

N1-guanyl-1,7-diaminoheptane enhances the chemosensitivity of NSCLC cells to cetuximab through inhibition of eukaryotic translation initiation factor 5A2 activation

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Abstract. – OBJECTIVE: N1-guanyl-1, 7-diaminoheptane (GC7), an inhibitor of deoxyhypusine synthase has been shown to exhibit significant anti-cancer activity. However, the biological role of eukaryotic translation initiation factor 5A2 activation (EIF5A2) and GC7 on drug resistance in non-small cell lung cancer (NSCLC) has not been investigated. In this study, we aimed to investigate the therapeutic effect of GC7 combined with cetuximab in NSCLC therapy.

MATERIALS AND METHODS: The current study used cell viability assays, EdU incorporation assays, and western blot to detect that the GC7 exhibited synergistic cytotoxicity with cetuximab in NSCLC.

RESULTS: CCK-8 assays showed that combined treatment with GC7 and cetuximab significantly inhibited the viabilities in three NSCLC cell lines. In addition, EdU incorporation assays also indicated that GC7 co-treatment remarkably enhanced the cetuximab sensitivity in NSCLC cells. Nevertheless, down-regulation of EIF5A2 diminished the regulatory role of GC7 in cetuximab cytotoxicity. Western blot showed that transfection of EIF5A2 siRNA significantly suppressed the protein expression of EIF5A2 in NSCLC cells.

CONCLUSIONS: These findings demonstrate that combined treatment with GC7 could enhance cetuximab sensitivity by inhibiting EIF5A2 in NSCLC cells, implying the potential clinical application of GC7 in cetuximab-based chemotherapy for NSCLC patients.

Key Words:

Non-small cell lung cancer, N1-guanyl-1,7-diaminoheptane, Eukaryotic translation initiation factor 5A2, Cetuximab.

Introduction

Lung cancer is the leading cause of cancer-related death worldwide, in which 80% of lung cancers are non-small cell lung cancer (NSCLC) with poor therapeutic efficacy when diagnosed¹. In addition to surgical resection, chemotherapy serves as one of the important adjuvant therapies for lung cancer². However, the drug resistance has become a major obstacle in NSCLC treatment, and drawn much attention in recent years^{3,4}. Therefore, exploration of novel therapeutic strategies is important to sensitize tumor cell to anti-cancer drugs.

Eukaryotic translation initiation factor (EIF5A) is the only known cellular protein to contain the post-translationally derived amino acid hypusine [Nepsilon-(4-amino-2-hydroxybutyl)lysine]⁵. Increasing evidences have linked EIF5A to cell proliferation, invasion, metastasis, and cancer progression, suggesting that EIF5A may be a novel oncogene and a potential molecular target in human cancers⁶⁻⁸. EIF5A2, one isoform of EIF5A, mainly acts as an elongation factor during mRNA translation step⁵. Recent studies have shown that aberrant expression of EIF5A2 may be responsible for the malignant behavior of cancer cells^{9,10}. In addition, inhibition of EIF5A2 by N1-guanyl-1, 7-diaminoheptane (GC7), an inhibitor of deoxyhypusine synthase (DHS), has been shown to exhibit significant anti-cancer property¹¹. However, the biological role of EIF5A2 and GC7 on drug resistance in NSCLC has not been investigated. In this study, we aimed to investigate the therapeutic effect of GC7 combined with cetuximab in NSCLC therapy.

Materials and Methods

Cell Culture

The human NSCLC cell lines, A549, HCC827, and NCI-H1299, were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). All cells were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA) and 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA). The cells were maintained at 37°C in a humidified atmosphere of 5% CO₂.

CCK-8 Assay

Cell viability was measured by Cell Counting Kit-8 (CCK8; Dojindo, Kumamoto, Japan) according to the manufacturer's instruction. To be brief, tumor cells at the density of 5 × 10³ cells/well were seeded into 96-well plates and cultured for 24 h. CCK-8 solution (10 μL/well) was added and absorbance was measured at 450 nm using an MRX II microplate reader (Dyex Technologies, Chantilly, VA, USA). Relative cell viability was calculated as a percentage of untreated controls.

