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Molecular predictive factors of response to anti-EGFR antibodies in colorectal cancer patients

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ABSTRACT

Monoclonal antibodies against EGFR represent one of the most important recent advancements in the treatment of colorectal cancer. Anyway, to date, only a subset of patients can really take advantage from this kind of drugs. The identification of predictive factors that are able to stratify patients who potentially can benefit by these biological treatment, is an important aim of anticancer research both in clinical and in pre-clinical fields. Many studies evaluating classical IHC analysis of protein expression failed in this purpose. So in the last years there was the need to look forward to a new class of molecular predictive factors. Indeed a number of biomarkers have been evaluated in their potential to predict the response to anti-EGFR-based therapies. These include marker related to EGFR amplification, activation and phosphorylation, EGFR polymorphisms, but also markers related to Ras/Raf/MAPK and the PI3K/Akt signalling pathways and angiogenesis. This review will focus the attention to these new genetic and molecular markers.

1. Epidermal growth factor (EGF) pathway

EGFR is a 170 kDa transmembrane glycoprotein composed of an extracellular ligand-binding domain, a single hydrophobic transmembrane domain and a cytoplasmic tyrosine kinase-containing domain.[1] It belongs to the ErbB family of tyrosine kinase receptors that includes four members, namely EGFR, ErbB-2, ErbB-3 and ErbB-4.[2] EGFR is known to homodimerise or heterodimerise with other ErbB family members, following the binding to the extracellular domain of the receptor of a set of specific ligands such as EGF, TGF-α, amphiregulin, betacellulin, heparin-binding EGF and epiregulin.[3,4] This dimerisation leads to auto- and trans-phosphorylation in tyrosine residues of the ErbB receptors, triggering different intracellular signalling cascades including the phosphatidylinositol 3-kinase (PI3K)/Akt, the ras/raf/MEK/mitogen-activated protein kinase (MAPK) and the signal transducer and activator of transcription (STAT) pathways.[5] The final effect of signalling through EGFR causes a variety of cellular responses, including cell division, survival, motility, invasion, adhesion and cellular repair.[6] Enhanced activity or overexpression of EGFR has been found to be associated with tumour progression and poor survival in various malignancies, such as head and neck, lung, breast, gastrointestinal tract and bladder cancers. In particular, overexpression of EGFR in colorectal cancer, that occurs in up to 80% of cases, can be associated to tumour stage and is able to predict a potential metastatic risk.[7–13] Starting from these evidences, pre-clinical and clinical studies have shown that targeting EGFR is a valid strategy for anticancer therapy. Currently, two different treatment strategies for targeting EGFR and blocking its downstream signalling pathways have been developed: monoclonal antibodies directed against the

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extracellular domain of EGFR and small molecules blocking tyrosine-kinase activation intracellularly (tyrosine-kinase inhibitors, TKIs). Anyway, to date, only two EGFR-specific monoclonal antibodies, cetuximab and panitumumab, have been approved in Europe and United States for the treatment of metastatic colorectal cancer patients (CRC).

2. EGFR status in immunohistochemistry and response to EGFR-targeted therapy

The early trials with cetuximab and panitumumab were based in part on the idea that EGFR status, as determined by immunohistochemistry, could help enrich or predict for activity. The data obtained from BOND study showed no correlation between EGFR receptor status and clinical response, as determined by immunohistochemistry.[14]

Other published data also suggest that patients not expressing the EGFR receptor, at least by immunohistochemistry, as well as patients who have a high expression of the receptor can respond.[15–17] Similarly other published data, also for panitumumab, show no difference in activity based on EGFR staining intensity.[18,19]

Several biological and technically possible reasons may explain these findings: the expression of EGFR that has been shown to be very heterogeneous within tumors; the level of expression can really vary depending upon the specific immunohistochemistry test used, and finally tissue handling, processing and storage, which may vary between laboratories, lead to a catalytic degradation of cell surface receptors, resulting in an altered protein expression.

