EGFR FISH in colorectal cancer: what is the current reality?

To our knowledge, the first study to assess epidermal growth factor receptor (EGFR) gene copy number, and KRAS and BRAF mutations as biomarkers of response to EGFR-targeted monoclonal antibodies for metastatic colorectal cancer was published in The Lancet Oncology in 2005. In this study, we reported that in patients treated with cetuximab or panitumumab, a correlation exists between clinical response and tumour EGFR copy number, and that KRAS or BRAF mutations occur mainly in patients with metastatic colorectal cancer resistant to treatment with these drugs. These findings paved the way to subsequent studies that confirmed the association of KRAS mutations and resistance with such compelling evidence that led to the approval of panitumumab in Europe for the treatment of KRAS wild-type only metastatic colorectal cancer. As for EGFR copy number, subsequent studies confirmed an association with clinical outcome. After almost 3 years, some comments should be made to point out the existing knowledge about EGFR fluorescence in-situ hybridisation (FISH) analysis in patients with metastatic colorectal cancer.

Our first comment is that a biological phenomenon, underlying the association between EGFR copy number and clinical outcome, certainly does exist. In our first study, we described the association between an increased EGFR copy number and tumour response in eight of nine responsive patients. Now, we know that most of the patients who respond have an increased EGFR copy number, but only a fraction of tumours with an increased gene copy number respond to treatment. Therefore, the high correlation between increased gene copy number and response to treatment was because of a high number of responsive patients selected in our first series in 2005. Actually, the crucial finding is that the non-increased, rather than the increased, EGFR copy number status is the most accurate predictive element for clinical outcome. Patients with low EGFR copy number are indeed unlikely to respond to treatment and have a worse time to progression and overall survival than patients with tumours with an increased gene copy number. In patients with tumours with an increased gene copy number, we described a 30% objective response, with six of 20 patients whose tumours had an increased EGFR copy number achieving an objective response compared with six of 58 patients in an unselected population. In responsive patients, tumour growth is probably mainly driven by the EGFR pathway and this biological characteristic is evoked by an increase in EGFR copy number. In non-responsive tumours that harbour an increased EGFR copy number, the resistance to treatment is probably because of constitutive activation of signalling pathways downstream of the receptor by mutations of oncogenes such as KRAS, BRAF, or PIK3C2A, or by the loss of a tumour suppressor gene such as PTEN.

Our second comment is that, in all studies, assessment of EGFR copy number by quantitative PCR resulted in no association with clinical outcome. In our experience, PCR analysis showed an increased EGFR copy number in a single patient with a high EGFR per cell count who had responsive disease, whereas detection of increased gene copy number in samples with a relatively low EGFR to chromosome enumeration probe 7 (CEP7) ratio was inconclusive. PCR inefficacy is probably because of tumour DNA dilution by healthy cells during DNA extraction. Colorectal cancer rarely presents with high polysomy or amplification of EGFR and, even in patients with increased gene copy number, the EGFR per cell count is not so much higher than that detected in healthy tissue.

Our third comment is that EGFR copy number has more predictive rather than prognostic usefulness in patients with metastatic colorectal cancer. In 2006, Lenz and colleagues postulated higher prognostic than predictive usefulness, based on a significant...
association of EGFR copy number (assessed by PCR not by FISH) with overall survival, but not with objective response or progression-free survival. To address this issue, in our 2007 series,\(^8\) we assessed patients treated with panitumumab compared with patients who received best supportive care only, and we detected an association between EGFR copy number and progression-free survival only in patients treated with panitumumab, thus suggesting predictive rather than prognostic usefulness of EGFR copy number.\(^6\)

Our final comment is that in metastatic colorectal cancer, EGFR FISH pattern is often not homogeneous, and has variable ratios of disomy versus polysomy or amplification. In these situations, scoring of EGFR signals and defining the EGFR pattern by FISH is sometimes difficult. To overcome this difficulty, we also assessed data as percentage of cells showing chromosome 7 polysomy (EGFR per nucleus ≥3) or EGFR amplification (EGFR to CEP7 ≥2).\(^1\) Applying this criteria, an increased EGFR copy number was significantly associated with better clinical outcome (figure).\(^6\) However, an exact definition of EGFR patterns and the reproducibility of data remain the largest obstacle for clinical applicability of the test; furthermore, although four studies\(^1,4,6,8\) have confirmed its predictive usefulness, methods of tissue processing and EGFR scoring systems were not standardised between these studies.

In conclusion, together with KRAS and BRAF mutations,\(^2,7\) the non-increased EGFR copy number detected by FISH is a predictive factor of resistance.\(^5,6,8\) Therefore, effort should be made to better define the low copy-number pattern, which is frequently homogeneous. In our series,\(^8\) chromosome 7 homogeneous disomy was described in 26 of 58 patients and was the most frequent pattern in tumours with non-increased EGFR copy number. Chromosome 7 disomy is easier to detect than an increase in EGFR copy number, and therefore, might enable a more reproducible FISH assay. However, further standardisation of methods is needed to reach better reproducibility and optimum sensitivity. From a clinical point of view, we can risk treating a non-responsive patient, but we cannot risk not treating a potentially responsive one.

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