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Effect of eIF3a on response of lung cancer patients to platinum-based chemotherapy by regulating DNA repair

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Abstract

Purpose: The purpose of this study is to test the hypothesis that eIF3a may regulate the expression of DNA repair proteins which in turn affects response of lung cancer patients to treatments by DNA-damaging anticancer drugs.

Experimental Design: Immunohistochemistry was used to determine the expression of eIF3a in 211 human lung cancer tissues followed by association analysis of eIF3a expression with patient's response to platinum-based chemotherapy. Ectopic over-expression and RNA interference knock down of eIF3a were performed in NIH3T3 and H1299 cell lines, respectively, to determine the effect of altered eIF3a expression on cellular response to cisplatin, doxorubicine, etoposide (VP-16), vincristine, and vinblastine using MTT assay. The DNA repair capacity of these cells was evaluated using host-cell reactivation assay. Real-time RT-PCR and Western Blot analyses were performed to determine the effect of eIF3a on the DNA repair genes using cells with altered eIF3a expression.

Results: eIF3a expression associates with response of lung cancer patients to platinum-based chemotherapy. eIF3a knockdown or over-expression increased and decreased, respectively, cellular resistance to cisplatin and anthrocycline anticancer drugs, DNA repair activity, and expression of DNA repair proteins.

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Conclusions: eIF3a plays an important role in regulating the expression of DNA repair proteins which, in turn, contributes to cellular response to DNA damaging anticancer drugs and patients response to platinum-based chemotherapy.

Keywords

DNA repair; drug resistance; eIF3a; nucleotide excision repair (NER); platinum

INTRODUCTION

Lung cancer is the most prevalent cancer worldwide and the 5-year survival rate is around 16% (1). Although many new targeted therapeutic drugs such as gefitinib, erlotinib, and bevacizumab (2–4) have been used for treating lung cancers, cytotoxic drugs such as cisplatin are still used as the first-line treatment (5, 6). One of the major problems in improving survival rate of lung cancer patients is the existence of drug resistance in chemotherapy. Platinum resistance has been investigated to a great extent and increased DNA repair is considered as one of the major mechanisms of platinum resistance (7). While there are several known DNA repair pathways (8), DNA damages induced by platinum anticancer drugs is primarily repaired by nucleotide excision repair (NER) mechanism (9). The NER pathway is a complicated multistep process involving multiple proteins including replication protein A (RPA) and xeroderma pigmentosum group proteins (e.g., XPA and XPC) (8). In fact, the increased expression level of these NER proteins has been associated with cisplatin resistance (9–15). However, the underlying mechanism for their increased expression in cisplatin-resistant cancers is not yet understood.

eIF3 is the most complex translation initiation factor, consisting of 13 putative subunits named as eIF3a-eIF3m (16, 17). eIF3 plays a critical role in all translation initiation steps. First, it is involved in dissociating the post-termination 80S ribosomes by binding with 40S ribosomal subunit and forming a complex to maintain the 40S ribosomal subunit in a dissociated state and to prevent re-association of free 40S and 60S subunits. Second, eIF3 participates in the 43S pre-initiation complex formation by binding to the 40S ribosome and facilitates recruiting GTP-eIF2-tRNA-methionine ternary complex. Third, eIF3 stimulates mRNA binding with 43S pre-initiation complex. It is also involved in the scanning and recognizing start codon. In addition to the above cap dependent translational process, eIF3 also plays an important role in cap independent translation regulation by binding to the putative internal ribosome entry site (IRES) element (18).

eIF3a, the largest subunit of eIF3 complex, has been shown to play a role in regulating synthesis of proteins including α -tubulin, ribonucleotide reductase M2 and p27 (19, 20) and in cell proliferation (19), cell cycle control (21), as well as cell differentiation (22). Over-expression of eIF3a has been found in many cancers such as cancers of lung (23), breast (23), cervix (24), stomach (25) and esophagus (26). eIF3a appeared to be essential for cancer cells to maintain malignant phenotype (19) and ectopic over-expression of eIF3a transformed NIH3T3 cells *in vitro* (27). Moreover, it has been observed previously that cervical and esophageal cancer patients with high eIF3a level had better relapse-free and

overall survival than that with low eIF3a expression (24, 26), suggesting that eIF3a may affect patient responses to treatments.

In this study, we tested the hypothesis that eIF3a plays an important role in cisplatin response in lung cancer treatments by regulating the expression of NER proteins. We found that eIF3a expression correlates with response of lung cancer patients to platinum-based chemotherapy. eIF3a knockdown or ectopic over-expression increased and decreased, respectively, cellular resistance to cisplatin and anthracycline anticancer drugs. Further investigation showed that eIF3a regulates the expression of NER proteins and NER activity which likely mediates the effect of eIF3a on cellular response to DNA-damaging anticancer drugs.

MATERIALS AND METHODS

Materials.

Cisplatin, doxorubicine, etoposide (VP-16), vincristine, vinblastine, and β -actin antibody were purchased from Sigma (St Louis, Missouri, USA). Antibodies against PCNA, XPA, XPC, RPA32 and RPA72 were from Santa Cruz Biotechnology (Santa Cruz, California, USA). Antibody against RPA14 was from Abcam (Cambridge, Massachusetts, USA). Cell culture media and reagents were obtained from Invitrogen (Carlsbad, California, USA). All other reagents were of molecular biology grade from Sigma or Fisher.

Selection of study population and acquisition of clinical information.

