# Circulating Hepatocyte Growth Factor Is Correlated with Resistance to Cetuximab in Metastatic Colorectal Cancer

KIMIO YONESAKA[1], TAROH SATOH[2], SHINYA UEDA[1], TAKESHI YOSHIDA[1], MASAYUKI TAKEDA[1], TOSHIO SHIMIZU[1], ISAMU OKAMOTO[3], KAZUTO NISHIO[4], TAKAO TAMURA[1] and KAZUHIKO NAKAGAWA[1]

[1]*Department of Medical Oncology, Kinki University School of Medicine, Osaka, Japan;*
[2]*Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan;*
[3]*Center for Clinical and Translational Research, Kyushu University, Fukuoka, Japan;*
[4]*Genome Biology, Kinki University School of Medicine, Osaka, Japan*

**Abstract.** *Background/Aim: The epidermal growth factor family (EGF) has been suggested to influence the sensitivity to anti-epidermal growth factor receptor therapy. We examined the correlation between circulating levels of the epidermal growth factors amphiregulin and transforming growth factor-α (TGF-α) and the MET ligand hepatocyte growth factor and sensitivity to the anti-epidermal growth factor receptor antibody in colorectal cancer (CRC) patients. Materials and Methods: Plasma levels of each ligand were measured by enzyme-linked immunosorbent assay in 51 patients with wild-type KRAS CRC. Results: Patients with high hepatocyte growth factor (HGF) levels had a significantly lower disease control rate (DCR) and shorter median progression-free survival (PFS) and overall survival (OS) than those with low expression levels. Amphiregulin was correlated with objective response rate (ORR) but not with PFS or OS. Cetuximab response and survival were not associated with TGF-α. Conclusion: Circulating HGF may help identify CRC patients most likely to benefit from anti-epidermal growth factor receptor antibody therapy.*

Epidermal growth factor receptor (EGFR) over-expression is observed in many cancers including colorectal cancer (CRC) and associated with tumor stage and poor prognosis in CRC (1). EGFR-targeting strategies are crucial in the treatment of EGFR-over-expressing cancers. EGFR-targeting agents are broadly categorized into EGFR-tyrosine kinase inhibitors (EGFR-TKIs) and anti-EGFR monoclonal antibodies (2). EGFR-TKIs, such as gefitinib and erlotinib, are one of the standard therapies for patients with non-small cell lung cancer (NSCLC) harboring *EGFR*-activating mutations (3). In contrast to EGFR-TKIs, anti-EGFR monoclonal antibodies, such as cetuximab and panitumumab, have been proven to provide survival benefit in patients with CRC and head and neck squamous cell carcinoma (4-7). A previous pre-clinical study showed that, in some cancers, EGFR is constitutively activated in an autocrine fashion upon binding to its ligands, such as amphiregulin and transforming growth factor (TGF)-α (8). Cancers with constitutively activated EGFR are sensitive to anti-EGFR therapies, including monoclonal antibodies and TKIs.

Expression of EGFR ligands has been shown to affect the response to anti-EGFR therapy. High tumor expression level of amphiregulin was found to be positively associated with sensitivity to EGFR-TKI therapy in patients with wild-type *EGFR* NSCLC (8). Furthermore, metastatic CRC patients with high tumor expression levels of the EGFR ligands amphiregulin and epiregulin were significantly more sensitive to anti-EGFR antibody treatment compared to those with low tumor expression levels of amphiregulin and epiregulin (9, 10). Thus, EGFR ligands may represent potential biomarkers for predicting the efficacy of anti-EGFR antibody therapy.

Several growth factors have also been shown to cause resistance to TKIs and monoclonal antibodies. The HER3 ligand heregulin has been shown to induce resistance to the anti-HER2 antibody trastuzumab and the EGFR-targeting agents cetuximab and gefitinib (11, 12). The EGFR ligand amphiregulin was found to cause resistance to the anaplastic lymphoma kinase inhibitor crizotinib in NSCLC (13). Hepatocyte growth factor (HGF), a MET receptor ligand (14, 15), regulates cell growth, cell motility and morphogenesis by activating an intracellular signaling cascade upon MET

*Correspondence to:* Kimio Yonesaka, Department of Medical Oncology, Kinki University School of Medicine, 377-2 Ohno-higashi Osaka-Sayamashi, Osaka 589-8511, Japan. Tel: +81 723660221, Fax: +81 723605000, e-mail: yonesaka@sakai.med.kindai.ac.jp

*Key Words:* Hepatocyte growth factor, amphiregulin, transforming growth factor-α, epidermal growth factor receptor, cetuximab, colorectal cancer.

