

IN THE SPOTLIGHT

Clonal Evolution: Multiregion Sequencing of Esophageal Adenocarcinoma Before and After Chemotherapy

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Summary: It is possible to decipher the clonal architecture of a tumor and the sequence in which cancer clones acquire genomic alterations through multiregion sequencing (M-seq). Serial evaluation of tumor specimens through M-seq can provide valuable information on the molecular basis of resistance to therapy. *Cancer Discov*; 5(8): 796–8. ©2015 AACR.

See related article by Murugaesu et al., p. 821 (3).

Cancer evolution can be viewed as a series of clonal expansions, where subclones carrying alterations that offer a survival advantage dominate and replace less fit subclones (1, 2). Tumors in which subclones fail to completely replace their predecessors or evolve in parallel show varying degrees of heterogeneity in their clonal architecture. Using samples obtained from different regions of a tumor [multiregion sequencing (M-seq)], it is now possible to study the clonal architecture and evolution in great detail through next-generation sequencing techniques (3–5). When the clonal architecture of a tumor is represented as a phylogenetic tree, founding clone alterations map to the “trunk,” whereas subclonal events (derived from the founding clone) map to the “branches.” Although spatial heterogeneity in solid tumors has been well studied in a wide variety of malignancies using multiregion sampling of primary tumors, very few studies to date have analyzed temporal heterogeneity, particularly following exposure to chemotherapy.

In this issue of *Cancer Discovery*, Murugaesu and colleagues report results from M-seq of 40 samples obtained from 8 patients with esophageal adenocarcinoma, before and after chemotherapy (3). The study offers insight into the genomic landscape, mutational processes, and evolution of these cancers prior to and following cytotoxic therapy. As observed in previous studies, there was an enrichment of thymine to guanine (T>G) mutations in a cytosine-thymine-thymine (CTT) context as early events—a mutational footprint of DNA damage sustained by oxidative stress from gastric reflux. *TP53* mutations, chromosomal instability, and genome duplication were observed in nearly all samples as early events. These findings support the results from a previous longitudinal case-control study that showed increased chromosomal instability, genome duplication, and genome

diversity in samples obtained from patients with Barrett’s esophagus as they approached the diagnosis of esophageal adenocarcinoma (6).

Nearly 47% of putative driver events in esophageal adenocarcinoma reported in the study by Murugaesu and colleagues occurred in the subclones. A greater number of driver events were identified through M-seq compared with single biopsy for each of the tumors studied (3). Furthermore, a number of putative driver mutations identified previously using single-region sequencing were present only subclonally in this study using the M-seq approach (7). Mutations that appeared clonally dominant in one region of the tumor were entirely absent from other regions, projecting an “illusion of clonality” (3, 4). Identifying such mutations can be helpful because targeting subclonal alterations would likely be less effective compared with therapies targeting true founding clone alterations. Assessing the fraction of subclonal alterations at different tumor locations also allows assessment of the extent of intratumor heterogeneity (ITH) in a cancer. Using this approach, a strong correlation between the ITH index (mean of the proportion of heterogeneous mutations relative to total number of mutations) and response to chemotherapy was reported in this study (3). ITH may be not just predictive of chemotherapy response but also a prognostic factor. A recent study of lung cancer demonstrated that tumors with a large number of subclonal mutations were associated with a higher likelihood of postsurgical relapse (5). However, larger well-designed prospective studies are required to confirm the potential utility of ITH as a predictive or prognostic biomarker.

The clonal composition of a cancer varies temporally in response to the selection pressure endured by the cancer cells secondary to natural and therapy-related events (2, 4, 5). For instance, in a study of lung cancer genomes from smokers, mutations acquired early were enriched for the typical cytosine to adenine (C>A) transversions induced by tobacco smoking, whereas later events were enriched for apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deaminase-associated cytosine to thymine (C>T) and cytosine to guanine (C>G) mutations (4, 5). These data suggest that although early initiating events are related to tobacco carcinogens, intrinsic cellular processes may shape further evolutionary changes in lung cancer.

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Similarly, although early mutations in esophageal adenocarcinoma showed a characteristic gastric reflux-related signature, later mutations showed a relative increase in C>T mutations in a CpG context—a mutational footprint of deamination, aging, and cell turnover (3). A better understanding of these processes is essential to design rational approaches for cancer therapy and prevention.

