acquire resistance to cisplatin and PARP inhibitors. Aside from allowing clinicians to anticipate such a response, if a patient should carry a tumor with this class of mutation, what could be the possible mechanism that gives rise to this? We do not know; however it does seem that these tumors tolerate the accumulation of DNA damage or that they are able to switch on compensatory repair pathways that require a BRCA1 function that is not affected by this mutation, or a combination of both. All in all, these studies provide new murine models for mutant *BRCA1*-driven breast carcinogensis, providing intriguing new results and an excellent platform for future studies.

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Many Tumors in One: A Daunting Therapeutic Prospect

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In this issue of *Cancer Cell*, Snuderl and coworkers demonstrate intratumoral genetic heterogeneity in glioblastoma based on in situ amplification of distinct genomic loci within individual cells in a mutually exclusive pattern. These findings may herald trouble for current targeted therapies but provide insights for future treatment strategies.

Glioblastoma multiforme (GBM) is among the most lethal of all human cancers with a median survival of about 14 months despite aggressive surgical resection and adjuvant chemotherapy with radiation (Stupp et al., 2005). Nevertheless, the clinical course of some GBM patients can be highly variable, which may, in part, be attributable to the recent identification of a least four molecular subtypes of GBM (Verhaak et al., 2010). These molecular subtypes, however, do not explain the diverse array of pathological findings within any given GBM including morphologically distinct tumor cells with various patterns of growth (e.g., bulky tumor to single cell invasion along white mater tracks) and variable effects on the host tissue that are pathognomonic of the disease and responsible for the designation "multiforme" (Bailey and Cushing, 1926).

One potential explanation for the diversity of intratumoral findings is that the phenotype of the GBM clonogenic or stem cell is plastic and variable, affected by both intracellular (e.g., stochastic) and extracellular (e.g., microenvironmental) stimuli. As a result, the variable phenotypes may represent different degrees of aberrant differentiation of a cancer stem cell. A more complex and therapeutically challenging explanation for the clinical and pathologic variability of GBMs, however, is that we are actually dealing with "different tumors" within a given patient.

The term "tumor heterogeneity" can be defined as the presence of subclones of cells, within a given tumor, with different genetic aberrations that mediate divergent biology that define the natural history of that particular tumor (Navin et al., 2011; Yachida et al., 2010). Although not a new idea, the recent advent of high-throughput molecular and genetic methodologies has begun to explore the nature of this phenomenon. To that end, several recent papers have used high-resolution chromosomal copy number analysis and next generation sequencing to show a range of genetically divergent tumor cell clones within leukemia (Stephens et al., 2011), breast cancer (Navin et al., 2011), and pancreatic cancer (Yachida et al., 2010).