Table I. *Patients' characteristics.*

| Parameter | Values |
|---|---|
| Age | |
| Median (range) | 62 (41-85) |
| Sex | |
| Male/Female (%) | 32/19 (63/37) |
| Primary | |
| Colon/Rectum (%) | 31/20 (61/39) |
| ECOG PS | |
| 1/2 (%) | 46/5 (90/10) |
| Regimen | |
| Cetuximab/Cetuximab+CPT-11 (%) | 11/40 (22/78) |
| Responses | |
| PR/SD/PD (%) | 13/19/19 (25/37/37) |

ECOG PS, Eastern Cooperative Oncology Group Performance Status; PR, partial response; SD, stable disease; PD, progressive disease.

Table II. *Patients' characteristics by serum levels of hepatocyte growth factor (HGF), amphiregulin and TGF-α.*

| Parameter | HGF median, pg/ml | Amphiregulin median, pg/ml | TGF-α median, pg/ml |
|---|---|---|---|
| Age | | | |
| <70 | 190 | 11.5 | 0 |
| >70 | 211 | 11.2 | 0 |
| Sex | | | |
| Male | 196 | 11.2 | 0 |
| Female | 207 | 15.9 | 0 |
| Primary | | | |
| Colon | 204 | 8.2 | 0 |
| Rectum | 202 | 12.1 | 0 |
| ECOG PS | | | |
| 1 | 194 | 9.7 | 0 |
| 2 | 218 | 21.8 | 0 |
| Regimen | | | |
| Cetuximab+CPT-11 | 190 | 8.8 | 0 |
| Cetuximab | 218 | 21.8 | 0 |

ECOG PS, Eastern Cooperative Oncology Group Performance Status; CPT-11, irinotecan.

Table III. *Objective response rate (ORR) and disease control rate (DCR) compared between high and low groups for serum levels of hepatocyte growth factor (HGF), amphiregulin and TGF-α.*

| | | HGF | | | Amphiregulin | | | TGF-alpha | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Total | Low (n=26) | High (n=25) | *p*-Value | Low (n=25) | High (n=26) | *p*-Value | Low (n=40) | High (n=11) | *p*-Value |
| PR | 13 | 8 | 5 | | 3 | 10 | | 9 | 4 | |
| SD | 19 | 12 | 7 | | 14 | 5 | | 16 | 3 | |
| PD | 19 | 6 | 13 | | 10 | 9 | | 15 | 4 | |
| ORR | 25% | 31% | 20% | 0.523 | 12% | 38% | 0.0225 | 23% | 36% | 0.4394 |
| DCR | 63% | 77% | 48% | 0.04476 | 68% | 58% | 1 | 63% | 64% | 1 |

PR, Partial response; SD, stable disease; PD, progressive disease; ORR,objective response rate; DCR, disease control rate.

binding. Yano *et al*. reported that HGF induces resistance to EGFR-TKI therapy in NSCLC harboring EGFR-activating mutations (16). Circulating HGF levels were found to be significantly correlated with resistance to EGFR-TKI therapy in patients with NSCLC (17). Cumulative evidence suggests that growth factors influence the susceptibility to anti-EGFR therapy. Therefore, we examined the association of circulating levels of HGF and the EGFR ligands amphiregulin and TGF-α with the efficacy of anti-EGFR antibody therapy in patients with metastatic CRC.

## Materials and Methods

*Patients and treatment*. The study included 51 patients who were treated for metastatic CRC at Kinki University School of Medicine between September 2010 and August 2012. Patients had received FOLFIRI (leucovorin, 5-fluorouracil and irinotecan) or FOLFOX (leucovorin, 5-fluorouracil and oxaliplatin) as first- or second-line chemotherapy. Most patients had received bevacizumab combined with chemotherapy. All patients were anti-EGFR antibody treatment naïve. Patients were treated with cetuximab plus irinotecan or cetuximab alone as third-line chemotherapy. Cetuximab was administered every 2 weeks. Unless dosage modification was indicated by the attending physician, cetuximab was administered at an initial dosage of 400 mg/m$^2$ and then reduced to 250 mg/m$^2$. Irrinotecan was also administered every 2 weeks. The study was approved by the Institutional Review Board of Kinki University School of Medicine. Written informed consent was obtained from all patients.