The study protocol was approved by the Ethics Committee of Xiangya School of Medicine, Central South University. All patients provided written informed consent in compliance with the code of ethics of the World Medical Association (Declaration of Helsinki). Eligible patients were from Hunan Provincial Tumor Hospital (Changsha, Hunan, China) or Xiangya Hospital (Changsha, Hunan, China) and diagnosed between March 2003 and March 2008. The eligible patients for the study had to meet the following criteria of (a) histologically or cytologically confirmed lung cancer; (b) receiving no radiotherapy and biological therapy before chemotherapy; (c) having been treated with more than two cycles of platinum-based chemotherapy as a first-line treatment; (d) with primary lung tumors; and (e) having undergone full follow-up at the hospital after treatment and been evaluated for response of chemotherapy using the Response Evaluation Criteria In Solid Tumor (RECIST) guidelines (28). For the purpose of our analysis, patients with complete (CR) and partial (PR) responses were considered as responders while patients with stable (SD) and progressive (PD) disease were identified as non responders. Exclusion criteria include: (a) pregnancy or lactation; (b) active infection; (c) symptomatic brain or leptomeningeal metastases; and (d) previous or other concomitant malignancies. The performance status was categorized using Eastern Cooperative Oncology Group criteria (ECOG) (29). All other demographic and clinical information were obtained from the two hospitals mentioned above.

Patient Characteristics.

Total 211 lung cancer patients including 165 males (78.2%) and 46 females (21.8%) with medium age of 56 years (range from 22 to 77 years) were collected in this retrospective

study and their major demographic and clinical characteristics are shown in Table S1. Most patients (66.82%) had a good performance status (0–1). At the time of diagnosis, 7.4% patients had early stage (I, II) while 42.0% and 50.6% patients had stage III and IV disease, respectively. Smoking history was available for all patients with 48.8% former smokers, 38.4% current smokers, and 12.8% never smokers. Among these patients, 46.0% are squamous cell carcinoma (SCC), 32.7% adenocarcinoma, 16.6% small cell lung cancer (SCLC), and 4.7% other types including large cell lung cancer (LCLC), bronchioloalveolar carcinoma and adenosquamous carcinoma. As a first-line treatment, all enrolled patients received platinum-based (including cisplatin, carboplatin and oxaliplatin) chemotherapy. Among them, 25.1% patients received paclitaxel doublets, 25.6% gemcitabine doublets, 17.5% docetaxel doublets, 17.5% etoposide doublets, 11.4% vinorelbine doublets, and 2.8% other regimens including platinum single-agent and Chinese herbal medicines.

Sample collection and immunohistochemistry.

All tissue specimens were collected *via* biopsy of bronchofiberscope or surgical resection and paraffin-embedded for immunohistochemistry (IHC) analysis in the Pathology Department of Hunan Provincial Tumor Hospital or Xiangya Hospital. Serial 4 μm -thick sections were cut from tissue blocks and mounted on slides. The slide containing maximum amount of tumors were selected for each case and one representative slide from each case were subjected to IHC analysis. Briefly, tumor sections on slides were first baked at 60°C for 30 min followed by incubation in xylene for 2 \times 10 minutes and rehydration through graded ethanol to distilled water. Antigen retrieval was performed by heating samples in 1 mM EDTA (pH 8.0) for 20 minutes. Nonspecific staining was blocked by 10% goat serum in PBS buffer for 20 min at room temperature and endogenous peroxidase activity was quenched by incubation in 3% H₂O₂ for 10 min. Slides were then incubated with rabbit polyclonal monospecific eIF3a antibody AbD or PBS control at 4°C overnight followed by incubation with biotinylated goat anti-rabbit antibody (Zymed, San Francisco, California, USA) and peroxidase-conjugated streptavidin. The staining was visualized using 3,3'-diaminobenzidine tetrahydrochloride substrate kit (Zhongshan goldenbridge, Beijing, China) according to manufacturer's instructions and all samples were counterstained with Hematoxylin and Eosin (HE) before viewing using a Leica DMI 4000B inverted microscope.

All IHC staining were evaluated independently by two pathologists in the Department of Pathology at Xiangya Hospital. Microscopic fields with the highest degree of immunoreactivity were chosen for analysis and at least 1,000 cells were analyzed in each case. The score of cells exhibiting staining in each case was evaluated semi quantitatively as previously described (30). Briefly, a numeric intensity score was set from 1 to 4 with 1 for no; 2 for weak; 3 for moderate; and 4 for strong staining. The fraction score (0%–100%) was based on the percentage of positive tumor cells per slide. Total score range of 0–400 was obtained by multiplying the intensity score and the fraction score. Scores of 0–200 were considered as low and scores of 201–400 were considered as high level of expression.

Cell culture and generation of stable cell lines.

NIH3T3 and H1299 cells were maintained in DMEM and RPMI-1640 media, respectively, supplemented with 10% fetal bovine serum, 200 units/ml penicillin and 100 µg/ml streptomycin. For transient eIF3a knockdown, H1299 cells were seeded in 6-well plates and transfected with 50 nM eIF3a or scrambled control siRNA using Lipofectamine 2000 as previously described (19, 20) and incubated for 48 hrs followed by cell lysis for further analysis. To establish stable clones with eIF3a over-expression, NIH3T3 cells in 12-well plate were transfected with eIF3a cDNA in pCβA (19, 20) or vector control using Lipofectamine2000 according to manufacturer's instructions. Forty-eight hrs following transfection, cells were collected and replated in 100mm dishes followed by selection with 600 µg/ml G418 (Invitrogen, Carlsbad, California, USA) for 2 weeks. The G418 resistant clones were selected and tested for eIF3a expression using Western blot. The positive stable clones were maintained in the presence of 350 µg/ml G418.

Survival assay.