EdU Incorporation Assay

Cell viability was calculated as a percentage of untreated control. Measurement of inhibitive rate of cell proliferation was carried out using a Click-iT EdU Imaging Kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instruction.

Cell Transfection

Cells were transfected with EIF5A2 siRNA or negative control siRNA using Lipofectamine 2000 (Invitrogen, Waltham, MA, USA) according to the manufacturer's protocol. The transfection medium (Opti-MEM; Gibco, Big Cabin, OK, USA) was replaced with complete medium 12 h after transfection, and the cells were incubated for the indicated times.

Western Blotting Analysis

Total cell extracts were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a nitrocellulose filter membrane. Then the membranes were incubated with primary antibodies against EIF5A2 and GAPDH. GAPDH was used as a loading control. The membranes were then incubated with the appropriate HRP-conjugated secondary antibodies for 1 h at room temperature.

The proteins were visualized by the enhanced chemiluminescence method and intensity of protein bands was quantified by densitometry.

Statistical Analysis

Each experiment was performed in triplicate, and repeated at least three times. All the data were presented as means ± SD and treated for statistics analysis by SPSS program (SPSS Inc., Chicago, IL, USA). Comparison between groups was made using ANOVA and statistically significant difference was defined as $p < 0.05$.

Results

Effects of GC7 on NSCLC Cells Viability

We first conducted CCK-8 assay to assess the sensitivity of NSCLC cells to GC7. Three NSCLC cells lines including A549, HCC827, and NCI-H1299 were incubated with a series of GC7 concentrations ranging from 0-80 μM. The CCK-8 assay showed that GC7 exhibited little cytotoxicity in cancer cells at the doses of 1, 2.5, and 5 μM. Meanwhile, higher concentrations of GC7 (10, 20, 40, 80 μM) remarkably suppressed the viability of NSCLC cells including A549 (Figure 1A), HCC827 (Figure 1B), and NCI-H1299 (Figure 1C). Thus, we chose 5 μM of GC7 for the following study.

Combined Treatment with GC7 Sensitizes NSCLC Cells to Cetuximab

To determine the synergistic effects of cetuximab combined with GC7, CCK-8 assay was conducted to measure NSCLC cell viability treated with cetuximab alone or in combination with GC7. Results showed that co-treatment with cetuximab and GC7 significantly decreased the viabilities in all three NSCLC cells including A549 (Figure 2A), HCC827 (Figure 2B), and NCI-H1299 (Figure 2C). In addition, EdU incorporation assay was performed to validate the inhibitory role of GC7 in NSCLC cells. We also found that combined treatment with GC7 enhanced the sensitivity of cancer cells to cetuximab (Figure 3A-C). Taken together, these results demonstrated that GC7 could potentiate the cetuximab sensitivity in NSCLC cells.

Knockdown of EIF5A2 Diminished the Regulatory role of GC7 on Drug Resistance in NSCLC Cells

In order to explore the role of EIF5A2 in the chemoresistance, RNAi was used to knockdown