*Measurement of HGF, amphiregulin and TGF-α plasma concentrations*. Plasma samples were obtained from the 51 CRC patients prior to anti-EGFR antibody-based therapy. Plasma concentrations of HGF, amphiregulin and TGF-α were measured using commercially available enzyme-linked immunosorbent assay

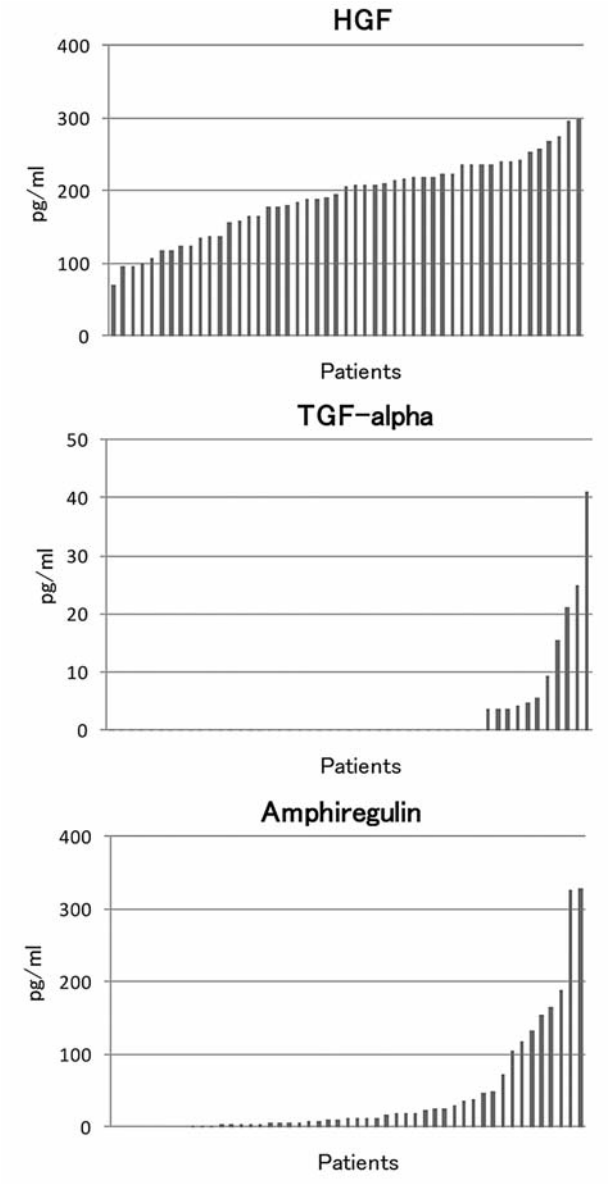


Figure 1. *Circulating HGF, amphiregulin and TGF-α levels in 51 patients with CRC. HGF, amphiregulin and TGF-α plasma levels were measured by ELISA. x-axis, patients. y-axis, concentration of growth factors. HGF: Hepatocyte growth factor, TGF-α: transforming growth factor-α, ELISA: enzyme-linked immunosorbent assay, CRC: colorectal cancer.*

(ELISA) kits (HGF ELISA Kit, Otsuka Pharmaceutical Co., Tokyo, Japan and human Amphiregulin and TGF-α Quantikine ELISA Kits, R&D Systems, Minneapolis, MN, USA) according to the manufacturers' instructions. Briefly, a 96-well microplate was coated with the capture antibody. The plate was washed and samples and standards were added. The plates were washed and detection antibody was added. After addition of the chromogen, color intensity was measured at 450 nm using a spectrophotometric plate reader. HGF, amphiregulin and TGF-α concentrations were determined by comparing to the standard curve.

*Assessment of cetuximab efficacy.* The objective response to cetuximab treatment was evaluated according to the Response Evaluation Criteria in Solid Tumors, ver. 1.1. Tumor response was evaluated every 2 to 3 months using computed tomography. Progression-free survival (PFS) was defined as the interval from initiation of anti-EGFR therapy to tumor progression or death without evidence of progression. Overall survival (OS) was defined as the interval from initiation of anti-EGFR therapy to death.

*Statistical analyses.* Differences in the distribution of variables were evaluated using the Fisher's exact test. PFS and OS analyses were performed using Cox proportional hazards models. All statistical analyses were performed using the StatView v.5.01 software (SAS Institute, Cary, NC, USA). All statistical tests were two-sided and a $p$-value<0.05 was considered statistically significant. Data were graphically displayed using GraphPad Prism v.5.0 for Windows (GraphPad Software, Inc., La Jolla, CA, USA).

## Results

*Patients' characteristics.* The characteristics of the 51 patients with metastatic CRC are shown in Table I. All patients had wild-type *KRAS*. Most patients were treated with cetuximab plus irrinotecan as third-line chemotherapy; however, 11 patients were treated with cetuximab-alone because of poor general condition or fluid retention. Thirteen patients achieved a partial response to cetuximab-based therapy and, thus, the overall response rate was 25%. Nineteen patients had stable disease for more than 8 weeks and 19 patients had short-term disease progression. The overall disease control rate (DCR) was 63%. Median PFS and OS were 141 days and 215 days, respectively. All patients discontinued treatment because of disease progression except for two patients who are still being treated. None of the patients discontinued treatment because of toxicity.

