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Co-survival of the fittest few: Mosaic amplification of receptor tyrosine kinases in glioblastoma

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RESEARCH HIGHLIGHT

Co-survival of the fittest few: mosaic amplification of receptor tyrosine kinases in glioblastoma

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Abstract

Mosaic amplification of receptor tyrosine kinases in glioblastoma suggests that tumor cells with different progression driver mutations may coevolve rather than compete during clonal evolution.

Keywords Clonal evolution, tumor heterogeneity, glioblastoma, mosaic amplification, receptor tyrosine kinases, progression driver mutations

Heterogeneity, long recognized as a hallmark for cancers, provides the platform for clonal evolution to occur. The advancement of genome biology, especially next generation DNA sequencing technology, has started to reveal tumor heterogeneity at the gene level and, increasingly, the nucleotide level. Deep sequencing of cancer genomes in combination with sophisticated clustering algorithms is being used to identify subclones with similar genomic variant profiles with increasing confidence and resolution. Comparisons of primary tumor samples with matched metastatic samples, xenografts and relapse samples have provided clues for delineating subclonal phylogenetic evolutionary history, rooted at the primary tumor and diverging into its various derivatives [1-7]. Ideally, such studies would use single cell sequencing, which provides the most detailed picture of tumor heterogeneity and clonal evolutionary history. Indeed, this challenging technique has recently been successfully used to infer the evolutionary history of breast cancer cells based on copy number variations [8]. However, the low genome coverage and the intrinsic error rate in current DNA sequencing technology limit the resolution with which genomic variants are detected in these samples. A recent study by Snuderl *et al.* [9] has revealed the mosaic amplification of multiple receptor tyrosine kinases (RTKs) in glioblastoma multiforme (GBM) (Figure 1) and achieved single-cell resolution, with regard to the selected target genes, in dissecting tumor heterogeneity. The discovery of mosaic amplification of RTKs in GBMs has broad implications for tumor clonal evolution, the concept of driver mutations, and treatment choices.

Mosaic amplification of receptor tyrosine kinases in glioblastoma

Amplification of RTK genes, including those encoding the epidermal growth factor receptor (*EGFR*), the vascular endothelial growth factor receptor (*VEGFR2*), the platelet-derived growth factor receptor A (*PDGFRA*), and the oncogenes *KIT* and *MET*, drives the tumorigenesis of GBM through the activation of the mitogen-activated protein kinase pathway. Usually, only one RTK shows high-level amplification in a given GBM sample. By analyzing The Cancer Genome Atlas (TCGA) project copy number data, Snuderl *et al.* [9] found, unexpectedly, that 6.3% (13 out of 206) of the GBM cases showed simultaneous amplification of multiple RTKs. The authors were able to confirm the coamplification of *MET* and *PDGFRA* within the same tumor cells from a single original TCGA case by fluorescent *in situ* hybridization (FISH). Snuderl *et al.* [9] further studied their own GBM cohort using FISH to detect gene amplification and immunofluorescent staining to detect increased protein production. They found that 4.5% (16 out of 350) of GBMs have more than one amplified RTK. A genome-wide copy number assessment, by array comparative genomic hybridization, showed that the amplicons were local, excluding the probability of coamplification due to unusually high chromosomal instability. Unlike the single TCGA case, these multiple RTK amplifications were present in intermingled subpopulations of tumor cells that are all mitotically active.

Nonlinear coevolution of tumor subclones

Snuderl *et al.* [9] found that in all cases with observed co-amplifications of RTKs, the *CDKN2A* and *TP53* genotypes were concordant in each of the subpopulations, suggesting that these subpopulations share the same precursor and can be considered subclones within each

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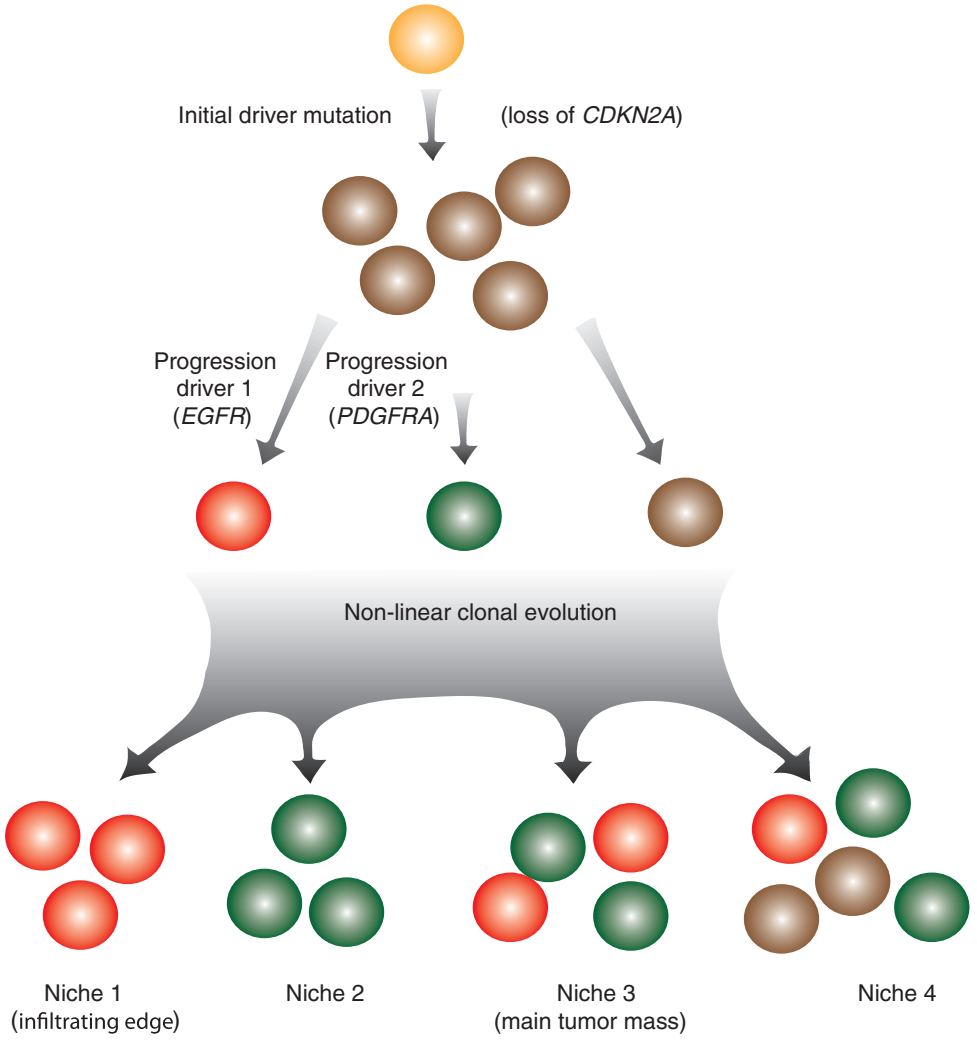

