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ORIGINAL ARTICLE

Cetuximab enhances the activities of irinotecan on gastric cancer cell lines through downregulating the EGFR pathway upregulated by irinotecan

Xin Liu · Wei-Jian Guo · Xiao-Wei Zhang · Xu Cai · Shu Tian · Jin Li

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Abstract
Purpose Treatment effects of advanced gastric cancer (AGC) are unsatisfactory, and novel therapeutic approaches are much needed. The epidermal growth factor receptor (EGFR) monoclonal antibody cetuximab inhibits the growth of several human cancer cells but has been tested rarely for the treatment of GC. The synergy between cetuximab and irinotecan has been reported in colorectal cancer, but the mechanisms are still not fully clarified. Consequently, we hypothesized cetuximab/irinotecan combination should enhance the antitumor activity of irinotecan in GC cells.
Methods The in vitro antiproliferative, pro-apoptotic, cell cycle arrest effects and induction of senescence were examined in SGC-7901 and MKN-45 GC cell lines. The effects of cetuximab or irinotecan as single agents or the combination on the expression of p53, p16, and EGFR signaling pathways were also studied.
Results The study revealed that cetuximab alone did not show any antiproliferative, pro-apoptotic, cell cycle arrest or cellular senescence effect on GC cells but when combined with irinotecan synergistically inhibits GC cell proliferation and induces apoptosis and G2/M phase arrest. Irinotecan increases phosphorylation of EGFR, MAPK, and AKT and decreases the expression of $P27^{Kip1}$, which could be all abrogated by its combination with cetuximab. The combination could also inhibit the expression of Cyclin D1 and phosphorylated mTOR while had no impact on p53, p16, PTEN, and HIF-1alpha.
Conclusions Cetuximab enhances the activities of irinotecan on GC cells via the downregulation of the EGFR pathway upregulated by irinotecan. Combination therapy with cetuximab and irinotecan, a novel therapeutic approach, warrants further study in GC.

Keywords Gastric cancer · Cetuximab · Irinotecan · Synergy · Epidermal growth factor receptor

X. Liu · W.-J. Guo · X.-W. Zhang · S. Tian · J. Li (✉)
Department of Medical Oncology, Fudan University Shanghai Cancer Center, 270 Dong-An Road, Shanghai 200032, China
e-mail: fudanlijin@163.com

X. Cai
Department of Pathology, Fudan University Shanghai Cancer Center, 270 Dong-An Road, Shanghai 200032, China

X. Liu · W.-J. Guo · X.-W. Zhang · X. Cai · S. Tian · J. Li
Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China

Background

Advanced gastric cancer (AGC), the fourth most common and the second most deadly cancer worldwide, is highly prevalent in developing nations with almost two-thirds of the cases occur in developing countries and 42% in China alone [1]. Despite the availability of chemotherapeutic agents that may improve survival or quality of life, including anthracyclines, taxanes, fluoropyrimidines, platinum derivatives, and topoisomerase I inhibitor (irinotecan), used either as single agent or in combination, there is no established standard regimen for AGC and the median overall survival remains less than 1 year [2]. Thus, agents with new mechanism of action and regimens deserve further investigation. Cetuximab is one of the very promising candidates.

Cetuximab, an anti-EGFR chimeric monoclonal antibody, has been used in combination with chemotherapy for treating colorectal cancer, squamous cell carcinoma of the head and neck, and non-small cell lung cancer (NSCLC)

[3]. In reported data, cetuximab had synergistic effects with chemotherapeutic agents especially irinotecan. When combined with irinotecan, it remained activity for patients with irinotecan-refractory colorectal cancer [4]. The synergistic activities of cetuximab with irinotecan in colorectal cancer make it possible to investigate the application of this strategy to gastric cancer (GC). The combination of lapatinib (dual EGFR/HER-2 tyrosine kinase inhibitor) and SN-38 (the active metabolite of irinotecan) revealed interactive synergism in the inhibition of GC cell proliferation [5]. Inhibition of the EGFR pathway and its synergy with irinotecan as reported previously present a novel approach of therapeutic modality for AGC. Several clinical trials are ongoing to investigate the possibility of the application of cetuximab to GC with different chemotherapeutic agents [6, 7]. In a phase II trial, Pinto C et al. found that cetuximab was active for first-line treatment to GC patients when combined with irinotecan [8].

It is not fully understood about the mechanism of the synergy between cetuximab and irinotecan. Irinotecan (CPT-11), an S-phase-specific semisynthetic derivative of camptothecin, interferes with DNA replication and cell division by inhibiting the enzyme topoisomerase I. Cetuximab, an anti-EGFR monoclonal antibody, binds to EGFR and blocks ligand-induced activation of EGFR. The inhibition of EGFR downregulates phosphoinositol-3-kinase (PI3 K)/AKT and RAS/mitogen-activated protein kinase (MAPK), which are the major intracellular pathways implicated following phosphorylation of EGFR, leading to a pro-apoptotic, cell cycle arrest or cellular senescent effect, which may sensitize the cancer cells to be more responsive to chemotherapeutic agents [9–11]. The aim of the present study was to investigate the combination effect of cetuximab and irinotecan on GC cells and to elucidate the biochemical mechanism of the synergistic interaction.

Methods

Reagents

Cetuximab (C225; Erbitux; kindly provided by Merck KGaA, Darmstadt, Germany) was given undiluted at concentration of 2 mg/mL. Irinotecan (Pharmacia and Upjohn Co., Kalamazoo, MI) was provided in a stock sterile saline solution of 20 mg/mL. For in vitro experiments, the two agents were combined in fixed drug ratio of 1:1.