*Circulating HGF, amphiregulin and TGF-α levels.* HGF, amphiregulin and TGF-α plasma concentrations in patients with metastatic CRC are shown in Figure 1. Median circulating levels of HGF, amphiregulin and TGF-α were 204 pg/ml (range=71-298 pg/ml), 11.2 pg/ml (range=0-327 pg/ml) and 0 pg/ml (range=0-41.1 pg/ml), respectively. TGF-α was detected in only approximately 20% of patients, whereas HGF and amphiregulin were detected in all patients. Interactions among HGF, amphiregulin and TGF-α levels were not observed. HGF, amphiregulin and TGF-α expression levels were not correlated with patients' characteristics including, sex, age, primary site, performance status and treatment regimen (Table II).

*Association of circulating HGF, amphiregulin and TGF-α levels with cetuximab response.* Next, we evaluated the association of HGF, amphiregulin and TGF-α plasma levels with anti-EGFR antibody response (Figure 2). Although mean circulating HGF and TGF-α levels tended to be higher in non-responders than in responders, these differences were

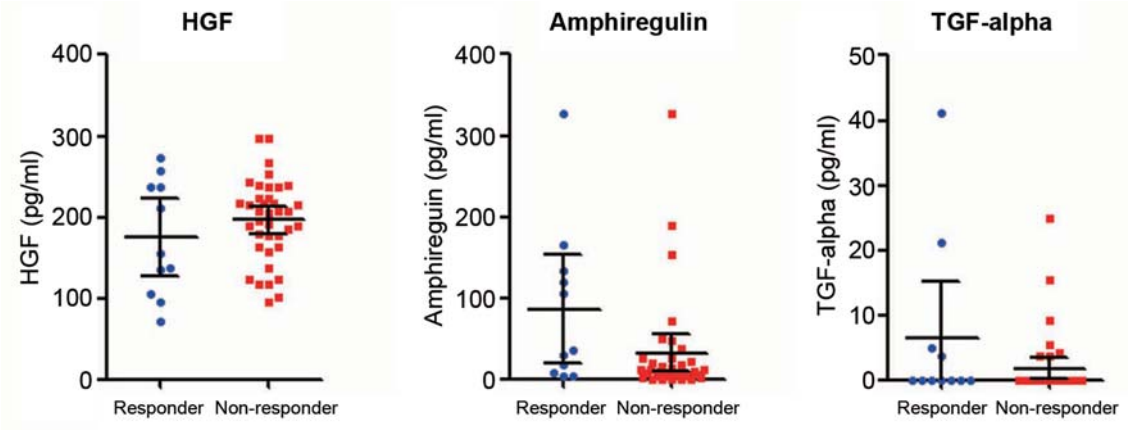


Figure 2. *Circulating HGF, amphiregulin and TGF-α levels in cetuximab responders and non-responders. HGF, amphiregulin, and TGF-α plasma levels were determined in cetuximab responders (n=13) and non-responders (n=38) using ELISA. Circulating amphiregulin level was significantly higher in responders than in non-responders (unpaired t-test, p=0.042). HGF: hepatocyte growth factor, TGF-α: transforming growth factor-α, ELISA: enzyme-linked immunosorbent assay.*

not significant. (174.4 *vs.* 196.3 pg/ml and 6.4 *vs.* 1.8 pg/ml, respectively). The mean circulating amphiregulin was significantly higher in responders compared with non-responders (85.8 *vs.* 31.9 pg/ml; $p$=0.042).

To further evaluate the effect of circulating HGF, amphiregulin and TGF-α levels on cetuximab response, patients were divided into high and low expression groups using the median values as cut-off points (HGF, 204 pg/ml; amphiregulin, 11.2 pg/ml; TGF-α, 0 pg/ml). Twenty-five and 26 patients were classified into the low and high HGF expression groups, 26 and 25 patients into the high and low amphiregulin expression groups and 11 and 40 patients into the high and low-TGF-α expression groups, respectively. Objective response rates (ORRs) and DCRs were compared between the high and low expression groups. The ORR was significantly higher in the high-amphiregulin expression group than in the low-amphiregulin expression group (12 *vs.* 38%; $p$=0.0225) (Table III). In contrast to amphiregulin, ORR was not significantly different between the high and low HGF and TGF-α expression groups. The high HGF expression group had a significantly lower DCR compared to the low HGF expression group (77 *vs.* 48%; $p$=0.0448). In contrast to HGF, DCR was not significantly different between the high and low amphiregulin and TGF-α expression groups.