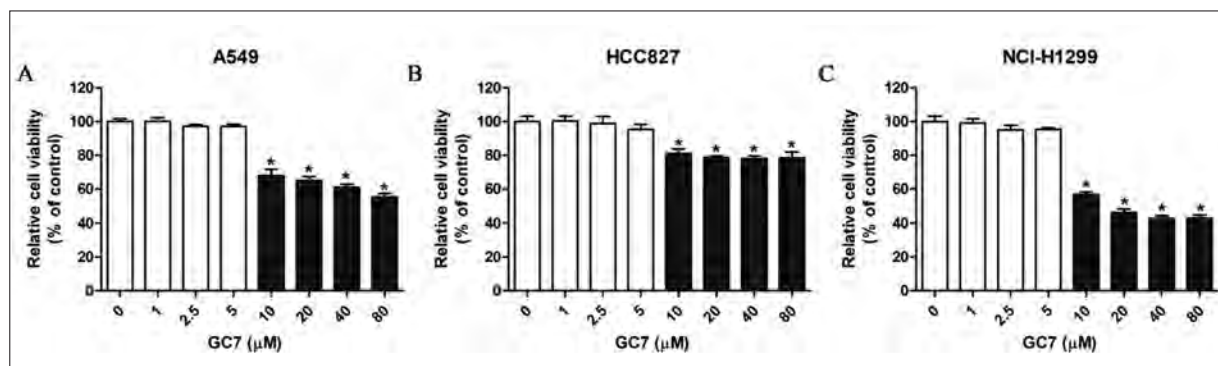


Figure 1. Effects of GC7 on NSCLC cells viability. Three NSCLC cell lines including A549 (A), HCC827 (B), and NCI-H1299 (C) were incubated with different concentrations of GC7 (1, 2.5, 5, 10, 20, 40, and 80 μM) for 48 h. The CCK8 values of the treated NSCLC cells were normalized to the control group with the absence of GC7. * $p < 0.05$.

the expression of EIF5A2 in NSCLC cells. The CCK-8 assay showed that the combined treatment with GC7 had little effects on cetuximab sensitivity in A549 (Figure 4A), HCC827 (Figure 4B), and NCI-H1299 (Figure 4C) transfected with EIF5A2 siRNA. Furthermore, Western

blot analysis revealed that transfection of EIF5A2 siRNA significantly suppressed the protein expression of EIF5A2 in NSCLC cells (Figure 4D). These results suggest that EIF5A2 plays an important role in the chemosensitivity in NSCLC cells.