Chemosensitivity was determined using methyl thiazolyl tetrazolium (MTT) assay as we described previously (31). Briefly, cells were seeded in 96-well plates and allowed to grow for 24 hrs followed by incubation with anticancer drugs for 96 hrs. Culture medium was then removed and cells were incubated with 1 mg/ml thiazolyl blue tetrazolium bromide (Sigma) for 4 hrs at 37°C. The formazan was solubilized in dimethyl sulfoxide and OD_{570nm} was measured using a Dynex MRX-TC Revelation microplate reader (Chantilly, VA, USA). The IC₅₀ was obtained from the dose-response curves using GraphPad Prism™ 5.0 program (GraphPad Software, Inc, La Jolla, California, USA).

Real-time RT-PCR.

Real time RT-PCR was performed as previously described (32). Briefly, total RNAs were isolated using RNeasy Mini Kit (Qiagen, Maryland, USA) and 1 µg of total RNAs were used for reverse transcription using iScript cDNA synthesis kit (Bio-Rad, Hercules, California, USA) according to manufacturer's instructions. Real-time PCR were performed in a 7500 Real-Time PCR System (Applied Biosystems, Foster City, California, USA) using Power SYBR® Green RNA-to-CT™ 1-Step Kit (Applied Biosystems, Foster City, California, USA) according to manufacturer's instructions. The threshold cycle (C_t) of each reaction was determined and normalized to that of β-actin internal control.

Protein sample preparation and Western Blot analysis.

Protein sample preparation and Western blot analyses were performed as previously described (19, 20, 31). Briefly, cells were lysed in RIPA lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1% NP-40, 1 mM sodium orthovanadate, 1 mM EDTA, 1mM sodium fluoride, 100 µg/ml PMSF, 100µg/ml DTT) at 4°C for 30 mins. The lysates were clarified by centrifugation at 10,000×g for 10 min at 4°C. To prepare nuclear extracts, cells were first lysed in buffer A (10 mM Hepes (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 1 mM PMSF, 1 mM sodium orthovanadate, 6% NP-40) and centrifuged for 5 min at 1,500g. The nuclear pellets

were then extracted using RIPA buffer. Protein concentration of the lysate samples was determined using the Bio-Rad Protein Assay kit.

For Western blot analysis, protein samples were separated by SDS-PAGE and transferred to PVDF membranes (Bio-Rad, Hercules, California, USA). The membranes were then blocked with 5% nonfat milk and incubated with primary antibodies overnight followed by washing and reaction with HRP-conjugated secondary antibody. The reaction was detected using ECL reagents (GE Healthcare, Buckinghamshire, UK) and the signals were captured using x-ray films.

Host-cell reactivation assay (HCR).

The firefly luciferase assay-based HCR was performed as previously described (33). Briefly, the pCMV *luc* plasmid (50 µg/ml) in a 24-well plate was UV irradiated on ice using a Stratalinker UV Crosslinker (Stratagene, La Jolla, California, USA). UV-induced damages were verified using PCR (34) with a forward primer of 5'-CGGTATCTTATCATGTCTG-3' and a reverse primer of 5'-TGCTTCTGACACAACAGT-3'. The UV-damaged or un-irradiated control plasmids (0.4 µg) were then used to transfect 4×10^4 cells in a 24-well plate using Lipofectamine (Invitrogen, Carlsbad, California, USA). A pRL-TK (Promega, Madison, Wisconsin, USA) plasmid encoding renilla luciferase was used as a control for transfection efficiency. Forty hours after transfection, cells were harvested and both firefly and renilla luciferase activities were determined using a Dual-Luciferase Reporter Assay System (Promega, Madison, Wisconsin, USA) on a luminometer (Berthold, Pforzheim, Germany).

Statistical analysis.

For comparison of differences between groups, Pearson's chi-square test or Fisher's exact test were used for qualitative variables, Student's t test or analysis of variance were used for continuous variables. $P < 0.05$ was considered statistically significant in the study.

RESULTS

Correlation of eIF3a expression with chemotherapy response.

The eIF3a expression level in cancer tissues of these patients was evaluated using IHC. Fig. 1 shows representative different grade of eIF3a staining in these cancer tissues. eIF3a staining did not appear to have any significant correlation with the clinical characteristics listed in Table S1. However, eIF3a staining had significant correlation with chemotherapy responses of SCC ($P=0.032$), adenocarcinoma ($P=0.038$) and SCLC ($P=0.025$) patients although this correlation was not observed with other histological types possibly due to their small sample size (Table 1 and S2). In general, chemotherapy responsive patients had higher eIF3a expression in their lung cancers. About 52.1%, 65.6%, and 65.0% of chemotherapy responders of SCC, adenocarcinoma and SCLC patients had high eIF3a level, respectively. These numbers go down to 47.9%, 34.4% and 35.0% of the non-responders. Similar significant correlation was also observed in total patients with 58.7% of responders and 41.3% of non-responders of all patients having high eIF3a expression ($P=0.001$). Thus,

the level of eIF3a expression correlates with chemotherapy responses of lung cancer patients and the increased eIF3a expression may increase chemo-sensitivity.

Effect of eIF3a knockdown on cellular response to anticancer drugs.

Since all enrolled patients received platinum-based chemotherapy as their first line of treatments, we next tested if eIF3a expression affects cisplatin sensitivity using cell lines. For this purpose, we first performed an experiment to knock down eIF3a in human lung cancer cell line H1299 and tested if these cells become more resistant to cisplatin. As shown in Fig. 2A, eIF3a expression was successfully reduced by siRNA as determined using Western blot and real-time RT-PCR analyses. Next, these cells were subjected to MTT assay in the absence or presence of different concentrations of cisplatin. As shown in Fig. 2B, H1299 cells with reduced eIF3a expression are significantly more resistant to cisplatin than the control scramble siRNA-transfected cells with a nearly doubled relative resistance factor.

