Synergistic Antitumor Activity of Cetuximab and Namitecan in Human Squamous Cell Carcinoma Models Relies on Cooperative Inhibition of EGFR Expression and Depends on High EGFR Gene Copy Number

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Abstract

Purpose: Despite the frequent overexpression of epidermal growth factor receptor (EGFR) in squamous cell carcinoma (SCC), the efficacy of cetuximab alone is limited. Given the marked activity of namitecan, a hydrophilic camptothecin, against SCC models, the present study was performed to explore the efficacy of the cetuximab–namitecan combination in a panel of SCC models.

Experimental Design: We examined the antiproliferative and antitumor activities of the cetuximab–namitecan combination in four SCC models characterized by a different EGFR gene copy number/EGFR protein level. We also assessed the effects of the combination on EGFR expression at both mRNA and protein levels and investigated the molecular basis of the interaction between the two agents.

Results: Cetuximab and namitecan exhibited synergistic effects, resulting in potentiation of cell growth inhibition and, most importantly, enhanced therapeutic efficacy, with high cure rates in three SCC models characterized by high EGFR gene copy number, without increasing toxicity. The synergistic antitumor effect was also observed with the cetuximab–irinotecan combination. At the molecular level, the two agents produced a cooperative effect resulting in complete downregulation of EGFR. Interestingly, when singly administered, the camptothecin was able to strongly decrease EGFR expression mainly by transcriptional inhibition.

Conclusions: Our results (i) demonstrate a marked efficacy of the cetuximab–namitecan combination, which reflects a complete abrogation of EGFR expression as a critical determinant of the therapeutic improvement, in SCC preclinical models, and (ii) suggest EGFR gene copy number as a possible marker to be used for patient selection in the clinical setting. Clin Cancer Res; 20(4); 995–1006. ©2013 AACR.
Translational Relevance

The epidermal growth factor receptor (EGFR) is a validated therapeutic target in many human cancers. In spite of the frequent EGFR overexpression in squamous cell carcinoma (SCC), the anti-EGFR antibody cetuximab, as a single agent, exhibits marginal efficacy. Our study shows that a combination including cetuximab and a topoisomerase I inhibitor, namitane or irinotecan, produced synergistic effects, resulting in complete regression of SCC preclinical models characterized by high EGFR gene copy number. The result was achieved at well-tolerated doses, thus indicating a good therapeutic index. The study also provides a molecular basis for a rational combination to be exploited for possible therapeutic applications in the clinical setting, and suggests EGFR gene copy number as a possible marker for patient selection.

inhibit EGFR: tyrosine kinase inhibitors (TKI) and anti-EGFR monoclonal antibodies (moAb). TKIs target the intracellular tyrosine kinase domain of the receptor by competing for the ATP-binding pocket, thus inhibiting phosphorylation of the receptor and its downstream targets. MoAbs, such as cetuximab, disrupt the EGFR signaling pathway through inhibition of ligand binding and induction of receptor internalization followed by degradation in lysosomes (16). In addition, cetuximab binding to the extracellular EGFR domain promotes the activation of anti-body-dependent cellular cytotoxicity (17). Despite the role of EGFR in the malignant phenotype, therapy with EGFR targeting agents only exhibited limited efficacy. Indeed, no clinical responses were reported following cetuximab monotherapy in cervical SCC (18) and a marginal efficacy of the moAb was observed in patients with head and neck SCC with metastatic disease (13% response rate; ref. 19). However, cetuximab is the first targeted therapeutic agent to show a significant improvement in the overall survival for patients with locally advanced head and neck SCC when used in combination with radiation (20) or for recurrent or metastatic disease when used in combination with chemotherapy (21).

We recently reported that namitane, a novel hydrophilic camptothecin analog, exhibited antitumor activity in a large panel of human tumor models (22, 23) and an excellent activity, superior to that of irinotecan, in the treatment of SCC models (23). Because EGFR expression has been implicated as a determinant of response to radiation and to chemotherapy (24–26), the present study was performed to explore the therapeutic potential of cetuximab–namitane combination against four SCC models characterized by different levels of EGFR expression and to elucidate the role of EGFR in response to the namitane-containing therapy. For comparative purpose, we also assessed the effect of cetuximab–irinotecan combination. The results provided evidence of a synergistic antitumor activity of cetuximab–topoisomerase I inhibitor (namitane or irinotecan) combination, with high cure rates, which was related to the level of EGFR protein expression/EGFR gene copy number of the tumor model. Ex vivo and cellular studies demonstrated that the main mechanism of such a synergistic interaction was the cooperative inhibition of EGFR expression induced by the two agents. Specifically, the EGFR downregulation induced by namitane and SN-38 seems to rely primarily on a marked and persistent transcriptional downregulation of gene expression. The early activation of p38 mitogen-activated protein kinase (MAPK), which in turn phosphorylates EGFR at Ser1046/1047, thereby inducing its degradation, also contributed to topoisomerase I inhibitor–induced EGFR inhibition.