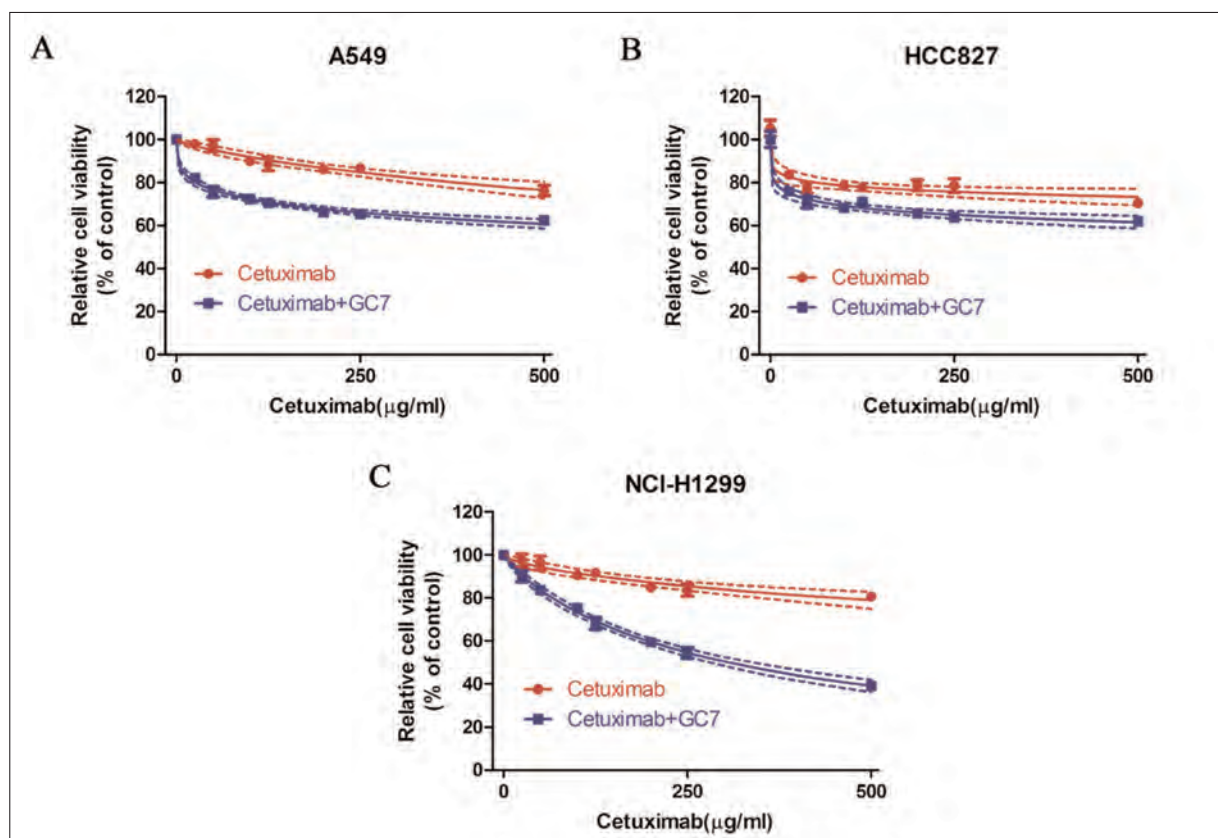


Figure 2. Cytotoxicity of cetuximab or cetuximab plus GC7 in NSCLC cells. Three NSCLC cell lines including A549 (A), HCC827 (B), and NCI-H1299 (C) were exposed to cetuximab alone or in combination with GC7 at the concentrations of 5 μM. CCK-8 assay was performed to determine cell viability treated with cetuximab alone or in combination with GC7.