In this issue of *Cancer Cell*, Snuderl and co-workers (2011) use fluorescence

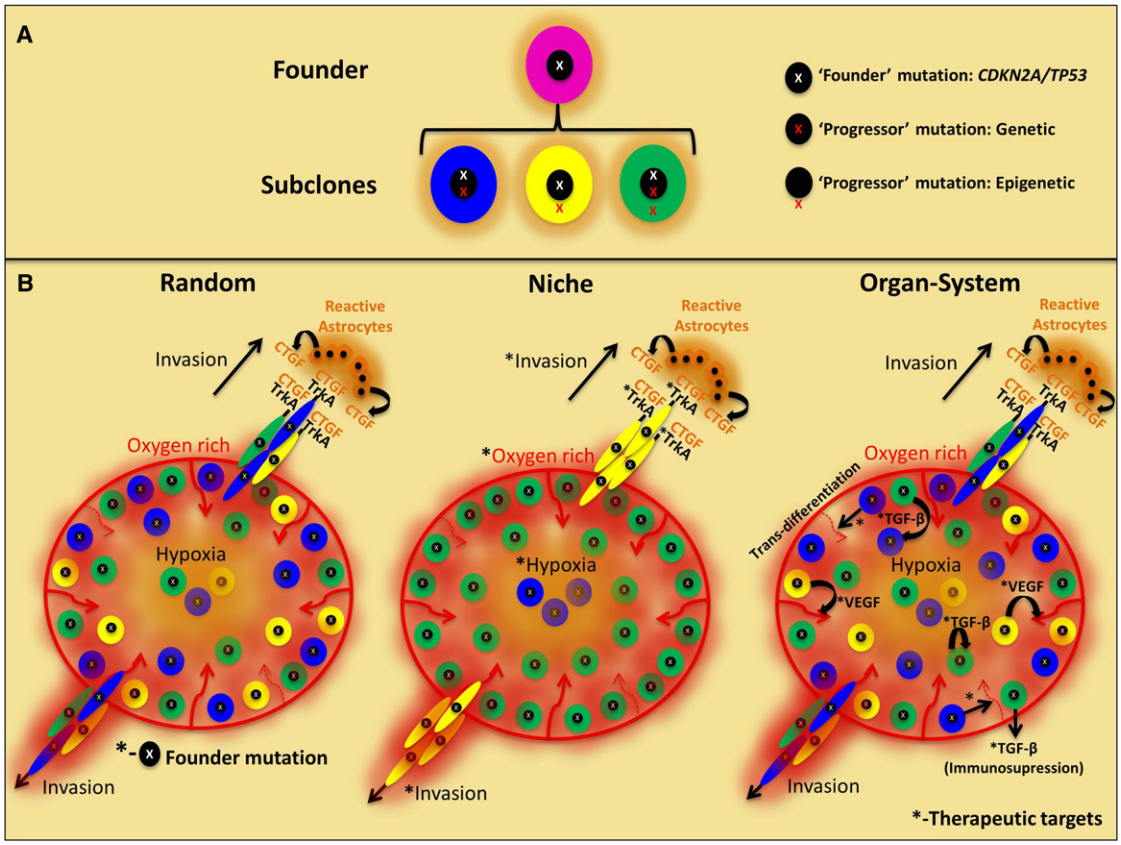

Figure 1. Intratumoral Clonal Heterogeneity
Three possible but nonmutually exclusive scenarios for interaction between subclones within tumors.
(A) "Founder" cell undergoes "progressor" mutations that generate three subclones: blue, yellow, and green.
(B) Random distribution of subclones within tumor body showing no association with specific niche or function. Niche-based distribution of subclones within the tumor body: hypoxic region (blue), oxygen rich regions (green), and invasive front (yellow). Organ system paradigm or function-based distribution of subclones within tumor body. Autocrine and paracrine TGF-β secretion (green), VEGF secretion (yellow), and endothelial cell trans-differentiation (blue). *Scenario-specific therapeutic targets.

in situ hybridization (FISH) to "genotype" individual tumor cells within a given GBM by probing for three of the most commonly amplified loci that encode receptor tyrosine kinase (RTK) in GBM (EGFR, PDGFRA and c-MET). Their results show that individual RTK and combinations of RTKs are amplified in different cells within a given tumor in a mutually exclusive pattern. Furthermore, they find evidence that many of the cells within these subclones express phosphohistone H3, indicating a proliferative state. Finally, they present evidence suggesting that the subclones are descendants of a common precursor based on the presence of *CDKN2A* deletion and/or a common *TP53* mutation.

The demonstration of these genomic subclones in GBM raises a plethora of new questions such as the number of subclones within a given tumor, the genomic relatedness of one clone to another (i.e., the "evolutionary tree" of the clones), and the mechanistic basis for the genomic heterogeneity. Answers to these questions may be tumor specific and will require methodologies with much higher genomic resolution than those used by Snuderl and co-workers such as next-generation sequencing (Navin et al., 2011).

One of the most important questions regarding these subclones is their individual and/or combined functional role during the pathogenesis of the tumor. One possibility is that each subclone is randomly generated and selected for through competition with some subclones and/or passive coexistence with others rather than direct interaction between subclones. A second possibility is that each subclone is selected for by its genetic and epigenetic predisposition to optimally occupy a specific niche within the variable microenvironment of the tumor. For instance, one subclone may thrive in the relatively oxygen rich environment of a highly angiogenic portion of the tumor, whereas another subclone may be preferentially suited for hypoxic regions (Figure 1). Likewise, other subclones may be enriched with signaling pathways allowing efficient invasion and migration into normal surrounding cerebral cortex coopting pre existing cerebral vasculature along the way (Figure 1). Finally, a third but not mutually exclusive possibility, envisions the GBM as an "organ system" and each subclone serves as a "tissue type" with a unique function within the life cycle of the tumor organ system. For instance, recent evidence suggests that some GBM stem cells can trans-differentiate into endothelial cells and contribute to tumor vasculogenesis (Ricci-Vitiani

et al., 2010). Other subclones may preferentially secrete angiogenic factors to support these new blood vessels (Figure 1). Also, some GBM subclones may express specific mitogenic receptors such as Notch (Wang et al., 2010), whereas others may express their ligands in a paracrine manner (Figure 1). Finally, subclones may also support the entire tumor through the expression of cytokines such as TGF-β that not only enhance GBM stem cell self-renewal but may provide an immunosuppressive microenvironment that protects the tumor from host immune surveillance and attack (Wu et al., 2010) (Figure 1).

The reality of both intertumoral and intratumoral heterogeneity have major therapeutic implications. The recent elucidation of four different gene expression-based GBM subtypes (e.g., intertumoral heterogeneity) (Verhaak et al., 2010) gives hope for being able to ultimately identify "targeted treatment" for molecular targets and pathways specific for those subtypes and to enrich for patient populations likely to benefit from any new targeted therapy in future clinical trials. By contrast, the concept of intratumoral genomic heterogeneity poses a daunting therapeutic prospect and successful strategies to target multiple subclones will depend on how those subclones interact within a tumor. For example, if the subclones are functionally dependent on each other within the context of the tumor "organ system" paradigm discussed above, then it may be possible to target one or two of the most critical subclones with the hope that their extinction will lead to the collapse of the tumor as a whole (Figure 1). Alternately, if the subclones exist and thrive in particular microenvironmental niches, then it may be possible to use a combination of agents that target those environments such as antiangiogenic agents, hypoxic drug sensitizers, and anti-invasion molecules (Figure 1).

Most problematic, however, is the scenario where subclones exist largely independent of each other making their elimination profoundly more difficult. In such a case, the hope would be to identify "founder mutations" (Yachida et al., 2010) that were present in the initial clonogenic tumor population and inherited by subsequent divergent subclones. Indeed, Snuderl and co-workers found evidence that deletion of *CDKN2A* and *TP53* mutations may be such founder mutations in the GBMs studied (Snuderl et al., 2011). Even if such founder mutations are found in all subclones, however, successful therapeutic targeting of these mutations requires that the divergent subclones retain dependence on these aberrant pathways and that the subsequent "progressor mutations" that define these divergent subclones do not instill functional independence from the initial founder mutations. If the latter were true, therapeutic success here would entail targeting each individual subclone, something that would be theoretically possible only if a few tumorigenic subclones exist. If, however, numerous mechanistically independent tumorigenic subclones coexist within a tumor, the prospect of identifying, selecting, and administrating multiple targeted therapies simultaneously to any given patient becomes much more challenging. In such a situation, it may be necessary to resort to our old paradigm of developing cytotoxic rather than targeted therapies. For our patients' sake, we can only hope this does not turn out to be the case given the disappointing record with such agents in the past (Stupp et al., 2005).

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