*Association of circulating HGF, amphiregulin and TGF-α levels with survival.* Next, we evaluated the effect of circulating HGF, amphiregulin and TGF-α levels on PFS and OS. Circulating HGF level was significantly correlated with PFS. Patients with high HGF expression had significantly shorter median PFS compared to patients with low HGF expression (161 *vs.* 76 days; hazard ratio (HR)=2.118, 95% confidence interval (CI) 1.551-2.686), $p$=0.0327). Circulating amphiregulin and TGF-α levels were not significantly correlated with PFS in patients treated with cetuximab. The median PFS was 168 days and 105 days in the high- and low-amphiregulin expression groups (HR=1.567, 95% CI=0.8644-2.840, $p$=0.1389) and 119 days and 141 days in the high and low TGF-α expression groups (HR=1.036, 95% CI=0.5198-2.064, $p$=0.9204), respectively.

Circulating HGF level was significantly correlated with OS. The high HGF expression group had a significantly shorter median OS compared with the low HGF expression group (166 *vs.* 371 days; HR=0.3325, 95% CI=0.1609-0.6873), $p$=0.003). Circulating amphiregulin and TGF-α levels were not significantly correlated with OS in patients treated with cetuximab. The median OS was 345 days and 200 days in the high- and low-amphiregulin expression groups (HR=1.154, 95% CI=0.5748-2.317, $p$=0.6869) and 317 days and 214 days in the high and low TGF-α expression groups (HR=1.075, 95% CI=0.4656-2.483, $p$=0.8653), respectively.

## Discussion

Biomarkers predictive of response to anticancer agents are important in identifying patients who will benefit from treatment. Previous studies have revealed that genomic alterations in tyrosine kinase receptors, such as EGFR and HER2, and their downstream kinases, such as KRAS and BRAF, are correlated with the efficacy of molecular-targeted agents and, therefore, have practical application as biomarkers (18-20). Furthermore, cumulative pre-clinical and clinical evidence suggests that growth factors can also influence the sensitivity or tolerance to kinase inhibitors and, thus, serve as potential biomarkers (11, 12, 21). Although our study was

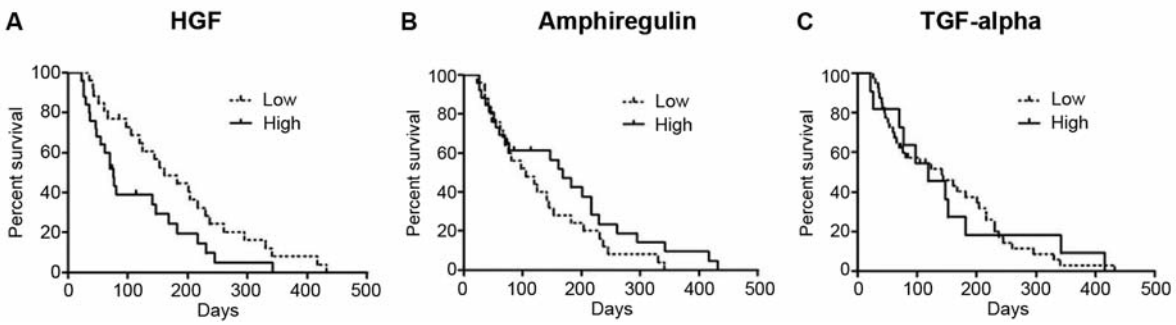


Figure 3. *Kaplan-Meier PFS curves in high and low HGF, amphiregulin and TGF-α expression groups. Plasma levels of HGF, amphiregulin, and TGF-α were measured by ELISA. Patients were divided into high and low amphiregulin, TGF-α and HGF expression groups using the median values as cut-off points. A. High and low HGF expression groups: Median PFS was 76 days and 161 days in the high and low HGF expression groups, respectively (HR=2.118, 95% CI=1.551-2.686, p=0.0327). B. High and low amphiregulin expression groups: Median PFS was 168 days and 105 days in the high and low amphiregulin expression groups, respectively (HR=2.190, 95% CI=0.05738-1.193, p=0.1389). C. High and low TGF-α expression groups: Median PFS was 119 days and 141 days in the high and low TGF-α expression groups, respectively (HR=1.185, 95% CI=0.6747-1.695, p=0.9204). HGF: Hepatocyte growth factor, TGF-α: transforming growth factor-α, ELISA: enzyme-linked immunosorbent assay, PFS: progression-free survival.*

