IN THE SPOTLIGHT

ERBB2 Emerges as a New Target for Colorectal Cancer

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Summary: *ERBB2* mutations and amplifications are present in 7% of colorectal cancers. The presence of these alterations may be a marker of resistance to anti-EGFR therapy and, more importantly, could help identify patients who would benefit from ERBB2-directed therapy. *Cancer Discov; 5(8); 799–801. ©2015 AACR.*

See related article by Kavuri et al., p. 832 (2).

Colorectal cancer is the second most common cause of cancer death in the United States. In the last two decades, advances in the treatment of these cancers have led to a clinically meaningful improvement in overall survival for patients with both metastatic and localized disease. However, progress in treatment has relied primarily on the development of refined combinations of empiric cytotoxic chemotherapy and has largely not been driven by insights into the molecular or genetic features of colorectal cancer. Today, the primary biologic agents used in colorectal cancer therapy are the anti-EGFR antibodies cetuximab and panitumumab and the VEGFA-directed antibody bevacizumab, both given in combination with cytotoxic chemotherapy. Although cetuximab is directed against the EGFR oncoprotein, its use in the second-line setting is guided by the absence of RAS alterations rather than any positive biomarker. Indeed, despite the wealth of molecular research on this disease, there are currently no targeted therapies in colorectal cancer guided by a positive predictive biomarker.

In 2012, The Cancer Genome Atlas (TCGA) Network published the most comprehensive systematic molecular characterization of colorectal cancer to date, revealing genomic amplifications or mutations of the tyrosine kinase–encoding gene *ERBB2* (also known as *HER2*) in 7% of colorectal tumors, suggesting a novel potential therapeutic target for this cancer (1). In both breast and gastroesophageal adenocarcinomas, patients with *ERBB2* amplification are routinely treated with a combination of the ERBB2-directed antibody trastuzumab and chemotherapy. However, the ERBB2 findings of the TCGA colorectal cancer study have not been translated into changes in clinical practice. In this issue of *Cancer Discovery*, Kavuri and colleagues (2) report on the functional significance of *ERBB2* somatic mutations in colorectal cancer. Introduction of the *ERBB2* mutations S310F, L755S, V777L, V842I, and L866M into immortalized mouse colon epithelial cells led to activation of downstream signaling pathways and promoted anchorage-independent cell growth, confirming their transforming capacity, similar to results when many of these mutations were also evaluated in nontransformed breast epithelial MCF10A cells (3). Further experiments in this report also address two specific clinical scenarios where the presence of *ERBB2* mutations may have relevance in guiding therapy: the potential for these mutations to serve as a negative marker for anti-EGFR therapy and, more significant, the potential of these alterations to identify patients who would benefit from ERBB2-directed therapy.

Although the most common known marker of intrinsic resistance to anti-EGFR therapy in colorectal cancer is the presence of *KRAS* mutations, there are a substantial number of patients with *KRAS* (or *NRAS*) wild-type tumors who fail to respond to EGFR antibodies. As the antibodies used in colorectal cancer do not inhibit ERBB2, a sibling of EGFR, the presence of somatic alterations leading to enhanced ERBB2 activity may serve as an additional negative predictor for these antibodies. Previous studies demonstrated that *ERBB2* amplification confers resistance to cetuximab in preclinical models (4, 5). Furthermore, these studies suggested an association between *ERBB2* amplification and clinical resistance to cetuximab. However, this latter analysis was limited by the small number of patients. Kavuri and colleagues present new data suggesting that *ERBB2* mutations may serve as a novel mechanism of resistance to EGFR antibodies, cetuximab and panitumumab, both *in vitro* and *in vivo*, including in patient-derived xenograft (PDX) models. The totality of these results, when coupled to our understanding of the activity of these signaling pathways, suggests that the presence of *ERBB2* somatic alterations may serve as another mediator of resistance to EGFR-targeted therapy in *RAS* wild-type colorectal cancer. This question should be evaluated further in large clinical cohorts, to determine if we could use ERBB2 status to spare additional patients the costs and toxicity of EGFR-directed therapy if there is no reasonable anticipation of benefit.

Besides their role as negative predictors of response to EGFR antibodies, the discovery of recurrent *ERBB2* mutations and amplifications provides an exciting opportunity to develop treatment strategies directly targeting genomic alterations in colorectal cancer. Kavuri and colleagues evaluate the effect of ERBB2-directed therapy in *ERBB2*-mutated colorectal cancer *in vitro* and *in vivo*. They first demonstrate that engineered intestinal cell lines harboring *ERBB2* mutations are highly sensitive to the irreversible EGFR/ERBB2 tyrosine kinase inhibitors neratinib and afatinib, with these inhibitors

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inducing effective inhibition of ERBB2 and its downstream pathways. In addition, xenografts from these cell lines were also sensitive to both neratinib and the combination of neratinib and trastuzumab. In contrast, single-agent neratinib in a PDX harboring *ERBB2* L866M mutation and amplification resulted in tumor stabilization, whereas the combination of trastuzumab and neratinib was required for tumor regression. In another PDX harboring *ERBB2* S310Y mutation, single-agent lapatinib or neratinib had a modest effect, slowing tumor growth. Again, the combination of trastuzumab with either lapatinib or neratinib produced tumor regression. Both PDX models were resistant to trastuzumab alone. Histologic examination of the tumors after treatment revealed decreased cell proliferation and phosphorylation of MAPK and S6 in the tyrosine kinase inhibitor monotherapy and combination group, whereas the trastuzumab monotherapy tumors did not show any evidence of decreased proliferation or downstream pathway inhibition.