Cell lines and culture conditions

Human GC cell lines SGC-7901 and MKN-45, previously shown to express EGFR[12], were obtained from Shanghai Institute of Digestive Surgery. SGC-7901 is moderately differentiated human gastric adenocarcinoma cell line with mutated P53 gene [13], and MKN-45 is poorly differentiated adenocarcinoma cell line with wild-type P53 [14]. The cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS).

Sequencing of K-Ras

DNA were extracted from SGC-7901 and MKN-45 cells and screened for *K-Ras* gene mutations in codons 12 and 13 of exon 2 with the use of a polymerase chain reaction method. Primers used were F, AGGCCTGCTGAAAATGACTG and R, TCAAAGAATGGTCCTGCACC.

Measurement of antiproliferative effects

SGC-7901 and MKN-45 cell lines were plated in 96-well tissue culture plates and treated with various concentrations of cetuximab or irinotecan or the combination of both agents in RPMI 1640 medium supplemented with 2% FBS for 72 h. Cell Counting Kit-8 (CCK-8) assay (Dojindo Laboratories, Kumamoto, Japan) was used to measure the number of metabolically active cells. The IC_{50} value of each drug was defined as the concentration needed for a 50% reduction in the proliferation. The combined effects were analyzed with the Calcusyn software (Biosoft, Cambridge, United Kingdom) that is based on the median effect principle developed by Chou and Talalay. Combination index (CI) is the ratio of the combined dose to the sum of the single-drug doses at an isoeffective level, and a $CI > 1$ was considered antagonism, <1 was considered synergism, and $CI = 1$ was considered additive interactions.

Measurement of apoptosis and cell cycle distribution

SGC-7901 and MKN-45 cells were treated with various concentrations of cetuximab or irinotecan or the combination of both agents. After 72 h, apoptosis was detected using an Annexin V-FITC Apoptosis Detection Kit (Merck). Cells were stained with annexin V-FITC and propidium iodide (PI) and analyzed by fluorescence-activated cell sorting scan flow cytometry (Becton–Dickinson, Mountain View, CA, USA). The results were expressed as the ratio of apoptotic to total cells. Cell cycle phase distribution was analyzed by flow cytometry using PI staining.

Measurement of cellular senescence

SGC-7901 and MKN-45 cells were exposed to cetuximab or irinotecan or the combination of both agents. After 24 h, cellular senescence was determined using senescence-associated beta galactosidase assay (Genmed, U.S.A), the

activity of which can be visualized by means of a cytochemical reaction resulting in blue precipitate. The results were expressed as the ratio of senescent to total cells.

Antibodies and Western blot analysis

SGC-7901 and MKN-45 cells were exposed to cetuximab or irinotecan or the combination of both agents for 16 h and stimulated with 10 ng/mL EGF for 15 min. Then, the cells were lysed with PhosphoSafe Extraction Reagent (Merck). Samples containing equal amounts of protein were electrophoretically separated on a SDS–polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA), which was probed with primary antibodies and hybridized with the appropriate horseradish peroxidase-conjugated secondary antibody (Cell Signaling Technology, Beverly, MA) and detected via chemiluminescence with the SuperSignal West Dura Extended Duration Substrate (Pierce, Rockford, IL).

Primary antibodies used were as follows: rabbit monoclonal phospho-EGFR (Tyr1068), EGFR, phospho-p44/42 MAPK (Thr202/Tyr204), p44/42 MAPK, phospho-AKT (Ser473), AKT and β-Actin antibody (from Cell Signaling Technology, Beverly, MA); and rabbit monoclonal $P27^{Kip1}$, phosphatase and tensin homologue deleted on chromosome 10 (PTEN) antibody, mouse monoclonal hypoxia inducible factor-1alpha (HIF-1alpha), Cyclin D1, P53 antibody, rabbit polyclonal phospho-mTOR (Ser2448) antibody (from Millipore, Bedford, MA).

Results

K-Ras mutation status

First, we studied the mutation status of *K-Ras* since activating mutations in the *K-Ras* gene could result in EGFR-independent activation of the MAPK pathway and impair the response to cetuximab [15]. The results showed both SGC-7901 and MKN-45 cell lines have wild-type *K-Ras* gene.

Cetuximab combined with irinotecan synergistically inhibits GC cell proliferation

The dose–response curves are presented in Fig. 1A, B. The CCK-8 assay revealed that irinotecan exhibited dose-