We next tested if eIF3a knockdown also affects the cellular response to other commonly used anticancer agents including doxorubicin, etoposide, vincristine, and vinblastine. As shown in Fig. 2C, knocking down eIF3a expression significantly increased cellular resistance to doxorubicin and etoposide, but not to vincristine and vinblastine. Together, these observations suggest that eIF3a expression may influence cellular responses to DNA-damaging anticancer drugs but not to vinca alkaloids.

Effect of eIF3a over-expression on cellular response to anticancer drugs.

To further determine the role of eIF3a expression in cellular response to drug treatments, we established two eIF3a over-expressing stable cell lines using NIH3T3 cells that express low level of endogenous eIF3a (19). Fig. 3A shows two stable clones that have over-expression of eIF3a at both mRNA and protein levels compared with the vector-transfected control clone. Next, these cells were subjected to MTT assay following treatments with different concentrations of cisplatin. As shown in Fig. 3B, the stable clones with eIF3a over-expression are significantly more sensitive to cisplatin than the vector-transfected control cells with more than 50% decrease in relative resistance factor. Other anticancer drugs were also tested with these clones. As shown in Fig. 3C, compared to the vector-transfected control clone the stable clones with eIF3a over-expression are significantly more sensitive to doxorubicin and etoposide but not to vincristine and vinblastine. This observation is consistent with the eIF3a knockdown studies (Fig. 2). Thus, we conclude that eIF3a expression contributes to cellular response to DNA-damaging anticancer drugs but not to vinca alkaloids.

Effect of eIF3a expression on cellular NER activity.

The above observation that eIF3a expression contributes to cellular response to DNA-damaging drugs suggest that eIF3a may affect DNA repair activities. To test this hypothesis, we analyzed if altering eIF3a expression affects NER activity which is responsible for repair of DNA damages induced by cisplatin using host cell reactivation (HCR) assay. For this purpose, a plasmid containing luciferase reporter was UV-irradiated to generate DNA adducts which were confirmed using PCR analysis (Fig. 4C). The damaged reporter plasmids were then transfected into H1299 cells with reduced eIF3a knockdown and

NIH3T3 cells with eIF3a over-expression followed by analysis of luciferase activity. As shown in Fig. 4A and 4B, the luciferase activity reduces with the increasing DNA damages due to higher doses of UV irradiation. However, the luciferase activity in H1299 cells with eIF3a knockdown is significantly higher than the control cells transfected with scrambled siRNA (Fig. 4A). The luciferase activity in NIH3T3 cells with eIF3a over-expression is reduced compared with the control clone (Fig. 4B). These findings suggest that eIF3a expression likely suppresses cellular NER activity.

Effect of eIF3a expression on NER protein level.

Previously, it has been shown that eIF3a plays an important role in regulating the translation of a subset of mRNAs including p27 and ribonucleotide reductase M2 (19, 20). Together with the above findings that eIF3a suppresses cellular NER activity, we propose that eIF3a may regulate the expression of proteins important for NER. To test this possibility, we examined if altering eIF3a level affects the expression of XPA, XPC, Rad23B, RPA, and PCNA, which are important proteins for NER using both H1299 cells with eIF3a knockdown and NIH3T3 cells with eIF3a over-expression. As shown in Fig. 5A and 5C, the protein level of XPA, XPC, RPA14, RPA32, RPA70, and Rad23B were all increased in H1299 cells with eIF3a knockdown compared with the vector-transfected control cells while the protein level of all these proteins in NIH3T3 stable clones with eIF3 over-expression decreased compared with the control clone. However, the protein level of PCNA in these cells was not changed.

To examine if the effect of eIF3a on the expression of NER proteins is at the transcriptional level, we determined the mRNA level of these proteins using real time RT-PCR. As shown in Fig. 5B and 5D, the mRNA level of none of these NER proteins was affected by either reducing eIF3a expression in H1299 cells or over-expressing eIF3a in NIH3T3 cells. Thus, we conclude that likely eIF3a regulates the synthesis of NER proteins XPA, XPC, RPA14, RPA32, RPA70, and Rad23B which, in turn, regulates the NER activity and cellular responses to DNA damages.

DISCUSSION

In this study, we found that eIF3a was highly expressed in responders compared to the non-responders of lung cancer patients to platinum-based chemotherapy. We further demonstrated that eIF3a regulates the expression of DNA repair enzymes which, in turn, contributes to DNA repair activities and cellular response to DNA damages. The eIF3a regulation of DNA repair protein expression is likely at their translational level.

Platinum-derived anticancer drugs have been used to treat many types of cancers including lung, cervical, and esophageal cancers. Unfortunately, resistance to platinum drugs frequently occurs and limits the efficacy of these drugs. The current finding that eIF3a expression correlates with clinical outcome and response to platinum-based chemotherapy of lung cancers and that eIF3a contributes to cellular response to cisplatin indicates that eIF3a may serve as an independent marker predicting patient drug response. Assessing eIF3a level may help design individualized treatment strategies for lung cancer patients. Since cisplatin is also a primary anti-cancer drug for many other cancers, such as cervical and esophageal

cancer, it is conceivable that eIF3a may contribute to cisplatin response and serve as an independent prognostic marker predicting drug response and help design individualized treatment strategies for these cancer patients as well. Because eIF3a also appears to affect cellular responses to other DNA-damaging anticancer drugs such as doxorubicin, it is tempting to speculate that eIF3a expression may also contribute response to these anticancer drugs in cancers that are treated with these drugs.

