Biomarkers for Predicting the Efficacy of Anti-Epidermal Growth Factor Receptor Antibody in the Treatment of Colorectal Cancer

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Key Words

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Abstract

Anti-epidermal growth factor receptor (EGFR) antibodies have been widely utilized as a standard treatment for metastatic colorectal cancer (CRC). Anti-EGFR antibodies bind competitively to EGFRs to inhibit receptor activation and subsequent signal transduction of the *RAS/RAF/MEK* pathway and *PI3K/AKT* pathway. By inhibiting EGFR-mediated signal transduction, anti-EGFR antibodies inhibit cell growth, invasion, metastasis and angiogenesis, and they induce apoptosis. The IgG1-type antibody cetuximab is also capable of inducing antibody-dependent cellular cytotoxicity. Several studies have shown that *KRAS* mutation is a useful biomarker for predicting the efficacy of anti-EGFR agents, and the major guidelines for the treatment of CRC recommend the use of anti-EGFR antibody only for the cancers with wild-type *KRAS*. Alterations of other genes, including *BRAF*, *NRAS*, *PTEN* and *AKT*, and EGFR expression/gene copy number have also been reported to be candidate biomarkers for predicting the efficacy of anti-EGFR agents. The predictive values of these biomarkers are still controversial and further investigations are required.

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Introduction

It is well recognized that epidermal growth factor receptor (EGFR), a receptor tyrosine kinase, is overexpressed in colorectal cancers (CRCs) and plays a pivotal role in CRC development. Anti-EGFR antibodies including cetuximab (Erbix[®]) and panitumumab (Vectibix[®]) have recently been developed and are currently used as standard first-, second- or third-line chemotherapy for the treatment of metastatic CRCs. However, it has been reported that these agents are effective only for CRC with wild-type *KRAS* and not for *KRAS* mutation, indicating that *KRAS* mutation can serve as a useful biomarker for predicting the efficacy of anti-EGFR agents. Aside from *KRAS* mutation, *BRAF*, *NRAS* and *PIK3CA* mutations have also been identified as candidate biomarkers for predicting anti-EGFR antibody efficacy. In this review, the mechanism of action of anti-EGFR agents and their role as candidate biomarkers for predicting the efficacy of anti-EGFR agents are summarized.

Mechanism of Anti-EGFR Antibodies

The EGFR is a 170-kDa transmembrane glycoprotein containing a tyrosine-specific kinase. Ligands known to bind with the EGFR include epidermal growth factor

(EGF), TGF- α , amphiregulin, epiregulin or heparin-binding EGF-like growth factor. Ligand binding to the EGFR induces dimerization of the receptor, which results in the autophosphorylation of tyrosine residue in the intracellular domain, and subsequently downstream signal transduction via the *RAS/RAF/MEK* (MAP kinase) pathway and *PI3K/AKT* pathway (fig. 1). EGFRs are expressed in 60–80% of CRCs [1]. Cancer cells secrete TGF- α , which binds to EGFRs on the surface of cancer cells and promotes their growth by activating signal transduction in an autocrine manner. The activation of EGFR signal transduction not only promotes cancer growth but also invasion, metastasis and neovascularization (angiogenesis) of cancer tissue.

Cetuximab and panitumumab are used clinically as EGFR antibodies. Their primary mechanism of antitumor action involves competitive binding to the extracellular domain of EGFRs, which leads to inhibition of EGFR activation and subsequent signaling via the *RAS/RAF/MEK/ERK* and *PI3K/AKT* pathways. Moreover, anti-EGFR antibodies induce EGFR downregulation through dimerization and internalization of the receptor. It has also been reported that cetuximab activates proapoptotic molecules in vitro [2]. Thus, anti-EGFR antibody drugs inhibit growth, invasion, metastasis and angiogenesis, and induce apoptosis in CRC. A secondary mechanism of action of cetuximab involves its ability to induce antibody-dependent cellular cytotoxicity (ADCC), since it is an IgG1 subclass antibody, unlike panitumumab, which is an IgG2 subclass antibody. Experimental evidence has demonstrated that cetuximab acts by an indirect mechanism on the immune system through a cytotoxic effect mediated by ADCC and effector cells such as monocytes and natural killer cells [3].