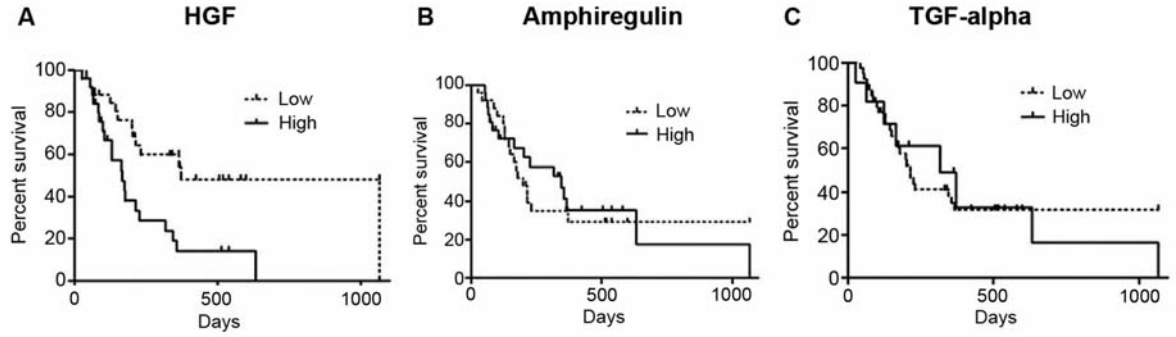


Figure 4. *Kaplan-Meier OS curves in the high and low HGF, amphiregulin and TGF-α expression groups. Plasma levels of HGF, amphiregulin and TGF-α were measured by ELISA. Patients were divided into high and low HGF, amphiregulin and TGF-α expression groups using the median values as cut-off points. A. High and low HGF expression groups: Median OS was 166 and 371 days in the high and low HGF expression groups, respectively (HR=2.235, 95% CI 1.737-2.732, p=0.0030). B. High and low amphiregulin expression groups: Median OS was 345 days and 200 days in the high and low amphiregulin expression groups, respectively (HR=0.5797, 95% CI 0.07446-1.085, p=0.6869). C. High and low TGF-α expression groups: Median OS was 317 days and 214 days in the high and low TGF-α expression groups, respectively (HR=0.6751, 95% CI 0.2240-1.126, p=0.8653 ). HGF: Hepatocyte growth factor, TGF-α: transforming growth factor-α, ELISA: enzyme-linked immunosorbent assay, OS: overall survival.*

limited by its small sample size and retrospective nature, we found that circulating HGF level is significantly correlated with tolerance to anti-EGFR therapy in patients with metastatic CRC. Our findings are consistent with those of a previous report by Takahashi *et al*. demonstrating the association of serum HGF level with shortened PFS and OS in metastatic CRC patients receiving anti-EGFR antibody treatment (22). Taken together, our study and the Takahashi *et al*. study indicate that circulating HGF may be a potential biomarker for predicting tolerance to anti-EGFR antibody therapy with cetuximab in CRC. We are currently conducting an ongoing prospective study to examine the effect of circulating HGF on survival in metastatic CRC patients treated with the anti-EGFR antibody panitumumab. Furthermore, other studies have reported that circulating HGF level is significantly correlated with poor prognosis in NSCLC patients harboring *EGFR*-activating mutations and melanoma patients harboring BRAF mutations who were treated with the EGFR-TKI gefitinib and the *BRAF* inhibitor vemurafenib, respectively (17, 21). This cumulative evidence implies that circulating HGF might be associated with resistance to a broad spectrum of kinase inhibitors in various types of cancer.

In the present study, we could not elucidate the mechanism by which HGF induces resistance to anti-EGFR

antibody therapy. Liska *et al*. reported that HGF-induced MET activation and accompanying mitogen-activated protein kinase and AKT signaling reactivation were associated with resistance to anti-EGFR antibody treatment in CRC cells (23). MET is broadly expressed in CRC (24); however, we did not evaluate MET expression and activation in CRC in our study. Furthermore, MET has been shown to be activated by genomic amplification. Thus, MET may induce resistance to anti-EGFR antibody treatment independent of HGF (25). MET amplification is rarely observed in CRC but appears after acquired resistance to anti-EGFR antibody treatment in CRC patients (25). Taken together, these findings suggest that circulating HGF might activate MET and its downstream signaling in cancer cells, thus promoting cancer cell survival and proliferation despite anti-EGFR antibody treatment.

In the present study, CRC patients with HGF over-expression had a significantly shorter OS. The reduced OS could be explained by HGF-mediated resistance to anti-EGFR antibody therapy. However, HGF might also have a negative prognostic effect independent of anti-EGFR antibody resistance. Previous studies have shown that HGF expression level is associated with poor prognosis in CRC (26). Circulating HGF might represent a potential target to overcome anti-EGFR antibody resistance and improve prognosis in CRC patients. A recent report demonstrated that the anti-HGF monoclonal antibodies rilotumumab plus panitumumab improve ORR in patients with wild-type *KRAS* metastatic CRC compared to panitumumab plus placebo (27). Furthermore, MET inhibitors could potentially be effective in blocking HGF-dependent MET activation in CRC patients with high circulating HGF levels.