Although we observed the association between eIF3a expression and patient responses to platinum-based chemotherapy, the correlation between eIF3a expression and lung cancer patients' survival is currently unknown. However, it has been observed previously that cervical and esophageal cancer patients with high eIF3a level had better relapse-free and overall survival than that with low eIF3a expression (24, 26). As mentioned above, cisplatin is one of the major chemotherapeutic drugs for both cervical and esophageal cancers. It is, thus, conceivable to speculate that high eIF3a expression level in lung cancer patients may contribute to better chemotherapy response and better survival of these patients. Based on these observations, it is also tempting to speculate that eIF3a may serve as an independent prognostic marker predicting lung cancer patients' survival. Clearly, more studies are needed to further investigate the prognostic role of eIF3a.

The role of eIF3a in cellular response to cisplatin is likely *via* its regulation of expression of NER proteins such as XPA and XPC, which in turn affects DNA repair activities and cellular response to cisplatin. For example, it has been shown that XPC plays a crucial role in cellular response to cisplatin treatment (13, 35). XPA level has also been shown to possibly contribute to platinum resistance in ovarian and lung cancers (9, 12). Thus, up- or down-regulating expression of these proteins by altering eIF3a level likely results in changes in cellular NER activity and response to cisplatin. Previously, eIF3a has been shown to function as a regulator of translation of a subset of mRNAs including α -tubulin, ribonucleotide reductase M2 and p27 (19, 20). The finding that altering eIF3a expression changed protein but not mRNA levels of XPA, XPC, RPA14, RPA32, RPA70, and Rad323B suggests that these mRNAs may also be under the translational control by eIF3a.

Previously, XPF/ERCC1 and PCNA have all been implicated in platinum induced DNA damage repair and ERCC1 has also been implicated as a prognosis marker of lung cancer patients (36, 37). Interestingly, eIF3a has no effect on the expression of PCNA (Fig. 5) and XPF/ERCC1 (unpublished observation). Thus, XPF/ERCC1 and PCNA, unlike XPA, XPC, RPA, and Rad23B, are not under translational regulation by eIF3a. Currently, the reason for this difference in eIF3a regulation of NER proteins is unknown. However, these findings suggest that combination of the two independent prognosis factors eIF3a and ERCC1 may better predict survival of lung cancer patients, which clearly requires further investigation. These findings also suggest that translational regulation of XPA, XPC, RPA, and Rad23B by eIF3a is likely specific and NER proteins are not under a general up-regulation pathway involving eIF3a. The mRNAs under eIF3a regulation may have a same element in their 5'- or 3'-UTRs, which can interact with eIF3a for reduced translation. We are currently testing this possibility.

eIF3a also contributes to cellular sensitivity to anthracycline anticancer drugs such as doxorubicin. Although these drugs are known topoisomerase inhibitors and cause double strand DNA breaks, they also act to some degree *via* inducing DNA adduct formation (38) and NER was thought to be used to repair anthracycline-DNA adducts (39). For example, it has been shown that reducing DNA repair by long-term XPC silencing led to an increased sensitivity to etoposide (40). It is, thus, possible that the effect of eIF3a on cellular sensitivity to anthracyclines may also be *via* its regulation of NER protein expression. It is, however, also possible that eIF3a may regulate syntheses of proteins important for repairs of double strand DNA breaks. These possibilities will be tested in future studies.

However, eIF3a appears to have no significant effect on cytotoxicity of non-DNA-damaging *vinca alkaloid* anti-cancer drugs that destabilize microtubules. This finding is surprising since we previously found that eIF3a up-regulates synthesis of α -tubulin (20) and knocking down eIF3a sensitized cells to G2/M arrest induced by nocodazole, a microtubule polymerization inhibitor (41). The reason for this difference is currently unknown and warrants further studies of eIF3a on cellular response to different microtubule modulators.

In summary, we showed that eIF3a expression level correlates with responses of lung cancer patients to platinum-based chemotherapy. We also showed that eIF3a may regulate expression of NER proteins which in turn affects cellular NER activity and response to DNA damaging anti-cancer drugs such as cisplatin. It is tempting to speculate that eIF3a may also regulate the expression of proteins important for other mechanisms of DNA repair such as repair of double strand DNA breaks. eIF3a may represent a new prognostic marker predicting drug response and a novel mechanism of resistance to DNA-damaging anticancer drugs such as cisplatin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of Translational Relevance:

Platinum-based chemotherapy is commonly used as the first-line treatment of lung cancer, the most prevalent cancer worldwide. However, successful treatment of lung cancer has been hindered by resistance to platinum-based chemotherapy. One of the possible causes to platinum-based chemotherapy has been thought to be increased expression of DNA repair proteins although the mechanism of up-regulation of DNA repair is not yet understood. In this report, we show that eIF3a, a translation initiation factor, plays a critical role in regulating the expression of DNA repair proteins which in turn may contribute to response of lung cancer to platinum-based chemotherapy. eIF3a, thus, may represent a new prognostic marker predicting drug response and a novel potential target for sensitizing lung cancer to DNA-damaging anticancer drugs in combinational chemotherapy.