Predictive Biomarkers

It is well accepted that *KRAS* mutation is a predictive marker for the efficacy of anti-EGFR agents in the treatment of CRC. Treatment guidelines for CRC published by the National Comprehensive Cancer Network (NCCN), European Society for Medical Oncology (ESMO) and Japanese Society for Cancer of the Colon and Rectum (JSCCR) recommend the use of anti-EGFR antibodies only for CRCs with wild-type. Several other gene alterations aside from *KRAS* have been identified as candidate biomarkers for predicting the efficacy of anti-EGFR treatment (table 1). Seven biomarkers are considered in turn in the following sections.

Fig. 1. EGFR signal transduction of the downstream pathway.

KRAS

KRAS is a small (21 kDa) GTP-binding protein. *KRAS* mutation is found in roughly 35–45% of CRCs; two hotspots – codons 12 and 13 – account for about 95% of all mutations (~80% for codon 12 and 15% for codon 13). Mutations in other regions, such as codons 61, 146 and 154, occur less frequently.

The predictive value of *KRAS* was first reported by Leivre et al. [4] who showed that *KRAS* mutant cancers were unresponsive to cetuximab and had a poorer overall survival (OS) compared with the *KRAS* wild-type cancers. Similarly, panitumumab was demonstrated to be effective only for *KRAS* wild-type cancers. Large randomized multicenter phase III clinical trials demonstrated the predictive value of *KRAS* for anti-EGFR therapy. van Cutsem et al. [5] performed a phase III trial to compare irinotecan, infusional fluorouracil and leucovorin (FOLFIRI) plus cetuximab versus FOLFIRI alone as a first-line chemotherapy for CRCs (CRYSTAL trial). The response rate for cetuximab treatment was 59.3% (102/172) in the *KRAS* wild-type group, which was significantly higher than that in the *KRAS* mutant group (36.2%, 38/105, $p = 0.03$). The median progression-free survival (PFS) in the *KRAS* wild-type group tended to be better than in the *KRAS* mutant group (9.9 vs. 7.6 months, $p = 0.07$). Bokemeyer et al. [6] performed a phase III trial to compare folinic acid-fluorouracil-oxaliplatin (FOLFOX) plus cetuximab versus FOLFOX alone as first-line chemothera-

Table 1. Predictive markers for anti-EGFR antibody agents

Marker	Therapy	Association with efficacy						Ref.
		RR, %		p value	PFS, months		p value	
		wild	mutant		wild	mutant		
<i>Recommended</i>								
<i>KRAS</i> mutation	FOLFIRI + Cmab	59.3	36.2	<0.004	9.9	7.6	<0.02	[5]
	FOLFOX + Cmab	61	33	<0.0027	7.7	5.5	<0.0064	[6]
	FOLFOX + Pmab	55	40	<0.07	9.6	7.3	<0.02	[7]
<i>Candidates</i>								
<i>BRAF</i> mutation	Cmab + CT	38	8.3	<0.0012	4.5	2.0	<0.0001	[11]
	Cmab + CT	48	39	0.43	11.4	6.5	0.0001	[12]
<i>PIK3CA</i> mutation Exon 9/20	Cmab/Pmab or Cmab/Pmab + CT	23	0	0.038			0.0035	[13]
	Cmab or Cmab + irinotecan	29.6	35.7	0.758	6	4.5	0.760	[14]
Exon 9	Cmab + CT	36.3	28.6	0.47	6	5.9	0.65	[11]
Exon 20		37	0	0.029	6	2.9	0.013	
<i>NRAS</i> mutation	Cmab + CT	38.1	7.7	0.013	6.5	3.5	0.06	[11]
		expression	loss		expression	loss		
PTEN expression	Cmab + irinotecan or CAPOX	62.5 (10/16)	0 (0/11)	0.001	N.D.	N.D.		[15]
	Cmab + CT	46.1 (41/89)	45.5 (10/22)	1	7.8	7.5	0.28	[17]
		high GCN	low GCN		high GCN	low GCN		
EGFR GCN	Cmab + irinotecan	60	9	0.02	7.7	2.9	0.04	[25]
	Cmab ± CT	71	37	0.015	8.5	7.0	0.28	[17]

RR = Relative risk; Cmab = cetuximab; Pmab = panitumumab; CT = chemotherapy; GCN = gene copy number; N.D. = not determined.

py for CRCs (OPUS trial). The trial found significant differences in response rate ($p = 0.011$) and PFS ($p = 0.0163$) between *KRAS* wild-type and mutant groups. Similarly, in a phase III trial to compare FOLFOX plus panitumumab versus FOLFOX alone as a first-line chemotherapy (PRIME trial) [7], significant differences in the response rate ($p = 0.02$) and PFS ($p = 0.02$) were noted between *KRAS* wild-type and mutant groups. A meta-analysis of 11 studies recently published showed that *KRAS* status was closely associated with the response rate ($p < 0.001$) and PFS ($p = 0.005$) [8].