Materials and Methods

Cell lines

Three human SCC lines derived from skin (A431) and uterine cervix (Caski and SiHa) and a topotecan-resistant subline of A431 (A431/topotecan; ref. 27) were used in the study. All the cell lines were cultured in RPMI-1640 media (Lonza) supplemented with 10% FBS and grown in a humidified incubator with 5% CO₂ at 37°C. Cell lines are periodically monitored for DNA profile of short tandem repeats analysis by the AmpFISTR Identifiler PCR amplification kit (Applied Biosystems).

Determination of c-MET and EGFR gene copy number variations

Genomic DNA was extracted using the iPrep Purification Instrument with the iPrep ChargeSwitch Forensic Kit (Invitrogen) according to the manufacturer’s protocol, and relative quantification was quantified using the Infinite 200 NanoQuant Spectrophotometer (Tecan).

MET and EGFR gene copy number variations were assessed using real-time PCR TaqMan Copy Number Assays (Applied Biosystems), using RNase P gene as endogenous control. Furthermore, MAD1L1 and CFTR genes, being located respectively on the p and q-arm of chromosome 7, were also used to exclude the presence of chromosome 7 polysomy. The assays were performed using the Applied Biosystems Viia 7 Real-Time PCR System (Applied Biosystems) according to the manufacturer’s protocol. Copy number variations of the target genes were determined as relative quantification (RQ) based on the ΔΔCt method and using control samples as calibrators with the ABI SDS software 1.1 (Applied Biosystems).

Mutational status analysis

Mutation status of KRAS, BRAF, KRAS, NRAS, PIK3CA, and EGFR genes was determined as detailed in Supplementary Methods.

Drugs

For in vitro studies, namitane (Sigma-Tau) and SN-38 and SB20580 and SB202190 (Sigma Chemical Company) were initially dissolved in dimethyl sulfoxide and then diluted in sterile saline before use. Cetuximab (Eribitux;
Merck Serono) was diluted in sterile saline before use. For in vivo studies, namitecan was dissolved using a magnetic stirrer in sodium lactate buffer (50 mmol/L) adjusted to pH 4.0 with the addition of hydrochloric acid. Irinotecan was dissolved in sterile distilled water. Both camtoptecins were administered i.v. in a volume of 10 mL/kg. Cetuximab was ready to use and was delivered intraperitoneally (i.p.) at the dose of 0.2 mL/mouse.

**Growth inhibition study**

The antiproliferative activity was evaluated after 72 hours of drug exposure by cell counting (27). Drug concentrations able to inhibit cell proliferation by 50% (IC\textsubscript{50}) and 20% (IC\textsubscript{20}) were calculated from dose–response curves.

**Antitumor activity study**

To generate tumor xenografts, exponentially growing cells (A431 and A431/topotecan, 10\textsuperscript{7} cells/mouse; SiHa 2.5 × 10\textsuperscript{7} cells/mouse, Caski 10\textsuperscript{7} cells/mouse) were s.c. injected into the mice flanks. For antitumor activity studies, groups of four/five mice bearing tumor implanted in both flanks were used. Tumor fragments were implanted on day 0, and tumor growth was followed by biweekly measurements of tumor diameters with a Vernier caliper. Tumor volume (TV) was calculated according to the formula TV (mm\textsuperscript{3}) = d × D/2, in which d and D are the shortest and the longest diameter, respectively. Treatment started 5 to 13 days after implant, when the tumors were just palpable, but established (TV > 80–90 mm\textsuperscript{3}). Namitecan, irinotecan, and cetuximab were administered every fourth day for four times. Cetuximab was given 1 hour after each administration of the camptothecin.

The efficacy of the drug treatment was assessed as (i) TV inhibition percentage (TV\textsubscript{I%}) in treated versus control mice, calculated as TV\textsubscript{I%} = 100 – [(mean TV treated/mean TV control) × 100]; (ii) complete responses (CR), i.e., complete disappearance of the tumors for at least 10 days. The toxicity of the drug treatment was determined as body weight loss and lethal toxicity. Deaths occurring in treated mice before the death of the first control mouse were ascribed to toxic effects.