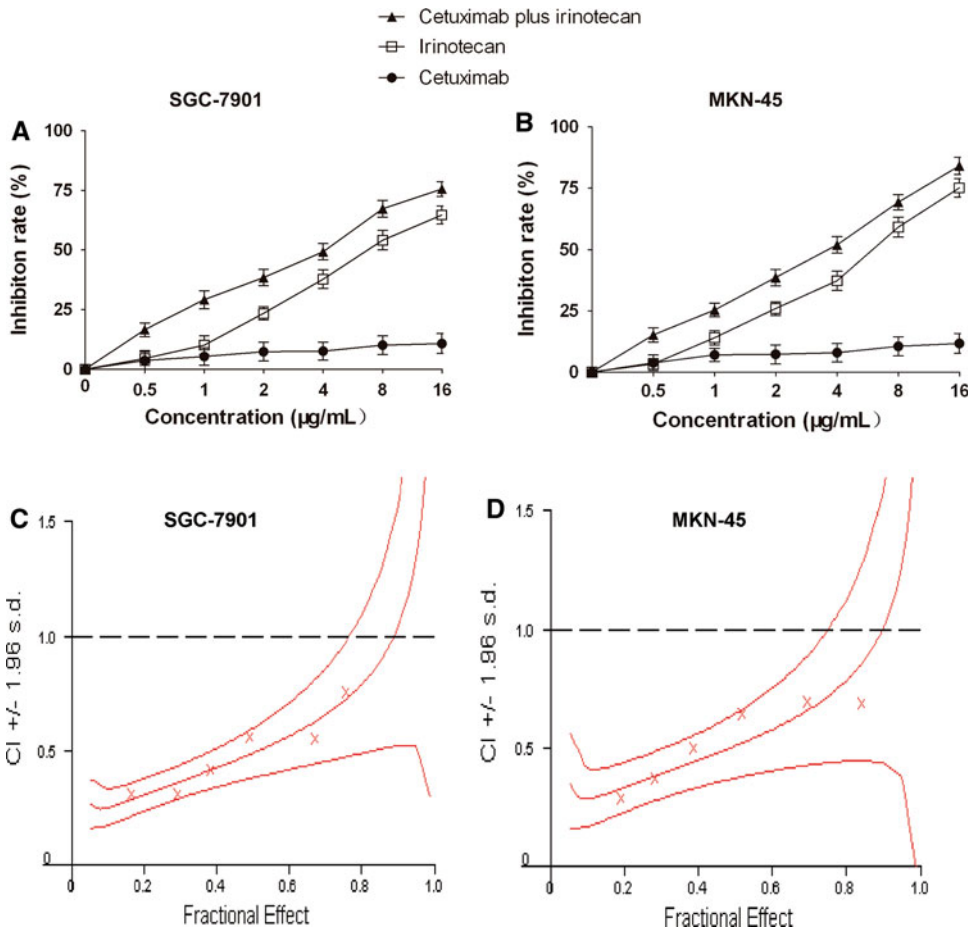

Fig. 1 Effect of the different concentrations of cetuximab, irinotecan, and combination of cetuximab and irinotecan on growth inhibition ratio of gastric cancer cells by CCK-8 assay. **a**, **b** Dose-dependent growth-inhibitory curves of SGC-7901 (**a**) and MKN-45 (**b**) cells treated with increasing doses of cetuximab and irinotecan alone or the combination. Data were expressed as the percentage of growth inhibition when compared with the growth of untreated control cells. The data shown represent the median values of triplicate experiments ± SD. **c**, **d** Fractional effect-combination index (Fa-CI) plots of interactions between cetuximab and irinotecan in SGC-7901 (**c**) and MKN-45 (**d**) cells. CI values were interpreted as follows: <1, synergism; = 1, additive; and >1, antagonism