Recently, De Roock et al. [9] reported that *KRAS* codon 13 mutants (G13D) treated with cetuximab showed significantly longer PFS and OS as compared with *KRAS* codon 12 mutants. However, this finding remains controversial and warrants further study.

BRAF

BRAF, a member of the serine/threonine kinase family, is directly downstream of *KRAS* in the MAP kinase cascade. Approximately 5–15% of CRCs are positive for *BRAF* mutation. More than 90% of the mutations are located at codon 600, where amino acid valine is substituted by glutamic acid (V600E) [10].

De Roock et al. [11] performed a retrospective analysis of 370 patients treated with cetuximab and found that *BRAF* mutation was present in 24 of 340 *KRAS* wild-type patients (6.5%). The response rate in patients with *BRAF* mutation was only 8.3%, which was significantly lower than the rate in patients without *BRAF* mutation (38%, $p < 0.01$). In addition, patients with *BRAF* mutation showed significantly worse PFS and OS than those with wild-type *KRAS* and *BRAF*. These results indicate that

BRAF mutation is capable of serving as a predictive and prognostic marker. However, Tol et al. [12] performed a retrospective analysis of *BRAF* mutation in a randomized controlled trial of patients receiving chemotherapy with (n = 227) or without cetuximab (n = 332) as first-line treatment. *BRAF* mutation was identified in 8.7% of all patients. The response rate for each group was not described in the study; however, in patients with *BRAF* mutation, there were no significant differences in PFS or OS between those treated with or without cetuximab (6.5 vs. 5.7 months for PFS and 12.9 vs. 12.8 months for OS). In contrast, the patients with *BRAF* mutation showed significantly worse PFS and OS than those with wild-type *KRAS* and *BRAF* irrespective of cetuximab treatment. The evidence to date indicates that *BRAF* mutation can serve as a prognostic biomarker, but its potential as a predictive biomarker for efficacy of anti-EGFR agents remains controversial.

PIK3CA (Exons 9 and 20)

The α -catalytic subunit of the phosphoinositol-3-kinase (*PIK3CA*) gene encodes the catalytic p110- α subunit of *PI3K*. It has been reported that *PIK3CA* mutation occurs in 10–20% of CRCs and can occur with *KRAS* or *BRAF* mutations. More than 80% of *PIK3CA* mutations occur in exon 9 (60–65%) or exon 20 (20–25%). Sartore-Bianchiet et al. [13] performed a retrospective analysis of 110 patients treated with anti-EGFR agent-based regimens and found *PIK3CA* mutations in 13.6% (15/110). None of the 15 patients with the *PIK3CA* mutation achieved an objective response with anti-EGFR agents compared with a relative risk of 23% in the 95 patients with wild-type *PIK3CA* ($p = 0.0337$). However, Prenen et al. [14] analyzed 200 patients treated with cetuximab and showed that 5 of 39 responders (13%) and 18 of 160 non-responders (11%) had *PIK3CA* mutations ($p = 0.781$). Recently, De Roock et al. [11] performed a retrospective analysis of 743 patients treated with cetuximab and found *PIK3CA* mutations in 14.5% (108/743); 68.5% (74/108) in exon 9 and 20.4% (22/108) in exon 20. They showed that *PIK3CA* exon 9 mutation had no effect, whereas exon 20 mutations were associated with a worse outcome compared with wild-types: i.e. respectively, a response rate of 0.0% (0/9) versus 36.8% (121/329, $p = 0.029$), a median PFS of 11.5 weeks versus 24 weeks ($p = 0.013$) and a median OS of 34 weeks versus 51 weeks ($p = 0.0057$). Thus, only *PIK3CA* mutations in exon 20 may be an effective marker for predicting treatment efficacy. However, since the incidence of *PIK3CA* exon 20 mutation is very low (2–5%), further investigations are required.

