Clinical Cancer Research

Paracrine Network: Another Step in the Complexity of Resistance to EGFR Blockade?

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Increased secretion of EGFR ligands amphiregulin and TGFα by limited *KRAS*-mutant clones is suggested as a paracrine resistance mechanism to anti-EGFR antibodies in colorectal cancer models. These findings are biologically sound but need to be replicated, including in the clinical setting, to foresee whether they are clinically relevant and therapeutically exploitable. *Clin Cancer Res; 20(24); 6227–9. ©2014 AACR.*

In this issue of *Clinical Cancer Research*, Hobor and colleagues (1) address the concept of resistance to EGFR inhibition in a novel manner. EGFR addiction, present in a subset of colorectal cancer cells, has been targeted with anti-EGFR mAbs for more than 10 years. Their therapeutic positioning in metastatic colorectal cancer has evolved from third-line monotherapy or doublets (panitumumab alone, cetuximab alone, or in combination with irinotecan) into first-line treatment in combination with the two most active chemotherapeutic regimens, *FOLFIRI* or *FOLFOX*. This therapeutic setting transition has been established as a consequence of a series of clinical trials designed for that purpose. More importantly, molecular markers have been introduced into this colorectal cancer setting, confirming that exon 2 *KRAS* hotspot mutations (mainly in codons 12 and 13) are predictive biomarkers for the lack of efficacy of anti-EGFR mAbs, and can, in fact, be harmful when combined with oxaliplatin-based chemotherapy. More recently, further subanalyses of the most relevant clinical trials have provided evidence of an additional predictive effect for other less frequent mutations in *KRAS* exons 3 and 4 and *NRAS* exons 2, 3, and 4 (2–4). With this extended *RAS* analysis, the benefit of the addition of anti-EGFR mAbs to standard first-line chemotherapy for the all-*RAS* wild-type population has translated into HRs for progression-free survival and overall survival (OS) of 0.72 and 0.78 in the phase III PRIME and 0.56 and 0.69 in the phase III CRYSTAL studies, respectively. In terms of absolute median OS gain, the addition of anti-

EGFR therapies increases survival by 5.8 to 8.2 months over the control population receiving only chemotherapy (2, 4).

Since the initial seminal publications by Misale and colleagues (5) and Diaz and colleagues (6), several studies have unequivocally shown that one of the most important mechanisms of secondary (acquired) resistance in patients with metastatic colorectal cancer to anti-EGFR mAbs is driven by the selection of cell clones that bear *RAS* or *RAF* mutations. It is notable that these mutant alleles, found in both tumor specimens and plasma samples when patients progress to previously active anti-EGFR treatments, are only observed in low numbers, suggesting that only a small fraction of tumor cells (0.4%–17%) bear *RAS/RAF* mutations (5). On the basis of this observation, Hobor and colleagues (1) tested the hypothesis that protective paracrine interactions exist between *RAS*-mutated (resistant) clones and the wild type (sensitive) clones in preclinical colorectal cancer cell models. In an elegant set of experiments using three paired cetuximab-sensitive and -resistant cell lines, the authors demonstrate that resistant clones are able to render wild-type clones more resistant to EGFR blockade. First, resistant cells *in vitro* produce more paracrine factors than their sensitive counterparts. Second, in spheroids derived from both resistant and sensitive OXCO colorectal cancer cell lines, resistant cells support the growth of the sensitive counterpart even in the presence of suppressive concentrations of the anti-EGFR mAb cetuximab. Third, these observations occur in the presence of complex regulation of the EGFR ligands. On the one hand, cetuximab-resistant cells secrete higher concentrations of TGFα and amphiregulin (AREG), although only AREG levels are further stimulated by cetuximab exposure in this model. On the other hand, TGFα, but not AREG, reduces the inhibitory effect of cetuximab in a dose-dependent manner. The observation that ligands for receptor tyrosine kinases can sustain resistance to targeted therapies has previously been proved for *EGFR*-mutated lung cancer cells (7). Concordantly, and based on their *in vitro* results, Hobor and colleagues (1) propose that microenvironmental concentrations of EGFR ligands—preferentially secreted by the subset of mutated cells—may well rise to finally counteract the inhibitory concentration of cetuximab in both mutated

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doi: 10.1158/1078-0432.CCR-14-1615

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and wild-type tumors. The originality of the approach and the novelty of the observations of Hobor and colleagues (1) must be acknowledged. Nonetheless, to grasp the potential impact of these exciting observations, recent clinical observations and a number of methodologic issues must be taken into account.

As for clinical findings, the variability of the effect of all-*RAS*–extended selection is high between trials. This can be explained by heterogeneity in sample sizes and mutation assays implemented in the distinct trials, each one with differing analytical sensitivity, ranging from 0.01% to 5%–10% (2–4). Some are more sensitive and pick up low numbers of mutated clones. However, the optimal sensitivity cutoff point for modeling prediction of response is currently unknown, with recently presented data suggesting that beyond a certain threshold the model does not improve and may even worsen predictive power (2). This could be explained by a minimum number of resistant clones beyond which their potential treatment-selected outgrowth is not relevant enough to influence the prediction modeling of the overall progression timing pattern, at least in the first-line setting of anti-EGFR mAbs combined with chemotherapy.

However, in this setting, a theoretically better patient selection does not improve individual long-term outcomes for the treated populations as patients who initially respond will ultimately become resistant and progress at some point. Paradoxically, the outgrowth of an initially small number of resistant clones, which does not affect the global prediction model, may have a relevant biologic impact on the subset of previously responsive individual patients. According to Hobor and colleagues (1), these rare mutated clones could partially exert their acquired resistance effect in a more subtle way through the autocrine and paracrine secretion of EGFR growth factors or ligands, as these authors suggest in their article.