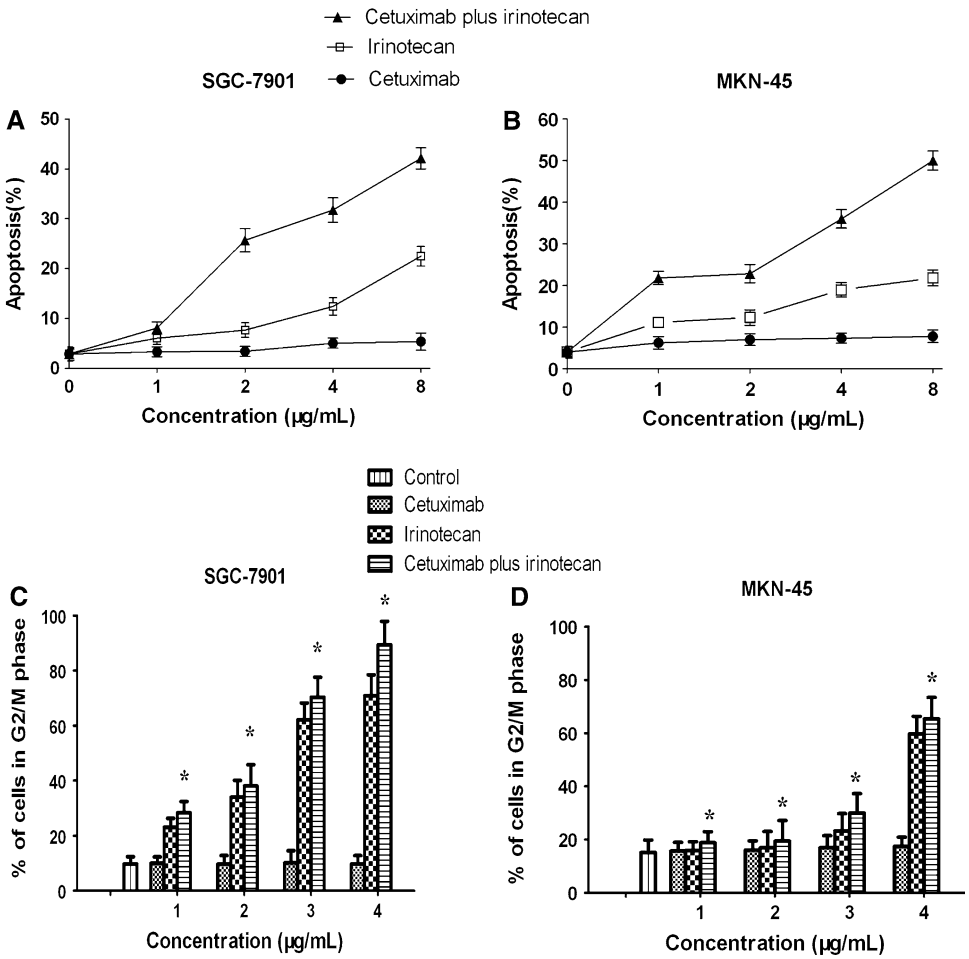

Fig. 2 Effect of the different concentrations of cetuximab, irinotecan, and combination of cetuximab and irinotecan on apoptosis and cell cycle arrest in gastric cancer cells. **a**, **b** Dose-dependent pro-apoptotic curves of SGC-7901 (**a**) and MKN-45 (**b**) cells treated with increasing doses of cetuximab and irinotecan alone or the combination. The results were expressed as the ratio of apoptotic to total cells. **c**, **d** The proportion of cells in G2/M phase after treatment with cetuximab and irinotecan alone or the combination in SGC-7901 **c** and MKN-45 **d** cells. The data shown represent the median values of triplicate experiments ± SD (*$P < 0.05$ for the comparison between combination therapy and irinotecan monodrug)

dependent growth-inhibitory activities while cetuximab exerted few effects, on cell proliferation, for both cell lines. However, it was observed that the addition of cetuximab enhances the inhibition effect of irinotecan to both cell lines, and the addition of cetuximab reduced the IC_{50} value of irinotecan from 7.35 to 3.61 μg/mL (51% reduction) and 5.83 to 2.97 μg/mL (49% reduction), in SGC-7901 and MKN-45 cells, respectively. Moreover, the fractional effect-combination index (Fa-CI) plots of interactions between cetuximab and irinotecan revealed synergistic interactions (as judged by calculated CI values of less than 1) in both cell lines as shown in Fig. 1C, D, suggesting cetuximab and irinotecan have a synergistic effect on GC cell proliferation.

Cetuximab enhances the pro-apoptotic, cell cycle arrest effects of irinotecan in GC cells

We next determined whether the synergistic growth-inhibitory effect of cetuximab and irinotecan could induce apoptosis and cell cycle arrest in GC cells. No significant apoptotic effect of cetuximab was observed in either cell line while irinotecan induced apoptotic cell death in a dose-dependent manner in both cell lines. The addition of cetuximab to irinotecan, however, increased the pro-apoptotic effect of irinotecan in all combinations tested in both cell lines (Fig. 2A, B). Treatment with cetuximab does not alter the cell cycle distribution compared to the untreated control in either cell line, while treatment with irinotecan was characterized by significant G2/M phase arrest in a dose-dependent manner. The combination of cetuximab and irinotecan resulted in a significant increase in cells arresting in G2/M phase when compared to either single agent alone (Fig. 2C, D).

Cetuximab does not enhance irinotecan-induced senescence in GC cells

Cetuximab had no effect on senescence for either cell line while irinotecan induced significant senescence in both cell lines compared to the untreated control cells. No significant synergy on senescence was noted with the addition of cetuximab to irinotecan at all dose level in both cell lines (Fig. 3).

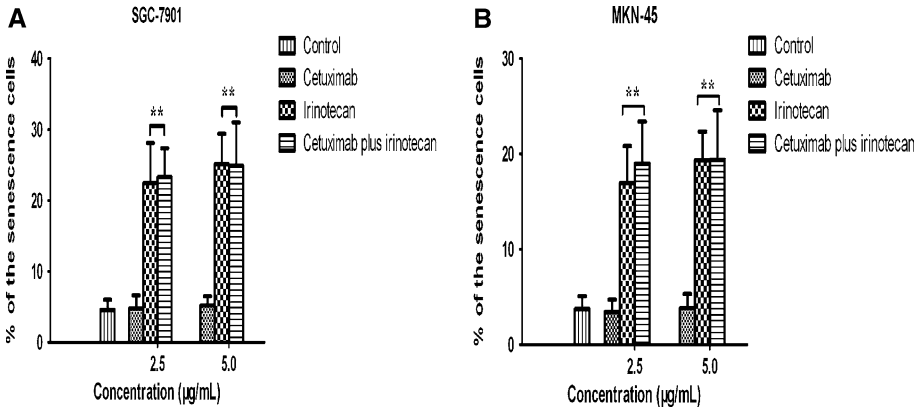

Fig. 3 Effect of the different concentrations of cetuximab, irinotecan and combination of cetuximab and irinotecan on senescence in SGC-7901 (**a**) and MKN-45 (**b**) cells. The results were expressed as the ratio of senescent to total cells. The data shown represent the median values of triplicate experiments ± SD (**$P > 0.05$ for the comparison between combination therapy and irinotecan monodrug which shows no significant difference)

Effects of cetuximab and irinotecan on p53, p16, and EGFR phosphorylation in GC cells

We examined the effect of cetuximab and irinotecan as single agent or combination on p53 and p16 expression, and EGFR phosphorylation level. The results indicate that either drug alone or in combination does not interfere the expression level of p53 and p16 in both cell lines (Fig. 4 and 5). In contrast, irinotecan significantly enhanced the phosphorylation levels of EGFR, which is the activated status of EGFR, in a dose-dependent manner, while cetuximab significantly decreases the phosphorylation levels of EGFR and abrogates the upregulation of phosphorylated EGFR induced by irinotecan in both cell lines (Fig. 4 and 5).

