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Studying clonal dynamics in response to cancer therapy using barcoding

The emergence of resistance to targeted cancer therapeutics is a significant problem clinically and is generally believed to result from genetic alterations in tumor cells. Whether resistance exists within a subpopulation of a tumor prior to treatment or develops de novo during treatment is a fundamental question that may significantly impact therapy. Answers to this question are elusive, as mutation assays generally lack the sensitivity to detect small (less than 0.1 percent) subpopulations. The authors described a novel approach to this question and provided evidence supporting the presence of a resistant population prior to therapy. They used cellular barcoding, in which lentiviral infection is used to insert a short (30 bp) semi-random DNA sequence into the genomes of a population of cells, to uniquely label approximately 106 individual cells. Following expansion, progeny of each of these labeled cells can be detected by next-generation sequencing. Using an erlotinib-sensitive non-small cell lung cancer cell line, the authors demonstrated that prolonged treatment with erlotinib led to the outgrowth of a resistant population of cells in each of eight replicate platings. Significantly, the same small group of barcoded cells was enriched in each replicate: 90 percent of enriched barcodes were present in more than one replicate and 40 percent were present in all eight replicates. This strongly suggests that a subpopulation of resistant cells, representing approximately 0.05 percent of the total, was present in the original population. Had the resistance developed de novo during treatment, it is extremely unlikely that the same group of barcoded cells would have been enriched in each replicate. In a second set of experiments, cells were treated with erlotinib and the c-Met inhibitor crizotinib. This combination treatment resulted in enrichment of a much smaller subpopulation (0.0005 percent) of only five barcodes, suggesting that the pre-existing erlotinib-resistant subclones are dependent on c-Met and were eliminated by treatment with crizotinib. In support of this, the erlotinib-only treated population was shown to have MET amplification, while the erlotinib plus crizotinib treated population did not. Using this same approach in a chronic myeloid leukemia cell line, the authors provided evidence of the pre-existence of a small kinase inhibitor-resistant subpopulation that is enriched upon treatment. Finally, with data from these experiments, they developed a mathematical model capable of recapitulating the observed growth dynamics of resistant populations and demonstrated that de novo mutations could not account for their observations. These experimental approaches, while not directly applicable in a clinical setting, provide novel and valuable information regarding the genesis of therapeutic resistance in tumor cell populations. These data will likely inform the rational development of more effective frontline therapeutic strategies, particularly those that involve targeting multiple resistance mechanisms.

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