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RESEARCH HIGHLIGHT

Resistance to EGFR blockade in colorectal cancer: liquid biopsies and latent subclones

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Two recent papers identify *KRAS* activation as a mechanism of acquired resistance to EGFR blockade in colorectal cancer. In doing so, they suggest that resistance to single-agent EGFR blockade will be unavoidable because these alterations exist as latent subclones within the tumor even prior to the initiation of therapy.

Among the important molecular alterations in colon cancer is constitutive activation of the epidermal growth factor (EGFR) receptor tyrosine kinase, which promotes constitutive cellular proliferation and tumor progression. Recently, cetuximab and panitumumab, two monoclonal antibodies against EGFR, have shown promising results in the treatment of metastatic colorectal cancer (mCRC) [1, 2]. However, only a subset of patients respond to these anti-EGFR therapies. Strikingly, mutations in *KRAS* — a signaling effector downstream of EGFR — predict a lack of response to EGFR blockade [3, 4]. In other words, in the presence of a downstream *KRAS* activating mutation, upstream signaling by EGFR is dispensable. This phenomenon — in which a patient never responds to a therapy — is termed intrinsic (or *de novo*) resistance.

However, even among patients with *KRAS* wild-type tumors, the efficacy of EGFR blockade is limited. Not all KRAS "wild type" patients respond, and those that do inevitably develop resistance to anti-EGFR therapy within a matter of months. This latter phenomenon of acquired — as opposed to intrinsic — drug resistance raises several important questions. What are the mechanisms of acquired resistance? Are they related to the observation of *KRAS* as a biomarker of intrinsic resistance? More generally, how does acquired resistance arise? Are new resistance-causing alterations generated during the course of treatment? Alternatively, do they pre-exist at low frequency in the initial tumor, expanding during the course of treatment due to the selective pressure of the therapy?

Two recent studies in *Nature* begin to address these and other questions [5, 6]. In a paper by Misale *et al.* [5], colorectal cancer cell lines initially sensitive to cetuximab were grown under continuous cetuximab treatment. The cetuximab-resistant daughter lines that ultimately emerged had gained either *KRAS* amplification or *KRAS* activating mutations — alterations sufficient to confer cetuximab resistance. Intriguingly, the authors showed that these alterations actually existed prior to treatment at low frequency in the parental cell lines, which suggests that the selective pressure of cetuximab treatment led to expansion of this previously latent sub-population and the emergence of resistance. To establish the clinical relevance of these findings, the authors queried whether *KRAS* alterations could be detected in previously *KRAS* wild-type mCRC patients following failure of cetuximab therapy. In 8/11 patients, cetuximab-resistant tumors had gained either *KRAS* mutation or amplification; moreover, by analyzing plasma samples for circulating tumor DNA (ctDNA), the authors were able to detect emergence of *KRAS*-mutant alleles up to 10 months before radiographic evidence of progression.

In contrast to Misale *et al.*'s translational approach, a study by Diaz *et al.* [6] began with a cohort of *KRAS* wild-type mCRC patients who received panitumumab. Starting prior to pantiumumab therapy, serum samples were collected from each patient monthly until disease progression. These so-called "liquid biopsies" enabled the investigators to establish that, among patients with initially *KRAS* wild-type tumors, 38% developed detectable *KRAS* mutations during the course of therapy.

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However, whereas a conventional post-relapse tumor biopsy allows only an after-the-fact analysis of resistance, this longitudinal collection of serum samples allowed the investigators to monitor the evolution of resistance over time. By relating computed tomography measurements of tumor volume to ctDNA read counts, and then monitoring the increase in *KRAS*-mutant ctDNA reads over time, the authors estimated the growth rate of these panitimumab-resistant subclones. This information, together with the length of time between the initiation of panitumumab therapy and first detection of *KRAS*-mutant ctDNA reads, allowed the authors to calculate that *KRAS* mutations preceded the initiation of therapy.

Cumulatively, these studies offer several insights. First, while it was previously known that *KRAS* mutations predicted intrinsic resistance to anti-EGFR therapy, the studies described here identify a causal role for *KRAS* in acquired resistance. Second, the use of ctDNA rather than conventional biopsy circumvents two critical problems: spatial bias of sample collection, and limited availability of matched pre-treatment and post-treatment tissue. This advantage was critical to the retrospective time course approach taken by Diaz *et al.* [6]; it also suggests tantalizing prospective applications for monitoring patients during therapy and anticipating clinical relapse. Third, these studies take markedly different approaches to the same question yet arrive at strikingly similar answers. Misale *et al.* [5] began with *in vitro* selection, followed by validation of their findings in patients; in contrast, Diaz *et al.* [6] began directly with patient samples and proceed to develop a mathematical argument for the pre-existence of *KRAS*-mutant resistant subpopulations. The convergence of these distinct approaches lends significant weight to both groups' results. Finally, both papers support the premise (now well-established) that many resistance mutations exist prior to the initiation of therapy. In this sense, they describe a mechanistic convergence of intrinsic and acquired resistance: resistance can simultaneously be acquired (from the perspective of the behavior of the overall tumor burden) and intrinsic (in that it was present early on, within a latent subclone of the tumor). Unfortunately, this also means that relapse following single-agent targeted therapy seems virtually assured, since treatment-resistant cells may be present in the tumor even prior to the initiation of therapy.

While these studies lay a solid foundation for understanding acquired resistance to EGFR blockade in mCRC, much future work remains. *KRAS* activation is unlikely to be the only mechanism of acquired resistance in this context. Indeed, both papers provide evidence that only a minority of the tumor cells within a relapsing lesion actually harbored mutant KRAS. Conceptually, this could imply that the relapsing subclone somehow "drives" rescue of the remaining "passenger" tumor tissue in a paracrine fashion, or that other mechanisms of resistance are also at play in the tumor as a whole. Under either model, future work must define the dynamics of interaction between these multiple subclones within a tumor as well as between the tumor and the circulation — particularly as ctDNA gains importance as a diagnostic tool. It will also be necessary to catalog the full spectrum of alterations that are sufficient to cause resistance. In particular, while the current studies focused on DNA-level changes — mutation and amplification — other mechanisms such as gene expression changes and epigenetic alterations must also be explored. The potential for stromal contribution to resistance adds yet another layer of complexity to this picture. Ultimately, as a more comprehensive view of mechanisms of resistance to EGFR blockade becomes available, it should become possible to group them by mechanism, identify therapeutic strategies to overcome these various classes of resistance, and achieve more durable responses for patients with mCRC.

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