3. EGFR amplification

Moroni and colleagues published interesting data on the possible predictive role of EGFR gene copy number in the treatment of metastatic colorectal cancer with cetuximab or panitumumab. EGFR gene amplification evaluated with FISH correlated with the objective response to treatment, with eight of nine responders having an increased copy number, versus only 1 of 21 non-responders.[20] Similar results were obtained by Lievre et al. who evaluated EGFR gene amplification in another series of 30 patients with chromogenic in situ hybridisation (CISH), showing that all gene-amplified patients were responders to cetuximab therapy.[21] Sartore-Bianchi and colleagues conducted a retrospective study with the aim to demonstrate the correlation between the EGFR copy number and outcomes in patients treated with panitumumab. This study showed that patients with a high EGFR copy number or chromosome 7 polysomy-amplification, another marker of EGFR level, had longer progression-free and overall survival times when treated with panitumumab.[22]

4. EGFR mutations

In the recent past, amongst patients with non-small-cell lung cancer, a subset of patients have been identified who exhibit EGFR gene mutations. The presence of these mutations predicted for the response to EGFR-targeting therapies.[23]

Although these EGFR gene mutations also have been identified in colorectal cancer, they seem to be very rare and failed in predicting the response to anti-EGFR treatment.[24–26]

5. EGFR phosphorylation

To date, a unique and small study conducted by Personeni et al., suggests that EGFR phosphorylation level might be useful as a biomarker of anti-EGFR therapy efficacy showing a trend towards higher disease control in patients with high levels of pEGFR who were treated with cetuximab with or without irinotecan.[27] These preliminary data need further demonstrations in clinical trials.

6. EGFR polymorphisms

The *EGFR* gene contains a highly polymorphic sequence in intron-1, which consists of a variable number of CA dinucleotide repeats ranging from 9 to 21.[28] This sequence has been shown to affect the efficiency of gene transcription such that subjects or cell lines with a greater number of CA repeats have lower levels of mRNA and protein expression.[29,30]

Graziano et al. demonstrated that, in 110 mCRC patients treated with cetuximab, EGFR intron-1 S/S polymorphism (lower number of CA repeat) in germ-line cells was associated with favourable overall survival and treatment response.[31] From a practical perspective, the assessment of the *EGFR* intron-1 $(CA)_n$ could represent an easy and reproducible marker, and does not change over time. On the other hand, its predictive role might be altered by genetic changes in cancer cells.

7. The Ras/Raf/MAPK and the PI3K/Akt signalling pathways and related mutations

7.1. *KRAS mutation*

KRAS, a human homologue of the Kirsten rat sarcoma 2 virus gene, encodes a signal transducer that operates downstream of the EGFR so when the KRAS gene is mutated, the KRAS protein is active regardless of EGFR activation.[32]

Nowadays, several retrospective studies have clearly stated the high prognostic and predictive value of *KRAS* mutations in metastatic CRC patients treated with anti-EGFR moABs-based therapy.

A small retrospective study conducted by Lievre et al. with 30 cetuximab plus irinotecan refractory patients showed that 40% of the patients had a KRAS mutation. On the contrary, patients with wild-type KRAS had a higher response rate and a much longer survival than patients with mutated KRAS.[33] Moreover, Khambata-Ford demonstrated, in a subsequent study, that the majority who achieved disease control following treatment with cetuximab monotherapy had wild-type *KRAS*.[34] A study conducted by De Roock et al. in a series of 20 patients with advanced CRC who were randomized to cetuximab with or without irinotecan showed that *KRAS* mutations were inversely correlated with objective responses to cetuximab and that none of the patients harbouring *KRAS* mutation achieved partial response.[35] Similarly, Di Fiore et al. demonstrated in 59 patients with chemotherapy-refrac-

tory metastatic CRC who were treated with cetuximab plus chemotherapy, that all of the 12 individuals who had objective clinical responses were of *KRAS* wild-type.[36]

A very recently published study including 113 chemorefractory colorectal cancer patients treated with cetuximab, showed a response rate of 30–35% in patients with wild-type KRAS following treatment with cetuximab plus irinotecan.[37]

Freeman and colleagues found, in an analysis of 62 tumour samples from patients treated with panitumumab in 3 phase II studies and a phase III study, that patients with wild-type *KRAS* mutational status are more likely to respond to treatment.[38] Amado et al. studied tumour samples from 427 patients who participated in a randomized phase III trial of panitumumab versus best supportive care and found an improved progression-free survival over best supportive care in patients with wild-type *KRAS* treated with panitunumab. Interestingly, in patients with mutated *KRAS* status the treatment with panitumumab was not superior to best supportive care.[39]

Given theses results, *KRAS* mutation status seems to be the most important candidate to become a standard biomarker for predicting response to anti-EGFR-based therapy in patients with mCRC, but its predictive value has to be confirmed by studies prospectively designed to evaluate outcomes by *KRAS* status since all data available to date came from retrospective analyses. Ongoing trials may provide this answer.[40–43]

7.2. *PTEN status*

PTEN is a tumor-suppressor gene located on chromosome 10. The lipid phosphatase and tensin homologue (PTEN) is a key tumour suppressor that normally regulates the activation of PI3K. Deficient *PTEN* expression leads to the activation of the phosphoinositide 3-kinase (PI3K)/Akt (pAkt) signalling pathway.[44]