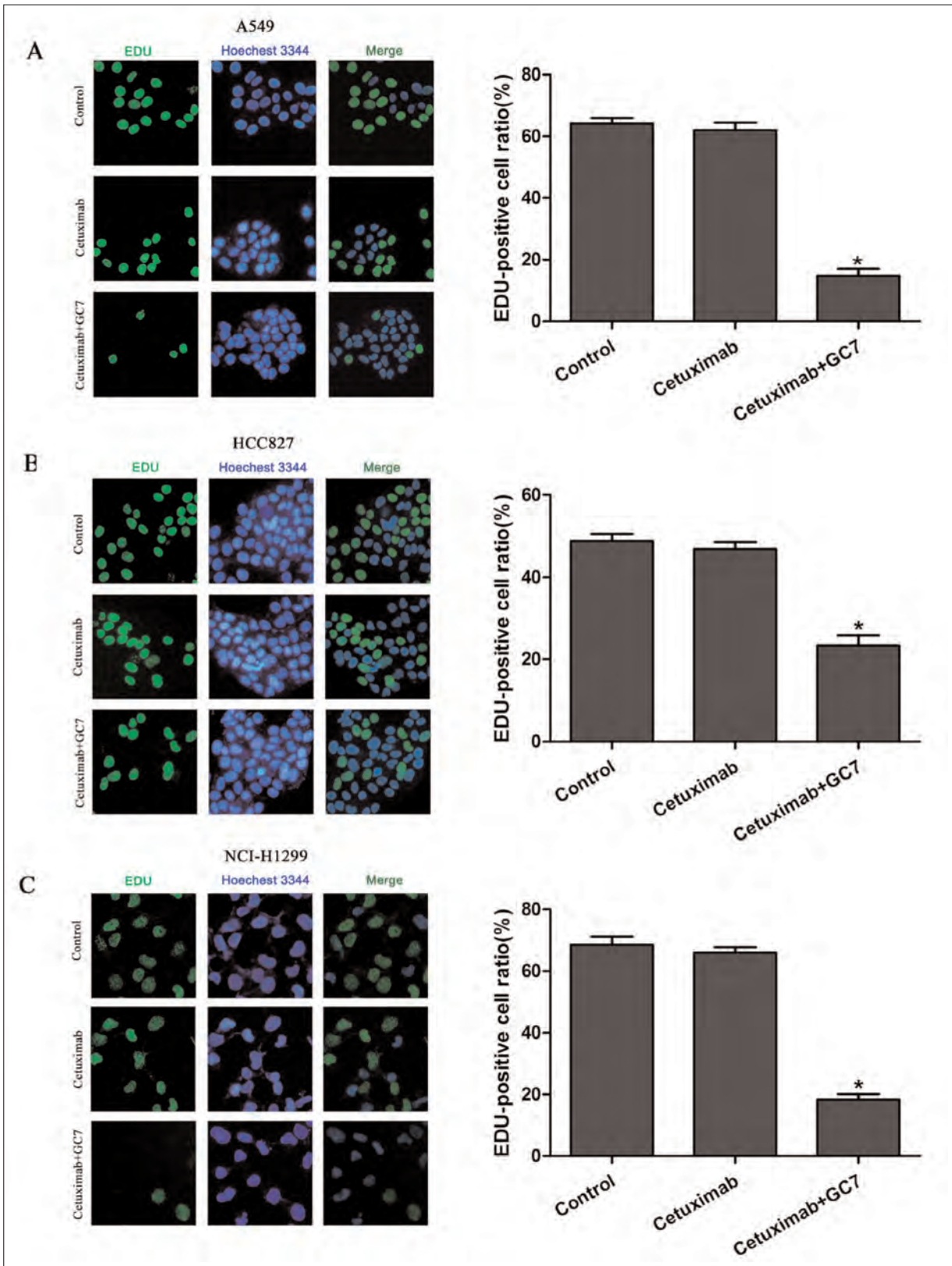


Figure 3. GC7 treatment increased the sensitivity of NSCLC cells to cetuximab. Photomicrographs and bar charts depict the EdU staining and relative EdU-positive ratio, respectively, of A549 (A), HCC827 (B), and NCI-H1299 (C) cell after treatment with cetuximab or cetuximab plus GC7 for 48 h. * $p < 0.05$.