This intriguing and somewhat speculative scenario is apparently at odds with some previous observations. Briefly, elevated gene expression of EGFR ligands AREG and epiregulin (EREG) has been consistently proposed for prediction of anti-EGFR blockade as a surrogate for EGFR addiction in patients with metastatic colorectal cancer treated with anti-EGFR mAbs. In 2007, Khambata-Ford and colleagues (8) reported that increased levels of EREG and AREG mRNA, but not of TGFα, were associated with better response to EGFR blockade irrespective of *KRAS* mutation status. These observations were further substantiated by Baker and colleagues (9) and Tabernero and colleagues (10), with the latter also suggesting that high TGFα expression was associated with a negative predictive effect. From a different perspective, Tian and colleagues (11) constructed a combined oncogenic pathway signature allowing the identification of patients with an active EGFR signaling pathway that predicted resistance to EGFR blockade irrespective of the mutation status. It is noteworthy that elevated levels of AREG and EREG clustered with the tumors harboring the wild-type signature that were sensitive to anti-EGFR blockade. In this report, no data on TGFα expression were available. In short, data on expression profiles obtained from tumor biopsies display elevated levels of EREG and AREG mRNA in sensitive tumors, likely reflecting the status of a significant proportion of the tumor cells analyzed. It is not straightforward to anticipate, in the presence of an evident overexpression of EGFR ligands before anti-EGFR blockade, the impact of a minority of mutant tumor cells further overexpressing the same or other ligands. Also relevant to mention is that none of the above-mentioned studies have analyzed protein levels in tumor specimens using sensitive, quantitative techniques precluding a direct, meaningful comparison between *in vitro* data and tumor analysis before and after treatment.
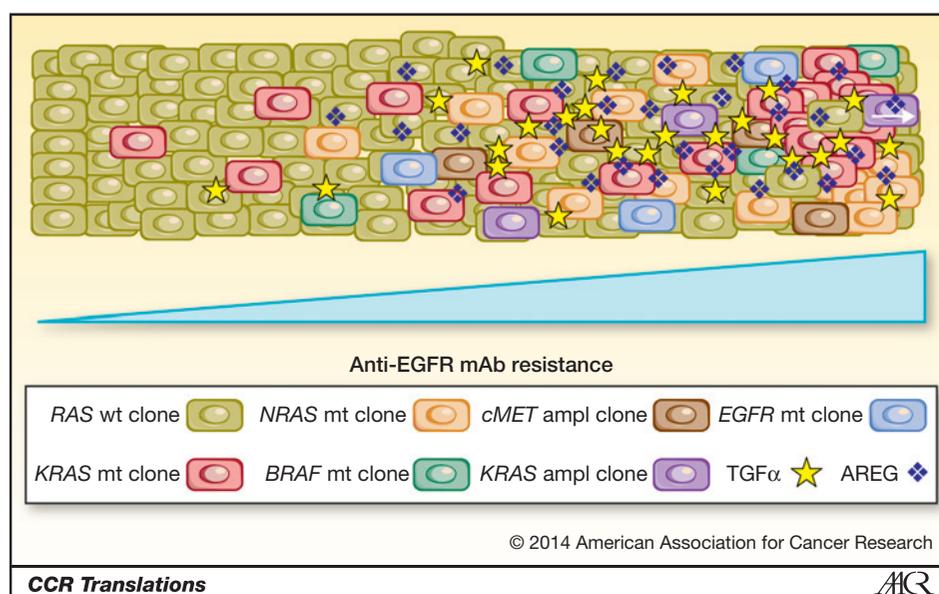

Figure 1. Mechanisms of acquired resistance to anti-EGFR mAbs in colorectal cancer cells. Ampl, amplified; mt, mutated; wt, wild type.

Regarding methodologic contributions, the *in vitro* spheroid model that mimics the complex cross-talk between resistant and sensitive clones is an interesting approach. Nonetheless, incomplete cell labeling and the fact that this approach only worked for one cell line limit its relevance. There is also significant heterogeneity among the three cells lines indicating a wide range of differences in EGFR signaling dependency. This heterogeneity is reinforced by the results of Khambata-Ford and colleagues (8), who reported that the cetuximab-resistant *KRAS*-mutated HCT116 colorectal cancer cell line overexpressed EREG, but not AREG or TGFα, after exposure to cetuximab. Thus, it may well be that a truly consistent ligand expression pattern will never emerge. Finally, the authors restricted their observation to the *in vitro* setting.

In addition to *RAS* and *BRAF* mutations, other mechanisms of clinically acquired resistance have been described. Acquired resistance to EGFR blockade has been linked to the emergence of polyclonal *KRAS*, *NRAS*, *BRAF*, and *EGFR* mutations in colorectal cancer tumor specimens and circulating tumor DNA from patients (12), along with other alternative mechanisms, such as *cMET* or wild-type *KRAS* amplification (5), that have been published in a recent series of breakthrough articles (Fig. 1).

Until *in vivo* observations are reported, we should rely on the clinical observations of adequately powered studies and consider these results very promising at the mechanistic level, although of yet unknown clinical relevance. However, in accordance with the distinct genetic alterations previously described in the literature, resistant cells consistently displayed MEK and ERK activation (5), an observation that should be taken into account when designing new clinical trials to evade, delay, or confront acquired resistance to anti-EGFR therapies.

Disclosure of Potential Conflicts of Interest

Authors' Contributions

Conception and design: R. Salazar, G. Capellà, J. Tabernero
Development of methodology: R. Salazar, G. Capellà
Writing, review, and/or revision of the manuscript: R. Salazar, G. Capellà, J. Tabernero

Acknowledgments

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