Sequencing tumor samples obtained before and after systemic therapy can provide crucial insights into how these interventions shape the cancer genomic landscape and lead to emergence of treatment resistance (8). Paired pre- and post-chemotherapy samples were available for analysis for 5 of 8 patients in the study reported by Murugaesu and colleagues (3). Combined analysis of the post-chemotherapy samples showed enrichment in C>A mutations in a cytosine-cytosine (CpC) context, a mutation pattern that has been previously described in *Caenorhabditis elegans* following platinum exposure, and a trend toward an increased number of indels (9). There was no significant change in the mutational burden of these tumors following exposure to therapy. Post-chemotherapy regions clustered separately from the pre-chemotherapy regions in two samples that showed a poor response to chemotherapy, indicating a common genomic event in a shared ancestor that conferred resistance to treatment. The other three tumors demonstrated pre- and post-chemotherapy regions clustering together, indicating the persistence of pre-chemotherapy founding clones. Persistence of the founding clones and continued presence of driver oncogene amplifications through neoadjuvant chemotherapy explain the suboptimal responses to chemotherapy in these tumors and present a clear opportunity to develop molecularly targeted therapies directed against these amplified oncogenes.

Similar observations were previously reported in a study that compared the genomic analyses of the samples from 8 patients with acute myelogenous leukemia (AML) procured at the time of their initial presentation and at the time of relapse (8). Relapsed AML was a consequence of either the founding clone gaining additional relapse-specific mutations or expansion of a treatment-resistant subclone. Initial therapy failed to eradicate the founding clone in all eight relapsed cases in this study. Apart from serving as a selection pressure that leads to the emergence of resistant clones, genotoxic chemotherapy can also serve as a source of genomic instability capable of shaping further clonal evolution. For instance, in the AML study, relapse-specific alterations showed an increased number of transversions as a result of exposure to genotoxic therapy (8). Similarly, a temozolomide-related mutation signature in driver genes has been reported in recurrent glioblastomas following treatment with temozolomide (10). Although enrichment for the platinum signature was reported in esophageal adenocarcinomas following chemotherapy in the present study, little is known about the specific genes and molecular pathways that are affected by this mutational footprint and to what extent these changes contribute to disease progression.

Although the study by Murugaesu and colleagues offers valuable insights into the dynamic nature of mutational processes in the evolution of esophageal adenocarcinoma, the role of ITH as a predictive biomarker, and the effect of

neoadjuvant chemotherapy on the clonal architecture of these tumors, additional studies are required to confirm these findings (3). Overall, findings from this study emphasize the importance of serial multiregional sequencing of tumors following therapy to fully understand the molecular mechanisms underlying disease progression. The presence of alterations that project an illusion of clonality and multiple subclonal driver events in most malignancies suggest that the proportion of the cancer cells with driver gene alterations must be considered in assessing responses to targeted therapies (11).

Although the findings from this and other studies make a compelling case for routine implementation of M-seq, procuring core biopsies from multiple regions of a lesion or from multiple sites in patients with solid tumors poses a significant challenge in the clinic. Developing radionuclide-imaging modalities that target and enable the quantification of tumor-expressed products, and the ability to sequence small amounts of DNA obtained through fine-needle biopsies as opposed to large-core biopsies, represent attractive, less invasive alternatives (12). Studies that incorporate “liquid biopsy” approaches are also likely to provide useful information regarding known mechanisms driving treatment resistance (like *EGFR*^{T790M} in *EGFR*-mutated non-small cell lung cancer) without the need for multiple invasive biopsies. However, longitudinal and multiregion sequencing of tumors can provide temporal and spatial insight into the serial evolution of the genomic (including neoantigenic) landscape of solid tumors, offer valuable insights into the predictors of response, and elucidate the mechanisms of resistance to therapies.

Disclosure of Potential Conflicts of Interest

R. Govindan is a consultant/advisory board member for Bayer, Boehringer Ingelheim, Celgene, Clovis, Helsinn, Merck, Pfizer, and Roche. No potential conflicts of interest were disclosed by the other author.

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