Effects of cetuximab and irinotecan on EGFR signaling in GC cells

Next, we analyzed the effects of cetuximab and irinotecan on the downstream signaling pathways of EGFR in the two GC cell lines. Cetuximab significantly inhibited the phosphorylation levels of AKT and MAPK at two different doses. On the contrary, irinotecan significantly enhanced these phosphorylations in a dose-dependent manner. However, these upregulations were abrogated when cetuximab was combined with irinotecan at both dose combinations. There were no significant changes in the total levels of EGFR, AKT, and MAPK noted with cetuximab or irinotecan as single agent or in combination, indicative of the exclusive influence on the activated proteins. There were also no significant changes in the level of PTEN, the inhibitor of AKT phosphorylation, with either drug alone or in combination (Fig. 4 and 5). These findings indicated cetuximab could inhibit the irinotecan-induced EGFR signaling pathway activation in GC cells.

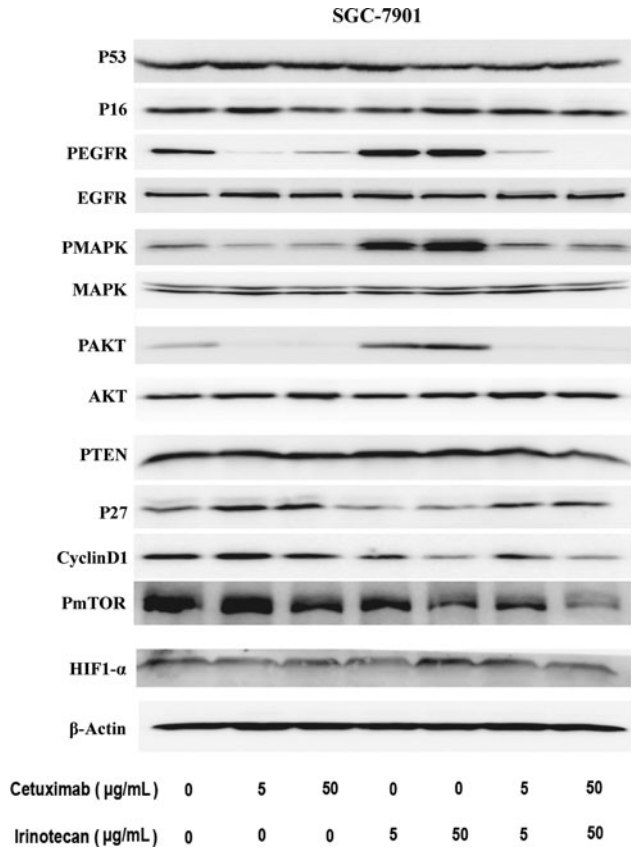

Fig. 4 Expression level of p53, p16, and EGFR phosphorylation and its downstream proteins by Western blot analysis for the effects of cetuximab or irinotecan as single agents or in combination in SGC-7901 cells. β-Actin was used as a loading control

Effects of cetuximab and irinotecan on downstream molecules of AKT signaling pathway in GC cells

Downstream molecules of AKT were investigated since the activation of AKT results in phosphorylation of a wide

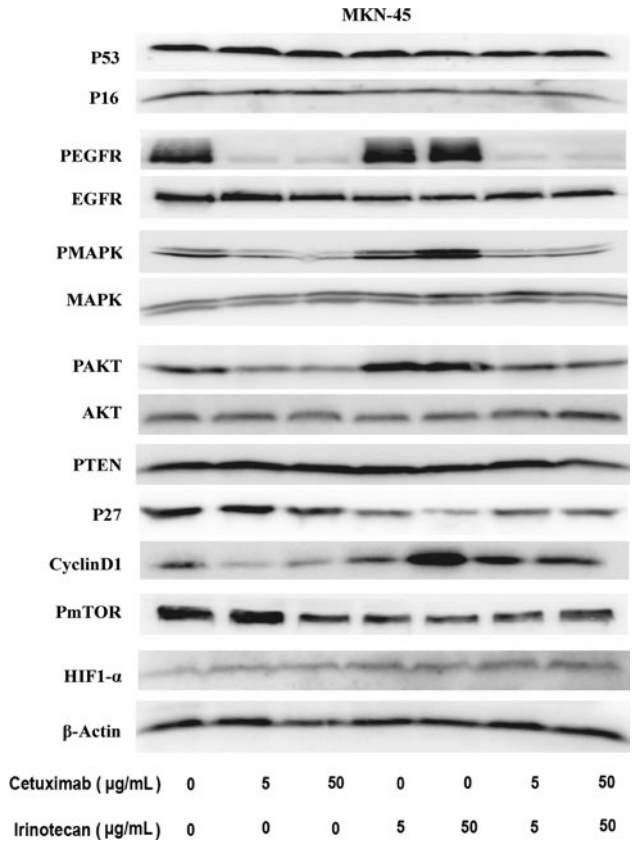

Fig. 5 Expression level of p53, p16, and EGFR phosphorylation and its downstream proteins by Western blot analysis for the effects of cetuximab or irinotecan as single agents or in combination in MKN-45 cells. *β*-Actin was used as a loading control

range of protein substrates. As shown in Fig. 4 and 5, the expression of $P27^{Kip1}$ was increased by treatment with cetuximab and decreased by treatment with irinotecan. However, the expression of $P27^{Kip1}$ could be restored when cetuximab was added to irinotecan in both cell lines. Cetuximab was found to inhibit the expression of Cyclin D1 in both cell lines, but the level of Cyclin D1 was noted to decrease in SGC-7901 cells and increase in MKN-45 cells after treatment with irinotecan. The phosphorylation levels of mammalian target of rapamycin (mTOR) were inhibited by cetuximab or irinotecan alone or the combination, and a more pronounced decrease was observed after combination treatment when compared to single agent treatment in both cell lines. There was no change in the expression level of HIF-1alpha.