**Antibodies and Western blot analysis**

The antibodies used in the study were anti-EGFR (Upstate Biotechnology); anti-vinculin (Sigma); anti-phospho EGFR (Ser1046/47), anti-phospho EGFR (Thr180/Tyr182; Cell Signaling Technology); anti-phospho p38 MAPK (Thr180/Tyr182; Cell Signaling Technology); anti-p38 MAPK and anti-c-Cbl (Santa Cruz Biotechnology). Western blot analysis was carried out as described previously (27) and as detailed in Supplementary Methods.

**Quantitative reverse-transcription PCR**

Total RNA was isolated from SCC cell lines using the RNAqueous-4PCR Kit (Ambion Europe Ltd.), according to the manufacturer’s instructions. EGFR mRNA expression was assessed by quantitative reverse-transcription-PCR (qRT-PCR) as detailed in Supplementary Methods.

**Immunofluorescence staining**

Immunofluorescence staining of EGFR and c-Cbl was carried out as detailed in Supplementary Methods.

**Data analyses**

In in vitro growth inhibition studies, the type of drug interaction was evaluated by the Chou and Talalay’s (28) method using the CalcuSyn software (Biosoft). Accordingly to it, combination index (CI) value (CI < 1, CI = 1, and >1) indicated synergism, additive effect, and antagonism, respectively. Synergism is further refined by CalcuSyn as synergism (CI = 0.3–0.7) and strong synergism (CI = 0.1–0.3). In antitumor activity studies, the Student t test and the Fisher exact test (two-tailed) were used for statistical comparison of TVs and complete responses, respectively, in mice.

**Results**

The panel of gynecologic SCC cell lines used in the study was characterized by different levels of EGFR expression at both protein (Fig. 1A) and mRNA (Fig. 1B) level. Specifically, strong EGFR overexpression was found in the A431 cell line and, although to a lesser extent, in its topotecan-resistant subline A431/topotecan, which was paralleled by a marked gain in EGFR gene copy number (Fig. 1B). Conversely, moderate or almost negligible EGFR protein levels were present in Caski and SiHa cell lines, respectively, in agreement with the small, although different increases in EGFR gene copy number (Fig. 1C). In addition, mutational analysis indicated that all cell lines harbored wild-type EGFR, KRAS, NRAS, BRAF, and PI3KCA genes, with the only exception of Caski cells in which a PI3KCA exon 9–activating mutation (p.E545K; ref. 29) was present (Fig. 1C). Finally, a similar MET copy number was observed in the different cell lines (Fig. 1C).

**The namitecan–cetuximab combination induced synergistic antitumor effects in SCC models as a function of EGFR gene copy number**

Given the hypersensitivity of SCC to namitecan (23), in the study we used a suboptimal dose of the drug (10 mg/kg, i.e., 1 of 3 of the maximum tolerated dose) to allow a comparison of single-drug treatment and combination of namitecan with the anti-EGFR antibody, cetuximab. Irinotecan was used as reference compound at 17 mg/kg (1 of 3 of the maximum tolerated dose). Under such treatment conditions, in the A431 model namitecan still produced a significant tumor growth inhibition (84%) with an appreciable number of complete tumor regressions (3 of 8). Cetuximab (1 mg/mouse) produced a good antitumor effect without evidence of complete tumor regression. The efficacy of the combination was impressive, because all animals exhibited complete tumor response with no evidence of disease at the end of the experiment (90 days after the last treatment; Fig. 2; Table 1). In an independent experiment, single-agent therapy with irinotecan resulted in a significant tumor growth inhibition (78%) with evidence of complete response in one tumor. Cetuximab showed a
good antitumor activity and induced 3 of 8 complete response. Combined cetuximab–irinotecan treatment resulted in complete tumor regression in all treated animals without any evidence of tumor regrowth until the end of experiment (90 days after the last treatment; Table 1; Supplementary Fig. S1).