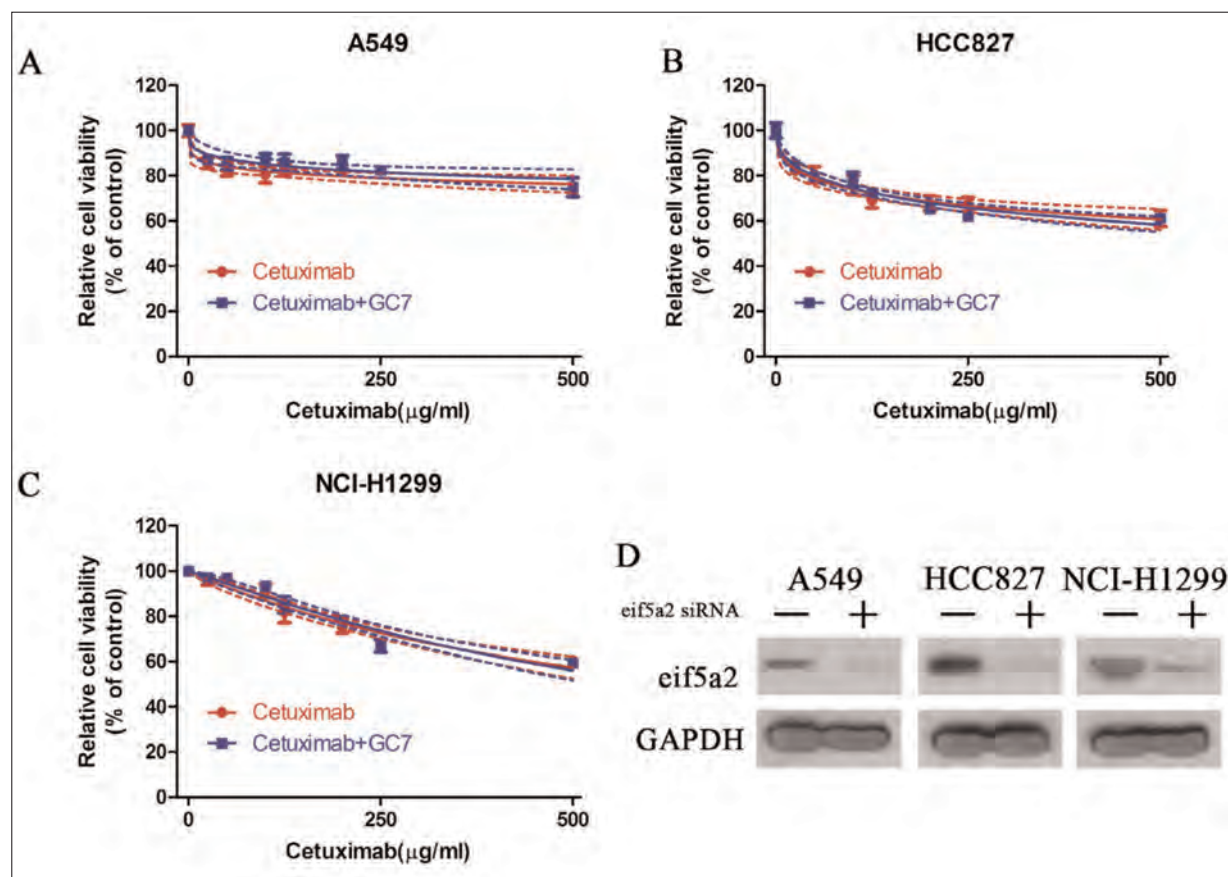


Figure 4. Cytotoxicity of cetuximab or cetuximab plus GC7 in eif5a2 siRNA-transfected NSCLC cells. Three NSCLC cell lines including A549 (**A**), HCC827 (**B**), and NCI-H1299 (**C**) transfected with eif5a2 siRNA were incubated with cetuximab alone or in combination with GC7 at the dose of 5 μM. Then, CCK-8 assay was conducted to measure cell viability treated with cetuximab alone or combined with GC7. (**D**) Western blot was used to detect the eif5a2 expression in three different cell lines.

Discussion

Nowadays, chemotherapy serves as an important component of postoperative treatment for cancer patients¹². However, acquisition of drug resistance to the conventional chemotherapeutics has become great challenges to the successful chemotherapy¹³. In this study, we found that combined treatment with GC7 enhanced the cetuximab cytotoxicity in human lung cancer cells through inhibition of EIF5A2.

It is suggested that GC7 exerts obvious anti-tumor effects in mammalian cancer cells¹¹. The enzymes DHS and deoxyhypusine hydroxylase (DOHH) are required to catalyze the post-translational modifications which lead to the activation of EIF5A2¹⁴. A recent study demonstrated that combined treatment with GC7 could enhanced the therapeutic efficacy of doxorubicin by inhibition of epithelial-mesenchymal transi-

tion in hepatocellular carcinoma cells and bladder cancer cells^{15,16}. In addition, the cisplatin sensitivity was elevated in NSCLC cells after incubation with GC7¹⁷.

Cetuximab, an anti-EGFR monoclonal antibody, is used to treat several cancers¹⁸. The Food and Drug Administration (FDA) has approved cetuximab for treatment of patients with colorectal cancer and squamous-cell carcinoma of the head and neck^{19,20}. A more recent study²² suggests the clinical benefit for the treatment of NSCLC²¹. Unfortunately, clinical data revealed that many patients who initially respond to cetuximab acquire resistance. And the synergistic cytotoxicity of cetuximab and GC7 has been unclear. Our study firstly found that combined treatment with GC7 significantly increased the cetuximab sensitivity in NSCLC cells, suggesting the potential clinical application of GC7 on patients acquired resistance to cetuximab.

Eif5a2 plays critical roles in cell proliferation, metastasis, and apoptosis, and has been considered as a novel oncogene²³. Inhibition of the activation of EIF5A2 by GC7 exhibits obvious anti-tumor effects in human cancers, such as hepatocellular carcinoma and glioblastoma^{11,24}. Accumulating evidences showed the up-regulated expression of EIF5A2 in many cancers, such as bladder cancer, hepatocellular carcinoma, and colon cancer²⁵⁻²⁷. In addition, over-expression of EIF5A2 could initiate cancer formation, enhance tumor cell proliferation and promote cell invasion²⁸. In our study, we transfected EIF5A2-siRNA into NSCLC cells to knockdown the expression of EIF5A2. Consequently, results showed that the GC7 co-administration had little effects on cetuximab cytotoxicity in NSCLC cells. These results indicate that GC7 sensitizes NSCLC cells to cetuximab through inhibition of EIF5A2 activity.

Conclusions

Our study demonstrates that combined treatment with GC7 enhances the cytotoxicity of cetuximab by inhibiting EIF5A2 in NSCLC cells. Thus, combination therapy with GC7 may lead to better therapeutic effects in cetuximab-based chemotherapy for NSCLC patients.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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