Figure 1. A nonlinear clonal evolution model derived from the observation of mosaic amplification of receptor tyrosine kinases (RTKs) in glioblastoma multiforme (GBM). The primary tumor and all of its derivatives carry the early driver mutation that initiates tumorigenesis. Multiple progression driver mutations can occur in a non-linear manner, creating subpopulations with variable levels of fitness. Many times, a dominant clone emerges before another round of diversification and selection pushes the clonal evolution forward. However, as illustrated by the coexistence of subclones with amplification of different RTKs in some GBMs, subclones may coexist because of either similar fitness levels or a cooperative relationship for enhanced tumor growth potential. Individual subclones and different combinations of them may occupy specific niches within the tumor microenvironment. Relevant genes and niches from the Snuderl *et al.* study [9] are indicated in parentheses.

tumor. Not surprisingly, the ratios of subclones with different amplified RTKs varied broadly among tumor samples. A particularly intriguing finding is the spatial distribution of the mosaic subclones within a complete GBM brain autopsy. Although the *EGFR*- and *PDGFRA*-amplified subclones are present at a 60:40 ratio in the main tumor mass, the portion infiltrating other tissues contained exclusively *EGFR*-amplified cells. The uneven distribution suggests that each population may occupy a distinct niche within the tumor microenvironment (Figure 1). Furthermore, the coexistence of two subpopulations with different RTK amplifications could mean a similar level of fitness for each of the subclones. It is even possible that these subclones may cooperate to achieve higher fitness than each of the subclones alone. This scenario is very different from a linear clonal evolution model in which subclones from the primary clone compete for dominance and further mutations in the dominant subclone initiate another round of selection. The hypothesized cooperation among the subclones warrants the survival of the fittest few. In fact, co-operation among different subpopulations of tumor cells is not unprecedented. In GBM tumors with both amplified wild-type *EGFR* (*wtEGFR*) and an activated

form of this RTK *(ΔEGFR*), the former is typically expressed at far greater abundance. Inda *et al.* [10] showed that cells with *ΔEGFR* secrete interleukin-6 and/or leukemia inhibitory factor, both of which activate the wtEGFR in neighboring cells through the receptor gp130, enhancing the rate of tumor growth. Thus, cooperation of tumor subclones can be achieved by paracrine signaling and can actively maintain tumor heterogeneity. Although other tumor types may conceivably have similar paracrine signaling among tumor subclones, direct evidence is still lacking. The scarcity of fibroblasts in GBMs may be a selection force for subclones that can provide mitogenic signals and other forms of support for tumor growth that are delivered by the stroma in other tumors.

Multiplicity of drivers and therapeutic implications

By using a clever combination of genomic data, FISH, immunofluorescent staining and other approaches, Snuderl *et al.* [9] have revealed the mosaic amplification of multiple RTKs in GBMs and delineated clonal evolution within these tumors in molecular and spatial detail. Clonal evolution is generally thought to be driven by the driver mutations that provide a selective growth advantage. Observations made in this study [9] highlight the division of driver mutations into early (initiating) drivers and late (progression) drivers. The tumor-initiating driver, apparently the loss of *CDKN2A* in the GBMs investigated in this study, is expected to be present in all tumor cells. The late (or progression) drivers, such as *EGFR*, *PDGFRA* and *MET* in this study, can be heterogeneous within a single tumor.

The authors [9] also identified a case of salivary duct carcinoma with amplification of *ERBB2* and *PDGFRA* in separate subclones, suggesting that the mosaic amplification of multiple RTKs is not limited to GBMs. The coamplification of multiple RTKs in GBMs and other tumors may demand treatments targeting multiple RTKs. However, if subclones with amplification of different RTKs depend on each other for survival and growth, hitting one partner may be sufficient to disrupt tumor growth. A better understanding of the complex relationships among tumor subclones with amplifications of different RTKs and other key genetic alterations would guide the selection of the weakest link to target, thus achieving the maximal therapeutic benefits with the fewest side effects.

Competing interests
The authors declare that they have no competing interests.

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