Irreversible EGFR/ERBB2 inhibitors have also shown efficacy in preclinical models of *ERBB2*-mutated breast and lung cancers (3, 6), results which have led to clinical trials evaluating neratinib in a variety of solid tumors harboring *ERBB2* mutations (Clinicaltrials.gov identifier NCT01953926). Furthermore, based on encouraging preclinical studies in *ERBB2*-amplified colorectal cancer (4, 5), a phase II clinical trial of dual ERBB2 blockade was conducted and recently presented at the American Society of Clinical Oncology Annual Meeting (7). Patients with *ERBB2*-amplified, *KRAS* exon 2 wild-type, metastatic colorectal cancer who progressed after multiple lines of therapy were treated with the combination of trastuzumab and lapatinib. Of 913 patients screened, 44 (4.8%) were found to be *ERBB2* amplified. Among 23 patients treated with dual anti-ERBB2 therapy, 8 (35%) patients had an objective response. These results are encouraging, particularly in this heavily pretreated population, and warrant further evaluation in earlier lines of treatment of patients with *ERBB2*-amplified colorectal cancer lacking RAS alterations. Similarly, based upon the data presented by Kavuri and colleagues, clinical studies evaluating patients with *ERBB2* mutations are also warranted. Again, based both on the clinical data in *ERBB2*-amplified colorectal cancer and the preclinical studies in this issue, combinations of small-molecule and antibody inhibitors may have the greatest potential.

Key questions remain about the genomic context in which somatic *ERBB2* alterations occur in colorectal cancer. In the original TCGA study, the *ERBB2* alterations were largely in the microsatellite-stable (MSS) population. In the TCGA study, the *ERBB2* mutations seen in patients with microsatellite-unstable (MSI) hypermutated cancers did not fall at the sites of hotspot codons where mutations are established to be activating. Indeed, Kavuri and colleagues' study in this issue focuses on the MSS population. More recently, however, *ERBB2* mutations at such hotspot loci were reported to be recurrent in MSI colorectal cancer. Kloth and colleagues (8) reported that 15% of *BRAF* wild-type MSI colorectal cancers harbor *ERBB2* mutations, with these tumors harboring highly recurrent L755S and V842I substitutions, as well as the new variants L726F, A848T, and G865R. In that study, MSI colorectal cancer cell lines harboring such *ERBB2* mutations were highly sensitive to irreversible EGFR/ERBB2 small-molecule tyrosine kinase inhibitors, highlighting their potential role in the treatment of *ERBB2*-mutated colorectal cancers regardless of mismatch repair status.

An additional and highly clinically and mechanistically relevant observation regarding the role of somatic *ERBB2* alterations in colorectal cancer relates to the lack of exclusivity of these events with canonical *KRAS* mutations. Kavuri and colleagues' study summarized previously sequenced colorectal cancer tumors with *ERBB2* mutation, finding that half (6 of 12) had a co-occurring *KRAS* mutation. Notably, *KRAS* mutations were observed in tumors harboring specific *ERBB2* codon mutations that are both found recurrently in this disease and also functionally demonstrating to be activating, such as the V842I alteration. Similarly, Kloth and colleagues reported that three of 14 of *ERBB2*-mutated MSI colorectal cancers also harbored *KRAS* mutation. Although this co-occurrence could be expected in hypermutated MSI tumors, it is a surprising finding in the nonhypermutated tumors. Whether these coexisting alterations reflect intratumor heterogeneity or if, indeed, individual tumor cells harbor both such events is unknown. Most notably, the functional studies to date have exclusively evaluated mutant or amplified *ERBB2* as a target in tumors or models lacking such *KRAS* alterations. Further studies will be needed to address both the etiology of this co-occurrence and the functional implications. However, even in the case of intratumor heterogeneity, the presence of *KRAS* mutations can readily be predicted to have implications for the treatment of these cancers, as they may confer resistance to ERBB2-directed therapies that are showing initial promise in the *KRAS* wild-type setting.

In conclusion, several lines of preclinical data are now converging to suggest that *ERBB2* activating mutations or amplifications may be new biomarkers that predict resistance to anti-EGFR therapy. More importantly, when these events occur in the absence of *KRAS* mutations, there is increasing enthusiasm that ERBB2 may represent a new target for biomarker-driven therapy in a subset of colorectal cancer patients. Although *in vitro* studies have demonstrated the potential for small-molecule kinase inhibitor sensitivity in these patients, the clinical and *in vivo* data support dual ERBB2 blockade as more effective compared with monotherapy. These observations provide the rationale for further evaluation of combination strategies to target ERBB2 in selected patients with colorectal cancer in clinical trials.

Disclosure of Potential Conflicts of Interest

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