In the present study, patients responsive to anti-EGFR antibody therapy had significantly higher circulating levels of amphiregulin. However, amphiregulin was not significantly correlated with PFS or OS. Our previous *in vitro* study showed that amphiregulin protein levels in culture medium is significantly correlated with sensitivity to cetuximab in cancer cell lines (8). Furthermore, a modest correlation between amphiregulin protein level by ELISA and amphiregulin (*AR*) mRNA level by quantitative polymerase chain reaction was observed. However, in the present study, we did not determine the correlation between circulating levels of amphiregulin and tumor expression of *AR* mRNA. Thus, the absence of a survival benefit might be explained by a lack of correlation between serum and tumor expression levels of amphiregulin.

## Acknowledgments

The Authors would like to thank Haruka Yamaguchi, Yume Shinkai, Michiko Kitano and Mami Kitano for their technical support.

## Conflicts of Interest

The Authors declare no financial relationships.

## References

1 Spano JP, Lagorce C, Atlan D, Milano G, Domont J, Benamouzig R, Attar A, Benichou J, Martin A, Morere JF, Raphael M, Penault-Llorca F, Breau JL, Fagard R, Khayat D and Wind P: Impact of EGFR expression on colorectal cancer patient prognosis and survival. Ann Oncol *16*: 102-108, 2005.

2 Hynes NE and Lane HA: ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer *5*: 341-354, 2005.

3 Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA and Fukuoka M: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med *361*: 947-957, 2009.

4 Jonker DJ, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ, Berry SR, Krahn M, Price T, Simes RJ, Tebbutt NC, van Hazel G, Wierzbicki R, Langer C and Moore MJ: Cetuximab for the treatment of colorectal cancer. N Engl J Med *357*: 2040-2048, 2007.

5 Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Oliner KS, Wolf M and Gansert J: Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol *28*: 4697-4705, 2010.

6 Van Cutsem E, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pintér T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J and Rougier P: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med *360*: 1408-1417, 2009.

7 Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, Erfan J, Zabolotnyy D, Kienzer HR, Cupissol D, Peyrade F, Benasso M, Vynnychenko I, De Raucourt D, Bokemeyer C, Schueler A, Amellal N and Hitt R: Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med *359*: 1116-1127, 2008.

8 Yonesaka K, Zejnullahu K, Lindeman N, Homes AJ, Jackman DM, Zhao F, Rogers AM, Johnson BE and Jänne PA: Autocrine production of amphiregulin predicts sensitivity to both gefitinib and cetuximab in EGFR wild-type cancers. Clin Cancer Res *14*: 6963-6973, 2008.

9 Jacobs B, De Roock W, Piessevaux H, Van Oirbeek R, Biesmans B, De Schutter J, Fieuws S, Vandesompele J, Peeters M, Van Laethem JL, Humblet Y, Pénault-Llorca F, De Hertogh G, Laurent-Puig P, Van Cutsem E and Tejpar S: Amphiregulin and epiregulin mRNA expression in primary tumors predicts outcome in metastatic colorectal cancer treated with cetuximab. J Clin Oncol *27*: 5068-5074, 2007.

10 Khambata-Ford S, Garrett CR, Meropol NJ, Basik M, Harbison CT, Wu S, Wong TW, Huang X, Takimoto CH, Godwin AK, Tan BR, Krishnamurthi SS, Burris HA 3rd, Poplin EA, Hidalgo M, Baselga J, Clark EA and Mauro DJ: Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol *25*: 3230-3237, 2007.

11 de Alava E, Ocaña A, Abad M, Montero JC, Esparís-Ogando A, Rodríguez CA, Otero AP, Hernández T, Cruz JJ and Pandiella A: Neuregulin expression modulates clinical response to trastuzumab in patients with metastatic breast cancer. J Clin Oncol *25*: 2656-2663, 2007.

12 Yonesaka K, Zejnullahu K, Okamoto I, Satoh T, Cappuzzo F, Souglakos J, Ercan D, Rogers A, Roncalli M, Takeda M, Fujisaka Y, Philips J, Shimizu T, Maenishi O, Cho Y, Sun J, Destro A, Taira K, Takeda K, Okabe T, Swanson J, Itoh H, Takada M, Lifshits E, Okuno K, Engelman JA, Shivdasani RA, Nishio K, Fukuoka M, Varella-Garcia M, Nakagawa K and Jänne PA: Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. Sci Transl Med *3*: 99ra86, 2011.

