Beyond the KRAS test

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The anti-epidermal growth factor receptor (EGFR) antibodies cetuximab and panitumumab have been demonstrated to be efficient in the treatment of irinotecan-resistant and chemorefractory metastatic colorectal cancer (mCRC) respectively. However, these costly and potentially toxic treatments are only efficient in a small proportion of patients. It is therefore necessary to identify markers better able to define which patients will benefit from these treatments. The major potential molecular predictive markers of response to cetuximab and/or panitumumab are involved more or less directly in the EGF signalling pathway. Among them, *KRAS* mutations, *EGFR* gene copy number and, more recently, expression of the PTEN protein andthe epiregulin and amphiregulin genes are those that appear to be the most relevant and which will have to be validated in future clinical trials before being incorporated in the therapeutic strategy of CRC.

KRAS mutations

Recently, several groups have shown that activating mutations in the Kirsten rat sarcoma viral oncogene homologue (KRAS) protein, which partially transduces the activation signal from EGFR, abrogates the therapeutic effect of anti-EGFR therapy [1–6]. This effect is seen in colorectal cancer patients regardless of the line of treatment or which anti-EGFR antibody is used. These results have changed the way in which anti-EGFR drugs are prescribed. For example, the European Medicine Agency has restricted drug prescription to the set of patients with wild-type *KRAS* tumours.

However, selection of patients on the basis of tumour *KRAS* status is not perfect. While the test for non-response is highly specific, (nearly 95% of the patients with somatic mutations of *KRAS* fail to respond to anti-EGFR therapy), it is not sensitive. In fact, 40–60% of patients with wild type *KRAS* fail to respond to the treatment [7]. This suggests that there are other important molecular determinants of response that have yet to be identified.

EGFR copy number

In colorectal cancer, a first study described an association between an increased *EGFR* copy number, analysed by fluorescent *in situ* hybridisation (FISH), and tumour response to cetuximab [8]. This association was reported both with cetuximab and panitumumab in subsequent studies [5,9–11]. However, tumour response was observed in colorectal tumours without an increase of *EGFR* copy number [12] and discrepant results were observed when *EGFR* copy number was assessed by quantitative polymerase chain reaction (PCR) and not by FISH [2,13,14]. The lack of sensitivity of the PCR technique for the detection of an increase of *EGFR* copy number, partly due to tumour DNA dilution, and the lack of reproducibility of FISH data, because of the absence of standardised EGFR scoring and the heterogeneity of FISH patterns, may explain these differences and render this molecular marker difficult to include in clinical practice [15,16].

Amphiregulin and epiregulin

Contrary to previous studies which focussed their research on one to several molecular markers, Khambata-Ford and colleagueshave used a more global genomic approach by analysing, on microarrays, the gene expression profiles of 95 tumours of metastatic CRC patients treated by cetuximab [2]. This approach showed that the expression of the EGFR ligands epiregulin (*EREG*) and amphiregulin (*AREG*) are very discriminate in distinguishing patients with disease control from those with progressive disease under cetuximab. The *EREG* and *AREG* gene expression level was significantly higher in the group of patients with disease control ($P = 0.000015$ and $P = 0.000025$, respectively). Moreover, patients with a high *EREG*

and *AREG* expression had longer progression-free survival than those with a low expression (*EREG:* median of 103.5 versus 57 days, $P = 0.0002$; *AREG:* median of 115.5 versus 57 days, $P < 0.0001$). Therefore, epiregulin and amphiregulin seem to be coordinately regulated. Although epiregulin binds more weakly to EGFR than EGF, it is known to be more efficient as it induces a more prolonged activation of the receptor. According to the authors an overexpression of epiregulin and/or amphiregulin could play a major role in tumour growth and survival by stimulating an autocrine loop through EGFR and, therefore, it could be possible to characterise an EGFR-dependent tumour potentially more sensitive to the receptor blockade by cetuximab.

PI3K and PTEN

As PTEN negatively regulates the PI3K/AKT pathway, it is easy to speculate that PTEN inactivation downstream of the EGFR could lead to a resistance to the EGFR inhibitors. Frattini and colleagues showed, in a series of 27 colorectal cancer patients, that PTEN expression by immunohistochemistry allows the distinction of responders to cetuximab from non responder patients [10]. With a cut-off of 50% of stained cells for positivity, all the responder patients were PTEN positive whereas only 35% of the non responders had a PTEN positive tumour. Another *in vitro* study examined the effect of cetuximab on several colon cancer cell lines and found that cell lines with loss of PTEN expression and/or *PI3KCA* mutation were resistant to cetuximab [17]. This later study underlines the implication of the PI3K/AKT pathway in the modulation of response to cetuximab and this was also recently suggested in a small series of colorectal cancers in which the activation of this pathway by the means of *PI3KCA* mutation and/or PTEN allelic loss was observed in 28% of cases, all being non responders to cetuximab [18].

Conflict of interest statement

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