The A431/topotecan subline, which was highly resistant to topotecan (27), was still responsive to namiecan at the low dose level, producing appreciable tumor growth inhibition but without complete tumor regression. Surprisingly, in spite of a reduced expression of the target EGFR, cetuximab was very effective in the control of tumor growth, resulting in 99% TVI and 6 of 8 complete responses. The addition of cetuximab to treatment with namitecan resulted in complete regression of all tumors, with no evidence of tumor regrowth in 8 of 8 animals at the end of the experiment (Fig. 2, Table 1). Irinotecan produced a 72% TVI without evidence of complete tumor regression. 8 of 8 animals treated with the combination irinotecan with cetuximab was less effective than the combination containing namitecan, at least in terms of complete response rate (Table 1 and Supplementary Fig. S1). This finding would suggest that the level of EGFR expression is more critical for irinotecan to achieve synergistic interaction.

The SiHa model, which is characterized by a very low level of EGFR expression, exhibited a lower responsiveness to both namitecan and cetuximab than other models. When animals were treated with the combination, only a slight not statistically significant increase in efficacy was observed, and no animal experienced tumor regression (Fig. 2, Table 1). It is important to emphasize that the curative efficacy of the cetuximab–namitecan (or irinotecan) combination was achieved at well-tolerated doses of each agent without evidence of appreciable toxicity (Table 1).
In vitro growth inhibition studies on the same SCC models showed a variable cellular sensitivity to cetuximab, with IC50 values ranging from 1.47 to 931 μg/mL (Supplementary Fig. S1A), which was directly correlated to EGFR protein expression/EGFR gene copy number of the tumor cell line. Conversely, no appreciable differences were observed in the sensitivity of the four cell lines to namitecan (Supplementary Fig. S2A). In combination studies, a synergistic interaction between the effects of cetuximab and namitecan, as determined by the Chou and Talalay method (28), was observed in A431, A431/topotecan, and Caski cells (Supplementary Fig. S2B). The extent of the synergistic effects was related to the level of EGFR protein expression/EGFR gene copy number of the cell line. Consistent with in vivo data, an almost complete abrogation of the protein expression was observed in all tumors exposed to the combined treatment (Fig. 3A).

Cetuximab and namitecan cooperate in inhibiting EGFR expression

In the search for possible molecular determinants of the cetuximab–namitecan synergistic interaction, we investigated therapy-induced changes in the expression levels of EGFR in A431, A431/topotecan, and SiHa xenografts. Western blot results showed that not only cetuximab but also namitecan, when singly administered, induced a marked reduction of EGFR levels, and that an almost complete abrogation of the protein expression was observed in all tumors exposed to the combined treatment (Fig. 3A).

Cellular studies were carried out to elucidate the mechanism through which namitecan inhibited EGFR expression. Consistent with in vivo findings, a 24-hour exposure to namitecan induced a dose-dependent decrease in EGFR expression in the different cell lines. In addition, results obtained in cells exposed to the drug combination confirmed a cooperative effect of namitecan and cetuximab in suppressing EGFR protein expression (Fig. 3B). Similar results were obtained when cells were treated with equimolar concentrations of SN-38 alone or in combination with cetuximab (Supplementary Fig. S3A).

Because it has been recently reported that topoisomerase I inhibition can trigger a transcriptional stress and consequently interfere with translation of specific genes, such as hypoxia-inducible factor-1α (HIF-1α), in human cancer cells (30), we assessed the ability of namitecan to modulate EGFR mRNA levels in treated cells. qRT-PCR
results showed that, in all cell lines, EGFR mRNA levels were decreased at 24 hours after treatment with namitecan. Conversely, no appreciable interference with EGFR mRNA levels was observed in cells exposed to cetuximab alone. In addition, the extent of the namitecan-induced reduction of EGFR mRNA levels was not significantly modified in the combination with cetuximab (Fig. 3C). As shown in Supplementary Fig. S3B, SN-38 was able to inhibit EGFR mRNA expression at a comparable extent. Overall, such data indicate that namitecan (or SN-38)-mediated topoisomerase I inhibition leads to EGFR mRNA downregulation.

Because the namitecan-induced effect on EGFR mRNA expression levels could not justify the almost complete abrogation of EGFR protein observed in treated SCC cells at 24 hours, we assessed the treatment-induced interference with EGFR cellular localization in A431 cells to define whether the decline of EGFR protein abundance was paralleled by an increased internalization and consequent ubiquitination of EGFR (Fig. 4A). As expected, EGFR was internalized after treatment with cetuximab (31). Interestingly, also namitecan showed the ability to induce EGFR internalization (Fig. 4A). Because it is known that the process of EGFR degradation is dependent on the ability of the E3 ubiquitin ligase c-Cbl to bind the receptor to its phosphorylated Tyr1045 residue (32), we examined whether, following drug-induced internalization, EGFR colocalizes with the c-Cbl protein in A431 cells. Unlike the well-characterized EGF-induced formation of EGFR–c-Cbl complexes (Supplementary Fig. S4), cetuximab and namitecan induced translocation of EGFR into intracellular vesicles, which did not colocalize with the c-Cbl protein (Fig. 4B), in accordance with recent evidence indicating that cetuximab induces internalization and subsequent ubiquitination of EGFR, recruiting an E3 ligase distinct from c-Cbl (31, 33).