Starting from the preclinical evidence that Akt activation is an important resistance factor for anti-EGFR therapy,[45] Frattini et al. conducted a study evaluating PTEN tumour expression in 27 cetuximab-treated mCRC patients and found that the patients who achieved a partial response were all PTEN expressors (10/10).[46] Moreover, Loupakis et al. demonstrated that loss of PTEN expression in colorectal metastasis, but not in primary tumor, helped in the identification of patients who cannot benefit from a cetuximab-based therapy.[47]

7.3. *BRAF mutations*

BRAF is a serine–threonine-specific protein kinase that is activated downstream of the small G-protein RAS. BRAF activates the MAP kinase extracellular signal-regulated kinase (MEK), which in turn activates the extracellular signal-regulated kinase (ERK). For this reason, BRAF is another candidate biomarker for resistance in colorectal cancer, although BRAF mutations occur in far fewer patients than KRAS mutations. A recent study by Benvenuti and colleagues suggested that BRAF status (activation) is associated with the lack of response to anti-EGFR mAb treatment in mCRCs patients.[48] These preliminary interesting data need further demonstrations in clinical trials.

7.4. *NF-κB expression*

NF-κB (nuclear factor-kappa B) is a protein complex that is a transcription factor. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, growth factors, free radicals, ultraviolet irradiation, oxidised LDL and bacterial or viral antigen. NF-κB target genes promote tumour cell proliferation, survival, migration, inflammation, and angiogenesis. Moreover, NF-κB is specifically activated by EGFR signalling.[49–51]

Starting from these evidences, Scartozzi et al. demonstrated that NF-κB expression evaluated in a series of 76 patients treated with cetuximab plus cpt 11 for metastatic colorectal cancer can be predictive of treatment efficacy. In particular, Scartozzi et al. demonstrated that response rate, median time to progression and overall survival were significantly better in NF-κB-negative patients than in positive ones.[52]

7.5. *EGFR ligands*

The EGF signal pathway is activated by several kinds of stimulations. In particular, elevated expression of epiregulin and/or amphiregulin may play an important role in tumour growth and survival by stimulating an autocrine loop through EGFR. For this reason, Khambata-Ford and colleagues published a study looking at a possible predictive role of the EGFR ligands epiregulin and amphiregulin. This author showed that patients with a high versus low epiregulin expression in metastatic biopsies have superior progression-free survival rates and are more likely to have disease control with cetuximab-based therapies.[49] Recently, Yamada et al. found that amphiregulin expression in primary lesions of colorectal cancer is also an important predictive marker of liver metastasis.[53]

7.6. *FcγRIIa polymorphism*

H/R polymorphism at position 131 of FcγRIIIa is associated with tumour response in follicular lymphoma patients treated with rituximab as first-line therapy, probably related to a different induction of an antibody-dependent cellular cytotoxicity (ADCC) response induced by the antibody.[53] Recently, Zhang et al. demonstrated that in 39 EGFR-expressing mCRC patients treated with cetuximab, FcγRIIa 131 H/H or H/R genotypes were associated with a better progression-free survival compared to patients with R/R genotype, suggesting that these two polymorphisms could represent useful molecular markers to predict clinical outcome in this setting of patients.[54]

7.7. *Angiogenesis and prediction of response*

Several mechanisms have been identified in pre-clinical models whereby cetuximab inhibits the growth and survival of EGFR-positive tumors. These also include inhibition of angiogenesis. The mechanisms by which EGFR signalling pathways regulate VEGF are unclear, but it has been demonstrated that up-regulation of these factors follows activation of the EGFR signalling pathways.[55] Vincenzi et al. demonstrated that in

45 advanced colorectal cancer patients who had undergone cetuximab plus irinotecan therapy, the modification of circulating level of VEGF during treatment can be predictive of cetuximab efficacy. Indeed, patients with reduced circulating levels of VEGF showed a better response rate, a longer median time to progression and a greater overall survival than those without them.[56–58]

8. Conclusions

From these data derives, in the near future, the imperative of selecting patients who really benefit from anti-EGFR moAbs, especially those who are potential candidates for secondary radical liver resection in which the tumour shrinkage is the major goal of treatment. For these reasons, future perspective studies may be aimed at evaluating the role of *KRAS* mutations and the role of other potential candidate molecular predictive factors in facilitating the choice of which biological factor (anti-EGFR, anti-VEGF or both) could be the best partner for up-front chemotherapy.

Conflict of interest statement

None declared.

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