NRAS (Codons 12, 13 and 61)

NRAS mutation accounts for only 3–5% of CRCs and mutation at codon 61 is the most commonly observed. *NRAS* mutation is exclusively detected to *KRAS* mutation, as is *BRAF* mutation. There has only been one study that investigated the relationship between *NRAS* mutation and the efficacy of anti-EGFR antibodies, conducted by De Roock et al. [11], in which *NRAS* mutation was observed in 4.3% (13/302 *KRAS* wild-type samples). *NRAS* mutants had a significantly lower response rate than wild-types [7.7% (1/13) vs. 38.1% (110/289), $p = 0.013$]. However, there were no significant differences between *NRAS* mutants and wild-types with respect to median PFS (14 vs. 26 weeks, $p = 0.055$) and median OS (38 vs. 50 weeks, $p = 0.051$). To date, there have been no studies of *NRAS* in a sizeable patient cohort.

PTEN and AKT

Several studies have investigated the relationship between *PTEN* and/or *AKT* protein expressions and the efficacy of treatment with anti-EGFR antibodies. Several studies have shown that *PTEN* loss is associated with resistance to cetuximab in patients with metastatic CRC [15, 16], although the studies were not uniform in evaluating the *PTEN* protein expression. Conversely, a study by Laurent-Puig et al. [17] reported that the loss of *PTEN* protein expression, which was detected in about 20% (22/111) of *KRAS* wild-type tumors, was not associated with tumor response or PFS, but it was associated with slightly worse OS ($p = 0.013$). Based on these studies, which differed with respect to the assay methodologies used, *PTEN* expression does not appear to have a clinically robust ability to predict the therapeutic response to cetuximab. Moreover, further standardization of *PTEN* expression assessment is a necessary challenge to confirm these data.

None of the four studies reported a statistically significant association between *AKT* expression and tumor response or survival [18, 19]. However, because these studies involved small sample numbers, further investigation is needed to determine the association between *AKT* expression and tumor response to anti-EGFR antibodies.

EGFR Expression

For initial clinical trials of anti-EGFR antibodies, only patients with metastatic CRC proven to be EGFR positive by immunohistochemistry were enrolled. However, the level of EGFR protein expression is not associated with sensitivity to anti-EGFR monoclonal antibodies [20, 21]. In fact, a therapeutic response to cetuximab has been observed in patients with EGFR-negative tumors, which in-

icates that determination of EGFR positivity by immunohistochemical evaluation is not a reliable marker for predicting the efficacy of anti-EGFR monoclonal antibody therapy [22]. Licitra et al. [23] analyzed data from the EXTREME and CRYSTAL trials and determined that even in patients with *KRAS* wild-type tumors, immunohistochemical determination of EGFR expression was not predictive of the efficacy of cetuximab in combination with chemotherapy.

The EGFR gene copy number evaluated by quantitative PCR does not appear to correlate with the clinical outcome of patients, whereas the results of analysis by fluorescence in situ hybridization, FISH, appears to be associated with higher than usual treatment response [17, 24, 25]. Although promising results have been seen with EGFR amplification, technical challenges, including the reproducibility of methods to assess gene copy number and interlaboratory scoring system variability, have limited its role as a predictive biomarker [26]. Therefore, further studies are required to assess increased EGFR gene copy number as a predictive biomarker of anti-EGFR therapy.

Amphiregulin, Epiregulin

The overexpression of the EGFR ligands amphiregulin (AREG) and epiregulin (EREG) may promote tumor growth and survival by an autocrine loop mechanism. Khambata-Ford et al. [27] reported that metastatic CRC patients with high expression of AREG and EREG who were treated with cetuximab showed a statistically longer PFS period. Jacobs et al. [28] observed that patients with

KRAS wild-type tumors that expressed high ligand levels had better outcomes with EGFR inhibitors, whereas *KRAS* wild-type tumors with low ligand expression behaved like *KRAS* mutant tumors.

Based upon these studies, AREG and EREG are candidate biomarkers for predicting the efficacy of anti-EGFR antibody treatment for patients with *KRAS* wild-type tumors. However, methods for measuring protein levels and gene expression for AREG and EREG are not standardized and further studies are needed.

Epilogue

Recent advances in molecular biology have made it possible to develop molecular targeting agents such as anti-EGFR antibodies. The *KRAS* gene was identified as a predictive biomarker and is currently being utilized in clinical trials. *BRAF* mutation is capable of serving as a predictive and prognostic marker. Regarding other candidate biomarkers, the predictive values are still controversial and further studies are required. In the near future, it is expected that new predictive biomarkers will be validated in clinical trials, and that more personalized treatment for CRC will be possible as a result.

Disclosure Statement

The authors have no conflicts of interest to declare.

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