Our fluorescence microscopy findings were further corroborated by immune coprecipitation assay results showing that namitecan and cetuximab did not induce EGFR binding to c-Cbl in spite of an increased EGFR ubiquitination (data not shown).

Because recent reports showed that some anticancer drugs cause EGFR degradation via phosphorylation at Ser1046/1047 residues by p38 MAPK in different tumor cell lines (32, 34, 35), and taking into account that irinotecan has been shown to activate p38 MAPK in HCT116 cell lines (36), we examined the effects induced by namitecan

<table>
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<th>Tumor</th>
<th>Drug</th>
<th>TVI%a</th>
<th>CRb</th>
<th>BWL%c (day)</th>
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<td>3/8</td>
<td>1</td>
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<tr>
<td></td>
<td>Cetuximab</td>
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<td>0/8</td>
<td>0</td>
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<td></td>
<td>Namitecan + cetuximab</td>
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<td>8/8</td>
<td>0</td>
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<td>3/8</td>
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<td>Irinotecan + cetuximab</td>
<td>100</td>
<td>8/8</td>
<td>3</td>
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<td>8/8</td>
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<td></td>
<td>Cetuximab</td>
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<td>0</td>
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<td></td>
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<td>8/8</td>
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<td>Namitecan + cetuximab</td>
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NOTE: Tumor fragments were implanted on both flanks at day 0. Treatment started when mean tumor volume was 80 to 90 mm³.

aTumor volume inhibition percentage in treated over control mice, determined 1 week after the last treatment.
bComplete responses, i.e., disappearance of tumor lasting at least 10 days.
cBody weight loss (BWL) percentage induced by treatment; the highest change is reported. No toxic death was observed.

⁎, P < 0.05; ⁎⁎, P < 0.01 by the Fisher exact test versus namitecan- and irinotecan-treated mice.

⁎⁎⁎, P < 0.001 by the Fisher exact test versus irinotecan-treated mice.
EGFR phosphorylation at Ser 1046/47 were observed when A431 cells were exposed to SN-38 (Supplementary Fig. S5A).

To better understand the role of activated p38 MAPK in namitecan-induced EGFR downmodulation, we used two specific inhibitors SB203580 (37) and SB202190 (36) in A431 and Caski cells. A 2-hour treatment of cells with SB203580 (10 \(\mu\)mol/L) or with SB202190 (5 \(\mu\)mol/L) before a 24-hour exposure to namitecan, alone or in association with cetuximab, strongly reduced EGFR phosphorylation at Ser1046/47 in A431 cells (Fig. 5B and Supplementary Fig. S5B and S5C). In addition, pretreatment with SB203580 or SB202190, which did not affect by itself EGFR expression levels, partially restored EGFR phosphorylation at Ser1046/47 in A431 cells (Fig. 5B and Supplementary Fig. S5B and S5C).
expression in namitecan-treated cells (Fig. 5B and Supplementary Fig. S5B and S5C). Similarly, pretreatment with p38 MAPK inhibitors was able to partially restore EGFR protein expression in A431 cells exposed to SN-38 for 24 hours (Supplementary Fig. S5B and S5C). Such a protective effect against namitecan-induced EGFR degradation was even more pronounced in Caski cells (Fig. 5C). Conversely, no appreciable effect of SB203580 and SB202190 pretreatment on cetuximab-induced EGFR downmodulation was found in either cell line (Fig. 5B and C and Supplementary Fig. S5B and S5C). Taken together, our findings support that namitecan and SN-38 promote the early activation of p38 MAPK, which in turn phosphorylates EGFR at Ser1046/47, thus inducing its degradation.