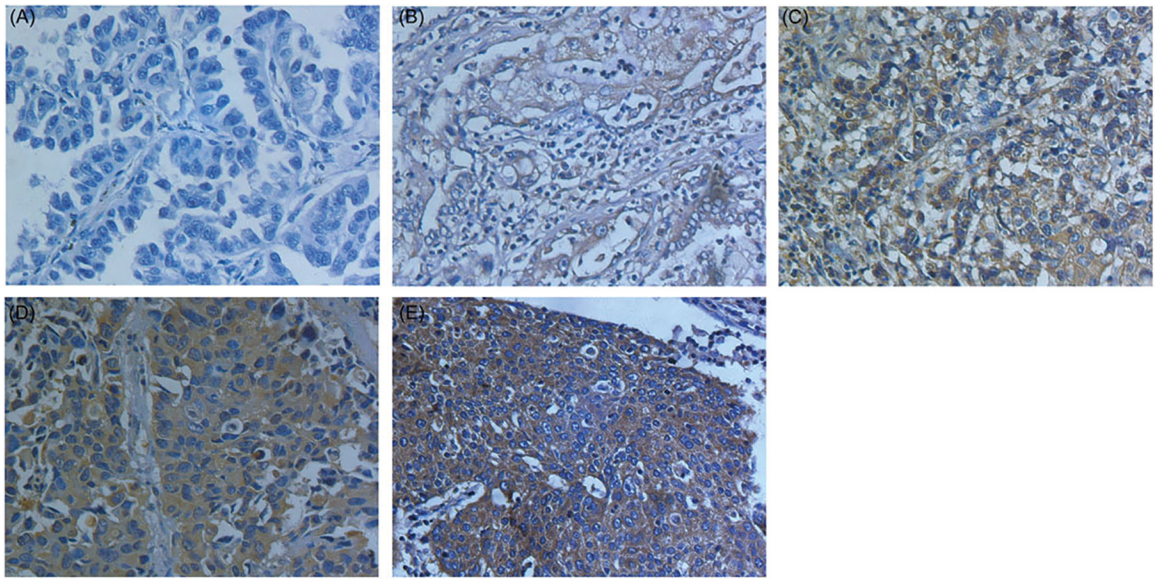


Figure 1. Representative immunohistochemistry staining of eIF3a expression in human lung tumor tissues.

Representative specimens of eIF3a staining with graded staining scores of 100 (B), 200 (C), 300 (D), and 400 (E) are shown. Panel A shows a representative negative staining control in the absence of eIF3a antibody AbD.

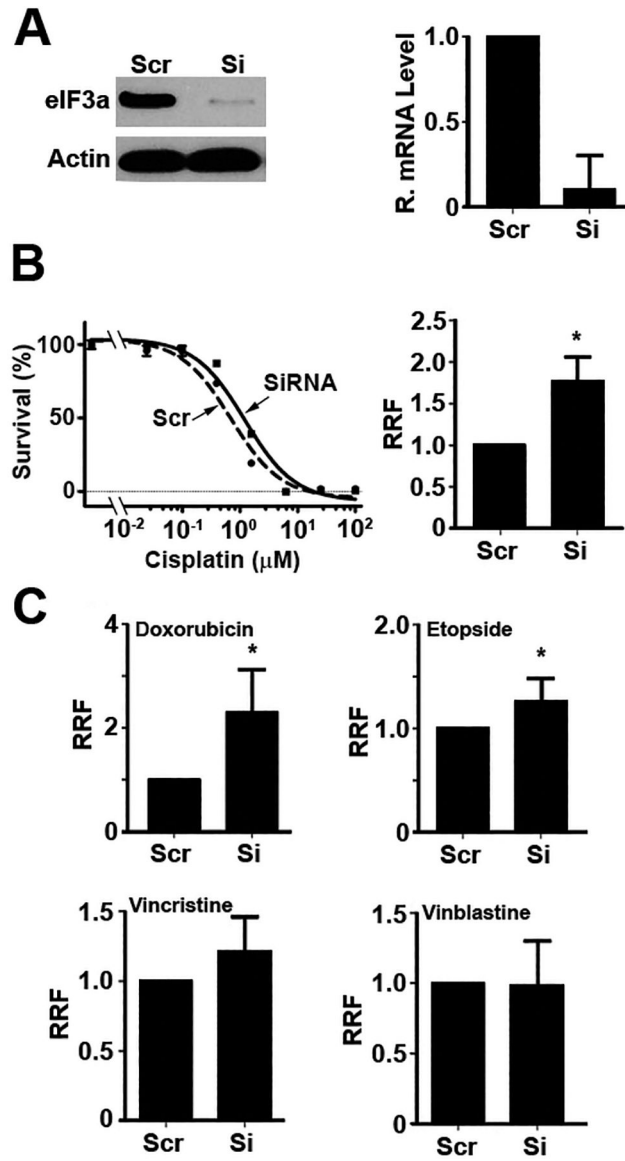


Figure 2. Effect of eIF3a knockdown on cellular response to anticancer drugs.

(A) H1299 cells were transiently transfected with eIF3a (Si) or scrambled control (Scr) siRNA followed by determination of eIF3a expression using Western blot and real-time RT-PCR. Actin was used as a loading control for Western blot. (B & C) H1299 cells transiently transfected with eIF3a (Si) or scrambled control (Scr) siRNAs were subjected to treatment with anticancer cancer drugs cisplatin, doxorubicin, etoposide, vinblastine, or vincristine at various concentrations. Half maximal inhibitory concentration (IC_{50}) was calculated using Graphpad 5.0 software from 3–5 independent experiments. Relative resistance factor (RRF) for each drug was derived by dividing the IC_{50} of the control cells by that of the cells with eIF3a knockdown. * indicates $P < 0.05$.

