The Moment that *KRAS* Mutation Started to Evolve into Precision Medicine in Metastatic Colorectal Cancer

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See related article by Benvenuti et al., Cancer Res 2007;67: 2643–8.

Almost a decade ago, *Cancer Research* published a translational article by Benvenuti and colleagues (1). It was straightforward with three major pieces of data: (i) among 48 metastatic colorectal cancer (mCRC) patients treated with anti-EGFR antibody, cetuximab or panitumumab, those with *KRAS* or *BRAF* mutations had a shorter time to progression; (ii) a colorectal cancer cell line transfected with mutant *KRAS* acquired resistance to cetuximab; and (iii) two colorectal cancer cell lines with *KRAS* mutation showed increased sensitivity to a combination of cetuximab and a MAPK/ERK inhibitor, PD98059.

The study thus confirmed that activating *KRAS* mutations (codons 12 and 13) impaired the response of mCRC to cetuximab and revealed that the activating *BRAF* mutation (V600E) may also be predictive and that inhibition of the MEK–ERK signaling cascade may improve anti-EGFR treatment in cancer cells with activating *KRAS* (and potentially *BRAF*) mutations. The study led to the advancement of multiple fields, as evidenced by its large number of citations (513 times as of October 10, 2016).

Development of *KRAS* Mutations into a Predictive Marker in Practice

The resistance of mCRCs with activating *KRAS* mutation (codons 12 and 13) to cetuximab was initially reported by Lievre and colleagues, also in *Cancer Research* (2), and its impact has been already commented on in this 75th anniversary series (3). The two reports in *Cancer Research* led to two large-scale studies of advanced colorectal cancer specimens collected in randomized control trials by Karapetis and colleagues and by Amado and colleagues (4, 5).

Both studies convincingly showed that patients with *KRAS* mutations in codons 12 and 13 do not benefit from anti-EGFR mAb therapy, namely cetuximab and panitumumab, with HRs of 0.99 for progression-free survival. Patients with wild-type *KRAS* in codons 12 and 13 showed greatly reduced risk with HR of 0.40 and 0.45. The European Medicines Agency and the Food Drug Administration were swift to bring the request of *KRAS* mutation analysis into the labels of cetuximab and panitumumab in 2008 and 2009, respectively (3, 6).

Knowledge from Basic Science in the Clinical Spotlight

Basic scientists had known from around 1980 that *KRAS* can be activated not only by mutations in codons 12 and 13, but also by those in codon 61 (7). All the mutant proteins showed impaired activity of GTP hydrolysis and resulted in constitutive activation of RAS-mediated signal. Further structural analysis revealed that Gly12 and Gln61 are located at the RAS–GAP binding interface, and their mutations therefore reduced the GTP hydrolysis. Basic scientists had also known the alternative roles of three *RAS* genes, *KRAS, HRAS*, and *NRAS*, for decades (7).

These pieces of knowledge in basic science were translated into clinically important information, namely that *KRAS* mutations at codon 61 also conferred resistance to cetuximab in mCRC patients (8). Also, mCRC patients with *NRAS* mutations had no benefit from anti-EGFR therapy (9, 10).

As *RAS* mutations constitutively activate GTPase and induce transformation and cell proliferation, it is reasonably expected that even treatment-naïve mCRC patients with activating *KRAS* mutations will not benefit from anti-EGFR therapy. Two large-scale clinical studies, PRIME and CRYSTAL trials, were conducted to incorporate anti-EGFR mAbs into the first-line doublet chemotherapy of mCRC patients (10, 11). As expected, anti-EGFR therapy showed clear survival benefit for patients with wild-type *KRAS* and *NRAS*. On the other hand, anti-EGFR therapy brought either worse or no benefit on overall survival [HR = 1.25; 95% confidence interval (CI), 1.02–1.55 for PRIME; HR = 1.05; 95% CI, 0.86–1.28 for CRYSTAL] for patients with mutant *KRAS* or *NRAS*. Now, cetuximab and panitumumab are indicated only for patients with wild-type *RAS* (12).