Discussion

EGFR plays an important role in GC. Kim et al. reported that a subgroup of GC cases with EGFR overexpression is associated with the presence of lymph node metastasis, a higher stage, lymphatic invasion, and unfavorable prognosis [16]. Thus, EGFR inhibitors, especially combined with cytotoxic agents, may have potential therapeutic use in AGC. Our findings revealed minimal effect of cetuximab alone on human GC cells in regard of proliferation, apoptosis, and cell cycle distribution. It is consistent with the studies in other tumor types such as colorectal cancer and thyroid carcinoma which found that cetuximab alone had very slight antiproliferative effect, but when given in vivo as single-agent therapy, cetuximab was able to produce significant inhibition of tumor growth [17, 18]. A possible explanation for this discordance between in vitro and in vivo activities of cetuximab may be the potential for cetuximab to induce antibody-dependent cellular cytotoxicity and antiangiogenic property.

The combination index method was used to assess the activity of cetuximab combined with irinotecan. The results showed that the concurrent use of the two agents interacted synergistically to produce their antiproliferative effects in GC cells. Furthermore, a significant potentiation of apoptosis and G2/M phase arrest was observed with the addition of cetuximab to irinotecan in both cell lines, which suggests that the enhanced antitumor effects of irinotecan by cetuximab are attributable to apoptosis and cell cycle arrest. A possible explanation for this may be that cetuximab-induced EGFR-mediated signaling blockade results in the impairment of effective DNA repair and recovery [19, 20], which amplified the effect of irinotecan on apoptotic and cell cycle arrest. In addition, accumulation of intracellular irinotecan through the inhibition of drug efflux following the inhibition of EGFR signaling pathway [21, 22] may partially explain the observed increases in apoptosis and cell cycle arrest with combination therapy.

Emerging evidence suggests cellular senescence acts as an in vivo tumor suppression mechanism by limiting aberrant proliferation and has been shown to play a central role in antitumor effect of anticancer agents and ionizing radiation [23, 24]. Hotta K et al. reported cellular senescence might be a major antitumor mechanism of gefitinib in NSCLC cells [25]. To our knowledge, we are the first to show that irinotecan could induce significant cellular senescence in human GC cells in a dose-dependent manner while cetuximab did not have a role in senescence, whether as single agent or in combination with irinotecan, which indicates that cellular senescence is not involved in the synergy between two agents.

P53 and P16 are important tumor suppressor proteins and important regulators of cell apoptosis, senescence, and cell cycle progression. The Western blot analysis showed both two agents did not exert their effects through these two proteins. In the present study, cetuximab could inhibit irinotecan-induced upregulation of the EGFR phosphorylation significantly. Kishida et al. also found EGF signaling

is enhanced by SN38 (the active metabolite of irinotecan) in GC cells which could be abrogated by combined with gefitinib (EGFR tyrosine kinase inhibitor). The SN38-triggered mechanisms include the generation of reactive oxygen species and the activation of protein kinase C, followed by metalloproteinase activation and the sequential ectodomain shedding of EGFR ligands [26].

Remarkable elevations and abrogations of the level of phosphorylated MAPK and AKT treated with irinotecan with and without cetuximab, respectively, were observed in our study. Cetuximab or irinotecan, alone or in combination, does not alter the expression level of PTEN, a tumor suppressor and the inhibitor of AKT phosphorylation [27], further suggesting that cetuximab and irinotecan might influence AKT phosphorylation through the EGFR-PI3 K-AKT pathway, but not PTEN. These findings suggested that upregulation of EGFR pathway might be an important mechanism of irinotecan drug resistance in GC cells, which could result in DNA repair, apoptosis blockage, cells survival and resistance to the inhibitory effect of irinotecan. Inhibition of the EGFR pathway by cetuximab could overcome this resistance by abrogating these effects and therefore sensitizes GC cells to irinotecan.

The further study was done to investigate which downstream pathways of AKT were involved in the synergistic response to cetuximab/irinotecan combination. It had been reported in hepatocellular cancer [28] and oral squamous carcinoma cells [29] that cetuximab could upregulate $P27^{Kip1}$, however, there is no study examined the influence of irinotecan on $P27^{Kip1}$, which is a cyclin-dependent kinase inhibitor and inhibits cell cycle progression and induces cell apoptosis [30]. Our data showed that downregulation of $P27^{Kip1}$ by irinotecan alone could be restored when cetuximab was added in GC cells. The downregulation of $P27^{Kip1}$ by irinotecan could promote cancer cell proliferation, block cancer cells apoptosis, and impair the effects of irinotecan. We concluded that the recovery of $P27^{Kip1}$ activity by the addition of cetuximab to irinotecan contributes to the synergistic interactions between the two agents and $P27^{Kip1}$ deserves further research as an attractive biomarker to predict the efficacy of cetuximab/irinotecan therapy.

The results from this study showed that the expression of Cyclin D1, which could promote cell cycle progression [31], decreased after treatment with cetuximab in both cell lines, but downregulated in SGC-7901 cells, and upregulated in MKN-45 cells after treatment with irinotecan. This difference might originate from genetic disparity between the two cell lines that were not explored in the present study. However, cetuximab combined with irinotecan produced a more pronounced decrease in Cyclin D1 in SGC-7901 cells and abrogated the irinotecan-induced upregulation of Cyclin D1 in MKN-45 cells, both result in synergistic inhibition of cell proliferation.