13 Sasaki T, Koivunen J, Ogino A, Yanagita M, Nikiforow S, Zheng W, Lathan C, Marcoux JP, Du J, Okuda K, Capelletti M, Shimamura T, Ercan D, Stumpfova M, Xiao Y, Weremowicz S, Butaney M, Heon S, Wilner K, Christensen JG, Eck MJ, Wong KK, Lindeman N, Gray NS, Rodig SJ and Jänne PA: A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. Cancer Res *71*: 6051-6060, 2011.

14 Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, Tashiro K and Shimizu S: Molecular cloning and expression of human hepatocyte growth factor. Nature *342*: 440-443, 1989.

15 Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF and Aaronson SA: Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. Science *251*: 802-804, 1991.

16 Yano S, Wang W, Li Q, Matsumoto K, Sakurama H, Nakamura T, Ogino H, Kakiuchi S, Hanibuchi M, Nishioka Y, Uehara H, Mitsudomi T, Yatabe Y, Nakamura T and Sone S: Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor–activating mutations. Cancer Res *68*: 9479-9487, 2008.

17 Kasahara K, Arao T, Sakai K, Matsumoto K, Sakai A, Kimura H, Sone T, Horiike A, Nishio M, Ohira T, Ikeda N, Yamanaka T, Saijo N and Nishio K: Impact of serum hepatocyte growth factor on treatment response to epidermal growth factor receptor tyrosine kinase inhibitors in patients with non-small cell lung adenocarcinoma. Clin Cancer Res *16*: 4616-4624, 2010.

18 Kobayashi S, Boggon TJ, Dayaram T, Jänne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG and Halmos B: EGFR mutation and resistance of non-small cell lung cancer to gefitinib. N Engl J Med *352*: 786-792, 2005.

19 Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ, Langer C, Moore MJ and Zalcberg JR: *K-ras* mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med *359*: 1757-1765, 2008.

20 Arteaga CL and Engelman JA: ERBB receptors: from oncogene discovery to basic science to mechanism-based cancer therapeutics. Cancer Cell *25*: 282-303, 2014.

21 Wilson TR, Fridlyand J, Yan Y, Penuel E, Burton L, Chan E, Peng J, Lin E, Wang Y, Sosman J, Ribas A, Li J, Moffat J, Sutherlin DP, Koeppen H, Merchant M, Neve R and Settleman J: Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. Nature *487*: 505-509, 2012.

22 Takahashi N, Yamada Y, Furuta K, Honma Y, Iwasa S, Takashima A, Kato K, Hamaguchi T and Shimada Y: Serum levels of hepatocyte growth factor and epiregulin are associated with the prognosis on anti-EGFR antibody treatment in KRAS wild-type metastatic colorectal cancer. Br J Cancer *110*: 2716-2727, 2014.

23 Liska D, Chen CT, Bachleitner-Hofmann T, Christensen JG and Weiser MR: HGF rescues colorectal cancer cells from EGFR inhibition via MET activation. Clin Cancer Res *17*: 472-482, 2011.

24 Takeuchi H, Bilchik A, Saha S, Turner R, Wiese D, Tanaka M, Kuo C, Wang HJ and Hoon DS: c-MET expression level in primary colon cancer: a predictor of tumor invasion and lymph node metastases. Clin Cancer Res *9*: 1480-1488, 2003.

25 Bardelli A, Corso S, Bertotti A, Hobor S, Valtorta E, Siravegna G, Sartore-Bianchi A, Scala E, Cassingena A, Zecchin D, Apicella M, Migliardi G, Galimi F, Lauricella C, Zanon C, Perera T, Veronese S, Corti G, Amatu A, Gambacorta M, Diaz LA Jr, Sausen M, Velculescu VE, Comoglio P, Trusolino L, Di Nicolantonio F, Giordano S and Siena S: Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov *3*: 658-673, 2013.

26 Toiyama Y, Miki C, Inoue Y, Okugawa Y, Tanaka K and Kusunoki M: Serum hepatocyte growth factor as a prognostic marker for stage II or III colorectal cancer patients. Int. J. Cancer *125*: 1657-1662, 2009.

27 Van Cutsem E, Eng C, Nowara E, Swieboda-Sadlej A, Tebbutt NC, Mitchell E, Davidenko I, Stephenson J, Elez E, Prenen H, Deng H, Tang R, McCaffery I, Oliner KS, Chen L, Gansert J, Loh E, Smethurst D and Tabernero J: Randomized phase Ib/II trial of rilotumumab or ganitumab with panitumumab versus panitumumab alone in patients with wild-type KRAS metastatic colorectal cancer. Clin Cancer Res *20*: 4240-4250, 2014.

*Received October 21, 2014*
*Revised November 4, 2014*
*Accepted November 7, 2014*