknown at the time of treatment. Another obvious question in this setting is the fate of EGFR amplified tumor cells that lack the mutant variant.

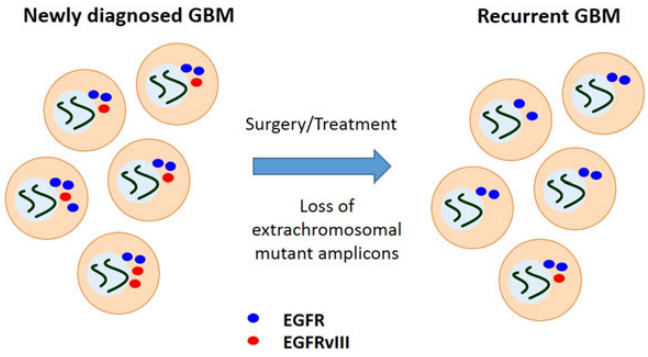

Fig. 1. Dynamic regulation of EGFR variants in recurrent glioblastoma. EGFR and EGFRvIII appear as extrachromosomal DNA elements in EGFR amplified glioblastoma. Interestingly mutant forms of the receptor are not fully retained in recurrent tumors, which may reflect the dynamic turnover of mutant amplicons and selective adaptation processes.

Mutation-Prone Double Minute Chromosomes

Oncogenic regions such as the EGFR locus are often amplified in tumor cells in the form of small paired chromosomal bodies termed double minute chromosomes.[15] These circular DNA elements, which lack a centromere and telomeres, are replicated during early S phase and segregate to daughter cells by "hitchhiking" on the chromosome arms.[16] Whether and how this process is regulated is largely unresolved. The segregation at mitosis is thought to occur randomly, which could at least partially explain the mosaic distribution of EGFR, EGFRvIII, and other receptor tyrosine kinases in glioblastoma. It does not explain, however, why certain amplicons (eg, EGFR) are maintained in daughter cells, while others (eg, EGFRvIII) are more scattered and are lost over time (in recurrent tumors). This must be linked to selective pressure on the tumor cell that favors a particular expression profile, and/or may reflect regulation by epigenetic mechanisms.[17] Interestingly a recent report suggests that extrachromosomal amplified DNA elements are prone to mutations, providing a mechanism for rapid mutational turnover and adaptation (eg, through loss of mutant amplicons) to a changing microenvironment[18] (Fig. 1).

In this regard, it should not be forgotten that besides EGFRvIII, other EGFR variants frequently appear in glioblastoma, always in association with EGFR gene amplification and often concomitantly with EGFRvIII expression.[19,20] The C-terminal deletion mutant, EGFRvV, and the EGFRvII mutant harboring an 83 amino acid deletion in the extracellular domain are among the more common variants, while other deletion mutants and point mutation variants represent more rare events.[20–22] Although the percentage of reported cases varies widely (10%–30% of mutants in an EGFR amplified background), these mutant forms also represent tumor-specific targets, and a better knowledge of their functional relevance and their expression pattern before and after treatment is warranted. Understanding the differential regulation of EGFR versus EGFR mutant expression will enhance our chances to gain therapeutic benefit from these rather elusive tumor-specific targets.

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