The mTOR, located downstream of the PI3 K/AKT signal, which plays a key role in cellular growth, is a target for anticancer therapy [32]. Our results showed both cetuximab and irinotecan could decrease the phosphorylation levels of mTOR, which suggests irinotecan interferes mTOR through diverse molecules besides AKT. And there was a more enhanced inhibition with combination treatment in both cell lines, which may also contribute to the synergy between cetuximab and irinotecan. mTOR positively regulates HIF-1alpha, a transcriptional regulator of VEGF expression. Although several studies showed cetuximab downregulates HIF-1alpha levels [33, 34], no remarkable changes in the HIF-1alpha levels was noted in our study, indicative of the existence of multiple modulating proteins other than mTOR for HIF-1alpha.

In conclusion, the present study showed that many molecules are involved in the synergistic antiproliferative, pro-apoptotic, cell cycle arrest effects between cetuximab and irinotecan in GC cells, including phosphorylated EGFR, MAPK, AKT, mTOR, $P27^{Kip1}$, and Cyclin D1. To date, there are no target agents that have been proved effective in GC. These findings suggest the combination of cetuximab with irinotecan may be a potential therapeutic approach for this fatal disease. Further studies are much needed, both basic and clinical. In our institute, a phase II clinical trial based on the results of the present study to evaluate the efficacy and safety of cetuximab plus irinotecan as second-line chemotherapy for AGC patients is ongoing (NCT00699881) and the potential biomarkers such as $P27^{Kip1}$ and Cyclin D1 will be investigated.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P (2005) Global cancer statistics, 2002. CA Cancer J Clin 55:74–108
2. Rivera F, Vega-Villegas ME, Lopez-Brea MF (2007) Chemotherapy of advanced gastric cancer. Cancer Treat Rev 33:315–324
3. Ciardiello F, Tortora G (2008) EGFR antagonists in cancer treatment. N Engl J Med 358:1160–1174
4. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351:337–345
5. LaBonte MJ, Manegold PC, Wilson PM, Fazzone W, Louie SG, Lenz HJ, Ladner RD (2009) The dual EGFR/HER-2 tyrosine kinase inhibitor lapatinib sensitizes colon and gastric cancer cells to the irinotecan active metabolite SN-38. Int J Cancer 125:2957–2969