Multiple Players in One Signaling Pathway

Constitutive activation of the RAS–RAF–MEK–MAPK/ERK pathway (ERK signaling) has been known to be critically involved in *RAS*-mutated cancers (13). It was therefore reasonable that Benvenuti and colleagues pointed out the importance of *BRAF* mutation, in addition to *KRAS* mutations, in the resistance to anti-EGFR therapy. Soon, Di Nicolantonio and colleagues showed that none of 11 *KRAS* wild-type and *BRAF*-mutated mCRC patients responded to cetuximab or panitumumab, whereas 22 of 68 *KRAS* and *BRAF* wild-type mCRC patients did (14). Now, the use of anti-VEGF therapy combined with triplet chemotherapy in the first-line setting is recommended for mCRC patients (12). This history again supports our belief that biological principles revealed by basic cancer research can lead to real clinical practice.

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From Intrinsic Resistance to Acquired Resistance

Acquisition of drug resistance during the course of treatment is a serious but almost inevitable issue. The identification of *KRAS* mutations as a cause for intrinsic resistance of colorectal cancers also contributed to the identification of a mechanism for the acquired resistance. Establishment and analysis of cetuximab-resistant colorectal cancer cell lines revealed that the resistant variants harbored *KRAS* point mutations or amplification, and the findings were confirmed in clinical specimens (15). Also, using circulating tumor DNA, the appearance of mutant *KRAS* was detected in 9 of 24 (38%) patients initially with wild-type *KRAS*, most of whom were initially sensitive to panitumumab but eventually developed the progressive disease (16).

In Vitro Treatment to Human Trials

Most importantly, Benvenuti and colleagues suggested a synergistic effect by the combination of cetuximab and a MAPK/ERK inhibitor. Afterward, Misale and colleagues conducted a systemic knockdown analysis of genes potentially involved in the resistance to anti-EGFR therapy and which were good candidates for therapy, namely *KRAS*, *NRAS*, *HRAS*, *BRAF*, *CRAF*, *MEK1/2*, *HER2*, *HER3*, *PIK3CA*, and *AKT1*, in colorectal cancer cell lines resistant to anti-EGFR therapy (17). Although the RAS–RAF–MEK–ERK pathway was activated in the resistant cell lines, the suppression of *MEK1/2* only marginally affected the growth of the resistant colorectal cancer cell lines. In contrast, concomitant inhibition of *MEK1/2* and *EGFR* suppressed growth of all the four resistant colorectal cancer cell lines examined.

Although the initial article by Benvenuti and colleagues employed a MAPK/ERK inhibitor along with cetuximab, the concept of simultaneous inhibition of EGFR and the RAS–RAF–MEK–ERK pathway remained valid. Now, multiple clinical trials to combine EGFR inhibition and MEK inhibition, such as "BRAF/MEK/EGFR Inhibitor Combination Study in Colorectal Cancer" (NCT01750918), are being conducted. According to a presentation at ESMO in 2016, a combination of three drugs, a BRAF inhibitor, a MEK inhibitor, and an EGFR inhibitor, appears to be most effective against *BRAF*-mutated mCRCs.

Recent success of cancer immunotherapy initiated multiple clinical trials involving an immune checkpoint inhibitor and some of the BRAF/MEK/EGFR inhibitors (NCT01750918). Again, a scientific rationale that remains valid for decades is important.

Reward for Cancer Science and for Cancer Patients

As initially mentioned, the study by Benvenuti and colleagues was brief. However, the article contained important messages and was highly cited. It is rewarding for scientists that their articles are cited many times. At the same time, being cited means that the finding is likely to have had clinical impact, making the interest of scientists and patients shared. We have to note that although results from large clinical trials are often frequently cited and change clinical practice, their real original ideas are often translational research on a small scale. *Cancer Research* should continue to serve the scientific community and, as a result, cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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