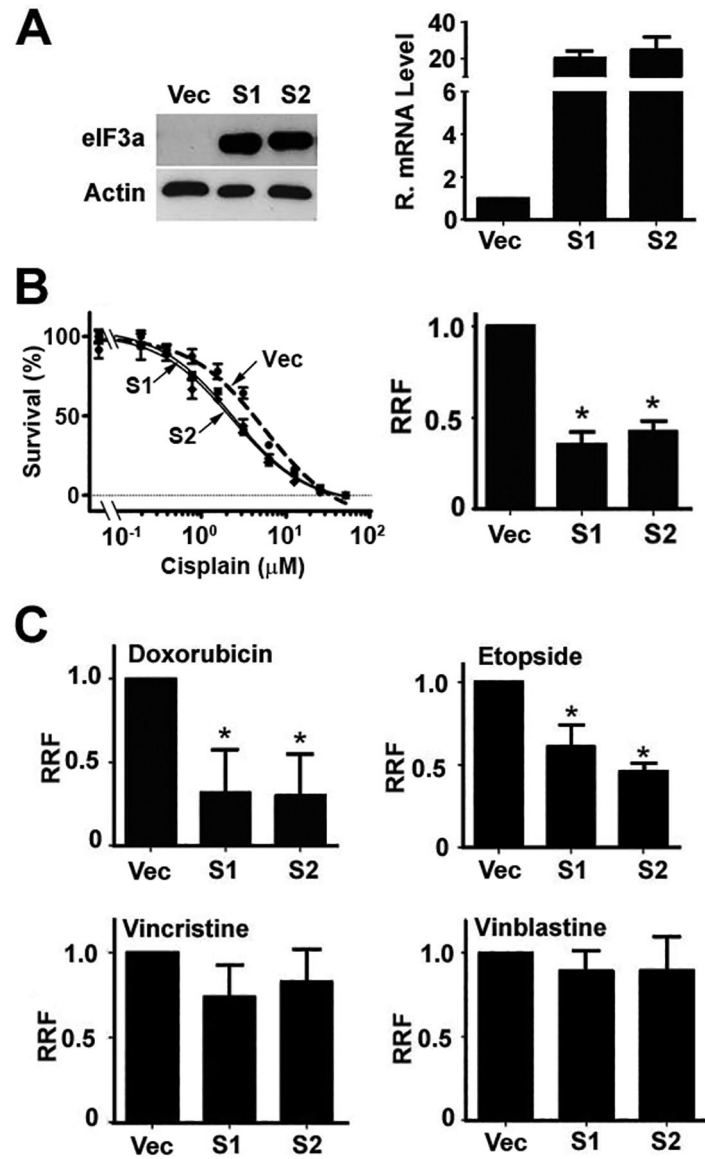


Figure 3: Effect of eIF3a over-expression on cellular response to anticancer drugs. (A) Stable NIH3T3 cells (S1 and S2) with eIF3a over-expression were subjected to Western blot and real-time RT-PCR analyses of eIF3a expression. Actin was used as a loading control for Western blot. Vec indicates the vector-transfected control cells. (B & C) Stable NIH3T3 cells (S1 and S2) with eIF3a over-expression or the vector-transfected control clone (Vec) were subjected to treatment with anticancer cancer drugs cisplatin, doxorubicin, etoposide, vinblastine, or vincristine at various concentrations. IC₅₀ for each drug was calculated using Graphpad 5.0 software from 3–5 independent experiments. Relative resistance factor (RRF) for each drug was derived by dividing the IC₅₀ of the control cells by that of the cells with eIF3a over-expression. * indicates P<0.05.

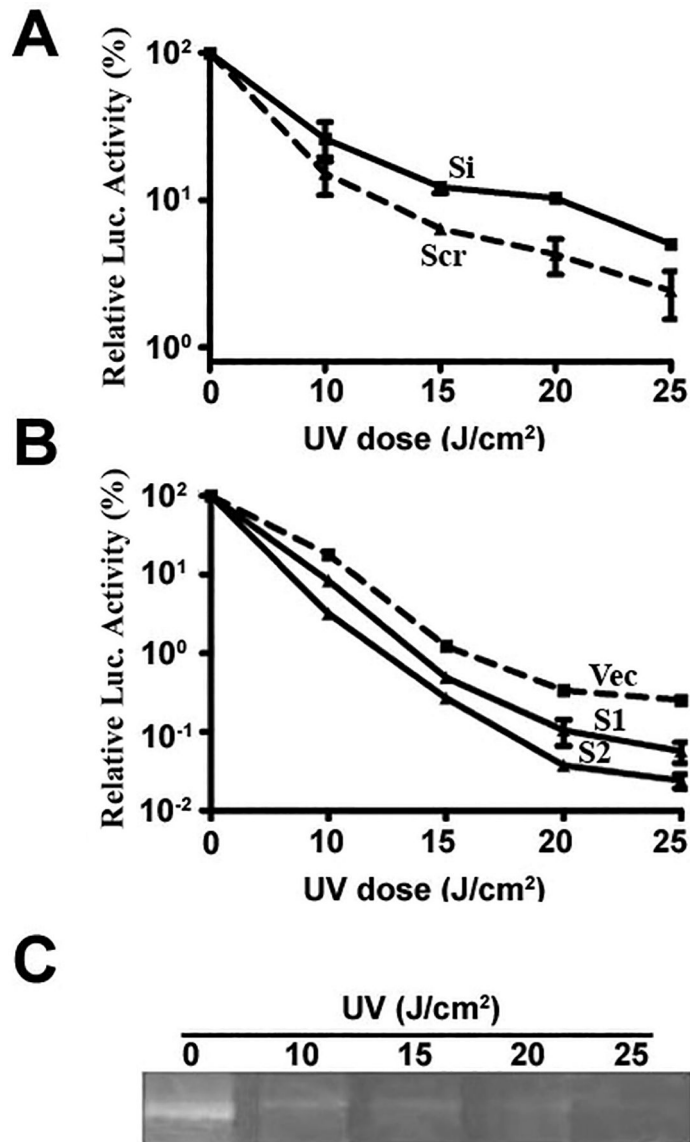


Figure 4: Effect of eIF3a expression on NER activities.