6. Han SW, Oh DY, Im SA, Park SR, Lee KW, Song HS, Lee NS, Lee KH, Choi IS, Lee MH, Kim MA, Kim WH, Bang YJ, Kim TY (2009) Phase II study and biomarker analysis of cetuximab combined with modified FOLFOX6 in advanced gastric cancer. Br J Cancer 100:298–304
7. Pinto C, Di Fabio F, Barone C, Siena S, Falcone A, Cascinu S, Rojas Llimpe FL, Stella G, Schinzari G, Artale S, Mutri V, Giaquinta S, Giannetta L, Bardelli A, Martoni AA (2009) Phase II study of cetuximab in combination with cisplatin and docetaxel in patients with untreated advanced gastric or gastro-oesophageal junction adenocarcinoma (DOCETUX study). Br J Cancer 101:1261–1268
8. Pinto C, Di Fabio F, Siena S, Cascinu S, Rojas Llimpe FL, Ceccarelli C, Mutri V, Giannetta L, Giaquinta S, Funaioli C, Berardi R, Longobardi C, Piana E, Martoni AA (2007) Phase II study of cetuximab in combination with FOLFIRI in patients with untreated advanced gastric or gastroesophageal junction adenocarcinoma (FOLCETUX study). Ann Oncol 18:510–517
9. Osaki M, Kase S, Adachi K, Takeda A, Hashimoto K, Ito H (2004) Inhibition of the PI3 K-Akt signaling pathway enhances the sensitivity of Fas-mediated apoptosis in human gastric carcinoma cell line, MKN-45. J Cancer Res Clin Oncol 130:8–14
10. Koizumi F, Kanzawa F, Ueda Y, Koh Y, Tsukiyama S, Taguchi F, Tamura T, Saijo N, Nishio K (2004) Synergistic interaction between the EGFR tyrosine kinase inhibitor gefitinib (“Iressa”) and the DNA topoisomerase I inhibitor CPT-11 (irinotecan) in human colorectal cancer cells. Int J Cancer 108:464–472
11. Horikawa Y, Otaka M, Komatsu K, Jin M, Odashima M, Wada I, Matsuhashi T, Ohba R, Oyake J, Hatakeyama N, Dubois RN, Watanabe S (2007) MEK activation suppresses CPT11-induced apoptosis in rat intestinal epithelial cells through a COX-2-dependent mechanism. Dig Dis Sci 52:2757–2765
12. Zhang J, Ji J, Yuan F, Ma T, Ye ZB, Yu YY, Liu BY, Zhu ZG (2009) EGFR-blockade by antibody Cetuximab inhibits the growth of human gastric cancer xenograft in nude mice and its possible mechanism. Zhonghua Zhong Liu Za Zhi 31:85–89
13. Lin CH, Fu ZM, Liu YL, Yang JL, Xu JF, Chen QS, Chen HM (1984) Investigation of SGC-7901 cell line established from human gastric carcinoma cells. Chin Med J (Engl) 97:831–834
14. Yokozaki H (2000) Molecular characteristics of eight gastric cancer cell lines established in Japan. Pathol Int 50:767–777
15. 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, Zalcberg JR (2008) K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 359:1757–1765
16. Kim MA, Lee HS, Lee HE, Jeon YK, Yang HK, Kim WH (2008) EGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number. Histopathology 52:738–746
17. Kim S, Prichard CN, Younes MN, Yazici YD, Jasser SA, Bekele BN, Myers JN (2006) Cetuximab and irinotecan interact synergistically to inhibit the growth of orthotopic anaplastic thyroid carcinoma xenografts in nude mice. Clin Cancer Res 12:600–607
18. Balin-Gauthier D, Delord JP, Rochaix P, Mallard V, Thomas F, Hennebelle I, Bugat R, Canal P, Allal C (2006) In vivo and in vitro antitumor activity of oxaliplatin in combination with cetuximab in human colorectal tumor cell lines expressing different level of EGFR. Cancer Chemother Pharmacol 57:709–718
19. Huang SM, Harari PM (2000) Modulation of radiation response after epidermal growth factor receptor blockade in squamous cell carcinomas: inhibition of damage repair, cell cycle kinetics, and tumor angiogenesis. Clin Cancer Res 6:2166–2174
20. Balin-Gauthier D, Delord JP, Pillaire MJ, Rochaix P, Hoffman JS, Bugat R, Cazaux C, Canal P, Allal BC (2008) Cetuximab potentiates oxaliplatin cytotoxic effect through a defect in NER and DNA replication initiation. Br J Cancer 98:120–128
21. Erlichman C, Boerner SA, Hallgren CG, Spieker R, Wang XY, James CD, Scheffer GL, Maliepaard M, Ross DD, Bible KC, Kaufmann SH (2001) The HER tyrosine kinase inhibitor CI1033 enhances cytotoxicity of 7-ethyl-10-hydroxycamptothecin and topotecan by inhibiting breast cancer resistance protein-mediated drug efflux. Cancer Res 61:739–748
22. Nakamura Y, Oka M, Soda H, Shiozawa K, Yoshikawa M, Itoh A, Ikegami Y, Tsurutani J, Nakatomi K, Kitazaki T, Doi S, Yoshida H, Kohno S (2005) Gefitinib (“Iressa”, ZD1839), an epidermal growth factor receptor tyrosine kinase inhibitor, reverses breast cancer resistance protein/ABCG2-mediated drug resistance. Cancer Res 65:1541–1546
23. Campisi J (2005) Aging, tumor suppression and cancer: high wire-act!. Mech Ageing Dev 126:51–58
24. Han Z, Wei W, Dunaway S, Darnowski JW, Calabresi P, Sedivy J, Hendrickson EA, Balan KV, Pantazis P, Wyche JH (2002) Role of p21 in apoptosis and senescence of human colon cancer cells treated with camptothecin. J Biol Chem 277:17154–17160
25. Hotta K, Tabata M, Kiura K, Kozuki T, Hisamoto A, Katayama H, Takigawa N, Fujimoto N, Fujiwara K, Ueoka H, Tanimoto M (2007) Gefitinib induces premature senescence in non-small cell lung cancer cells with or without EGFR gene mutation. Oncol Rep 17:313–317
26. Kishida O, Miyazaki Y, Murayama Y, Ogasa M, Miyazaki T, Yamamoto T, Watabe K, Tsutsui S, Kiyohara T, Shimomura I, Shinomura Y (2005) Gefitinib (“Iressa”, ZD1839) inhibits SN38-triggered EGF signals and IL-8 production in gastric cancer cells. Cancer Chemother Pharmacol 55:393–403
27. Keniry M, Parsons R (2008) The role of PTEN signaling perturbations in cancer and in targeted therapy. Oncogene 27:5477–5485
28. Kiyota A, Shintani S, Mihara M, Nakahara Y, Ueyama Y, Matsumura T, Tachikawa T, Wong DT (2002) Anti-epidermal growth factor receptor monoclonal antibody 225 upregulates p27(KIP1) and p15(INK4B) and induces G1 arrest in oral squamous carcinoma cell lines. Oncology 63:92–98
29. Huether A, Hopfner M, Baradari V, Schuppan D, Scherubl H (2005) EGFR blockade by cetuximab alone or as combination therapy for growth control of hepatocellular cancer. Biochem Pharmacol 70:1568–1578
30. Grimmler M, Wang Y, Mund T, Cilensek Z, Keidel EM, Waddell MB, Jakel H, Kullmann M, Kriwacki RW, Hengst L (2007) Cdk-inhibitory activity and stability of p27Kip1 are directly regulated by oncogenic tyrosine kinases. Cell 128:269–280
31. Kim JK, Diehl JA (2009) Nuclear cyclin D1: an oncogenic driver in human cancer. J Cell Physiol 220:292–296
32. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307:1098–1101
33. Luwor RB, Lu Y, Li X, Mendelsohn J, Fan Z (2005) The anti-epidermal growth factor receptor monoclonal antibody cetuximab/C225 reduces hypoxia-inducible factor-1 alpha, leading to transcriptional inhibition of vascular endothelial growth factor expression. Oncogene 24:4433–4441
34. Li X, Lu Y, Liang K, Pan T, Mendelsohn J, Fan Z (2008) Requirement of hypoxia-inducible factor-1alpha down-regulation in mediating the antitumor activity of the anti-epidermal growth factor receptor monoclonal antibody cetuximab. Mol Cancer Ther 7:1207–1217