However, the contribution of drug-induced p38 MAPK activation to the overall inhibition of EGFR expression and consequently to the cetuximab–topoisomerase I inhibitor synergistic interaction seems to be limited. In fact, in A431 cells, a 48-hour exposure to namitecan (or SN-38) alone or in association with cetuximab was able to induce a decrease in EGFR mRNA levels of about 90% (Fig. 5D), which was accompanied by a complete abrogation of EGFR protein expression (Fig. 5E). Due to the lack of substrate available to be phosphorylated by p38 MAPK, pretreatment with the inhibitor SB203580 failed to rescue EGFR protein
expression in camptothecin-treated cells (Fig. 5E). In addition, the pretreatment with p38 inhibitor SB203580 failed to impair namitecan–cetuximab synergistic interaction (Supplementary Fig. S6).

Discussion

Although single-agent therapy with cetuximab shows a limited efficacy in the treatment of SCC (18), an improved benefit is expected when it is combined with conventional
cytotoxic agents (21, 38). In this context, novel rational strategies to improve the efficacy of combination treatments and to reduce drug-induced side effects are needed.

Our study provides evidence that cetuximab, when used in combination with namitene, induced a synergistic effect that resulted in potentiation of cell growth inhibition in 3 of the 4 SCC cell lines used in the study. The most interesting observation was the enhanced therapeutic efficacy seen in the treatment of SCC xenografts, in which the combination produced a curative effect in most treated animals. Consistent with in vitro findings, a synergistic effect of the combination was appreciable in tumor models characterized by a high EGFR gene copy number. In fact, only the SiHa tumor, which carries the lowest number of EGFR gene copies, did not exhibit a significant therapeutic benefit by the combination treatment, thus suggesting a marginal impact of the receptor tyrosine kinase in the growth of the tumor. A synergistic antitumor effect was also observed when cetuximab was combined with irinotecan only in the two highly EGFR expressing tumor models. The substantial improvement with the combined therapy was achieved without an increase in toxicity, because well-tolerated doses of each agent were used. SCC is known to be responsive to namicene, but curative efficacy requires treatment with maximum tolerated doses, which are associated with significant toxicity (22, 23).

The present study also provides valuable information on the molecular/cellular bases of the synergistic drug interaction. Specifically, the combination of cetuximab and namicene produced a cooperative effect resulting in a complete downregulation of EGFR. Although the effect of cetuximab on EGFR protein levels has been already described (31), the ability of topoisomerase I inhibitors to downregulate EGFR expression is somewhat unexpected. In a previous study, Liu and colleagues (45) found that only the serine residues are critical for EGFR internalization and degradation. Different agents such as UV radiation (46) and cisplatin (47), as well as oxidative stress (48), can induce internalization of the EGFR but not degradation. On the contrary, gemcitabine was found to cause ligand-independent internalization and degradation of EGFR (49).

Here, we report that both namicene and SN-38 induced an early p38 MAPK activation and EGFR phosphorylation at Ser1046/47 in SCC cells, consistent with a recent observation indicating that irinotecan treatment activates p38 MAPK in HCT 116 cell lines (36). The role of p38 MAPK as an early event in EGFR downregulation was confirmed through inhibition of p38 MAPK with the specific inhibitor SB203580 and SB202190, which by themselves did not affect the EGFR levels but partially or almost completely restored EGFR expression in namicene- and SN-38–treated SCC cells at 24 hours, without affecting cetuximab-induced EGFR downmodulation. At later time points when a complete abrogation of EGFR protein expression, as a consequence of the marked transcriptional downregulation of the gene, was observed, the relevance of p38 MAPK seemed negligible. Indeed, the pretreatment with p38 MAPK inhibitor SB203580 failed to rescue EGFR protein expression in namicene- and SN-38–treated cells. In addition, the marginal role of p38 MAPK in the drug synergistic interaction was confirmed by inability of SB203580 to impair the synergism. Conversely, cell exposure to SB203580 enhanced namicene cytotoxic activity, in keeping with
Synergistic Antitumor Activity of Cetuximab and Namitecan

The combination therapy, including cetuximab and a camptothecin, may be a promising approach for the therapy of SCC, with special reference to patients with tumor characterized by a high EGFR gene copy number.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: C. Pisano, F. Zunino, N. Zaffaroni, V. Zuco
Development of methodology: M. De Cesare, C. Lauricella, S.M. Veronese, D. Cominetti, V. Zuco
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. De Cesare, C. Lauricella, S.M. Veronese, D. Cominetti, V. Zuco
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. De Cesare, S.M. Veronese, F. Zunino, N. Zaffaroni, V. Zuco
Writing, review, and/or revision of the manuscript: S.M. Veronese, F. Zunino, N. Zaffaroni, V. Zuco
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): V. Zuco
Study supervision: F. Zunino, N. Zaffaroni

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