(A and B) H1299 cells with eIF3a knockdown (Si) or its scrambled control (Scr) (A) and NIH3T3 stable clones with eIF3a over-expression (S1 and S2) or vector (Vec) control (B) were transiently transfected with a firefly luciferase reporter plasmids irradiated with different doses of UV followed by analysis of luciferase activity. A plasmid encoding renilla luciferase was co-transfected for normalization of transfection efficiency. Relative firefly luciferase activity was generated by first normalizing to renilla luciferase activity and then to that of transfection with the control cells transfected with intact firefly luciferase plasmids. (C) PCR analysis of UV damaged plasmids. Lane 1: unirradiated intact plasmid control, Lane 2–4: plasmids irradiated by 10, 15, 20 and 25 J/cm² UV.

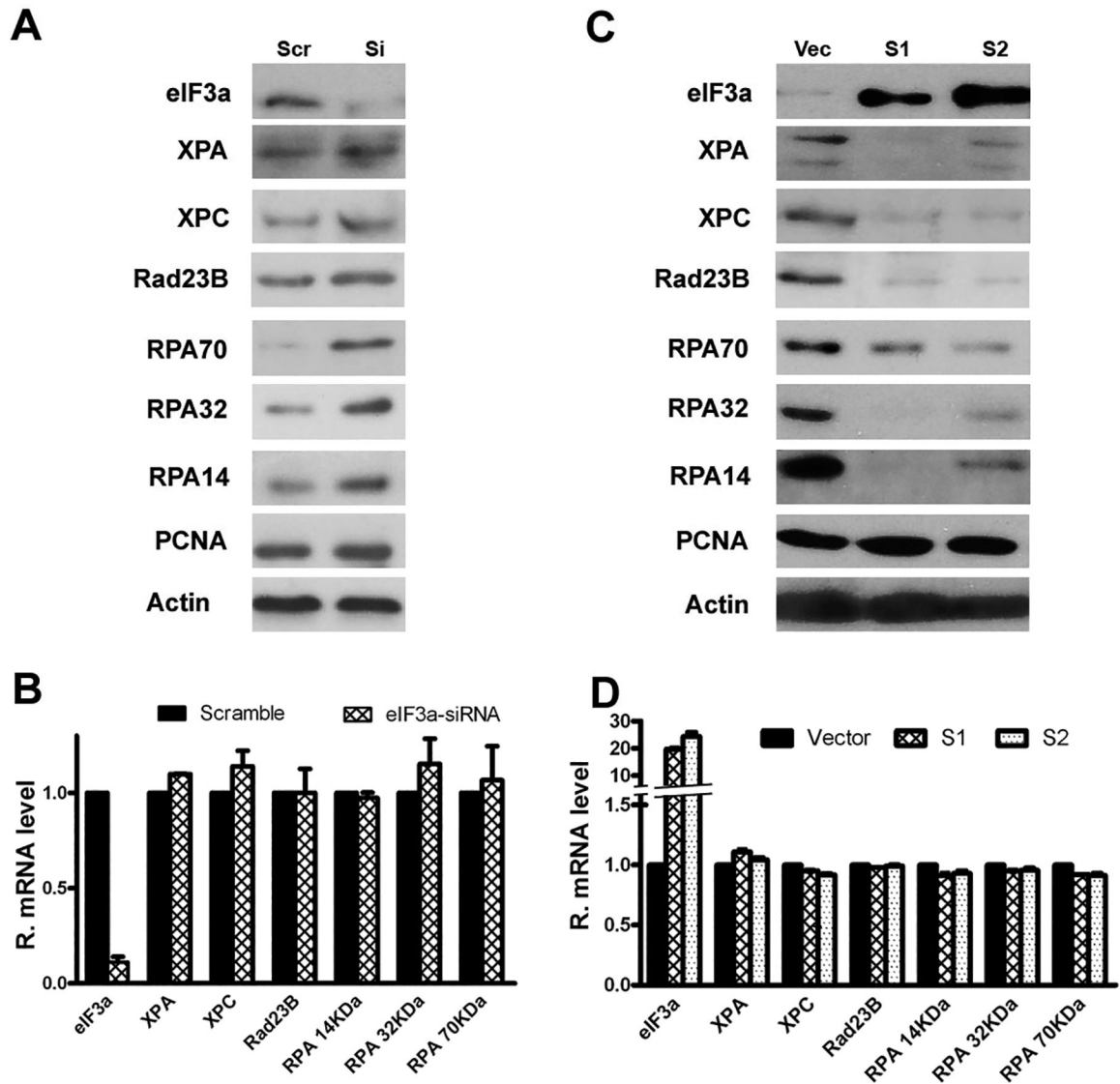


Figure 5: Effect of eIF3a expression on NER protein level.

H1299 cells transfected with eIF3a (Si) or scrambled control (Scr) siRNAs (A) and NIH3T3 stable clones with eIF3a over-expression (S1 and S2) or vector (Vec) control (B) were subjected to Western blot (A and C) or real-time RT-PCR analyses (B and D) of eIF3a, XPA, XPC, Rad23B, RPA70, RPA32, RPA14, and PCNA. Actin was used as a loading control for Western blot.

Table 1

Chemotherapy response and eIF3a expression

Chemotherapy Response	eIF3a level			P value
	Low (%)	High (%)	Ratio (high/low)	
SCC				
Responders	15 (30.6)	25 (52.1)	1.7	0.032
Non-responders	34 (69.4)	23 (47.9)	0.7	
Adenocarcinoma				
Responders	15 (40.5)	21 (65.6)	1.4	0.038
Non-responders	22 (59.5)	11 (34.4)	0.5	
SCLC				
Responders	4 (26.7)	13 (65.0)	3.3	0.025
Non-responders	11 (73.3)	7 (35.0)	0.6	
Others				
Responders	4 (66.7)	2 (50.0)	0.5	1.000
Non-responders	2 (33.3)	2 (50.0)	1.0	
Total				
Responders	38 (35.5)	61 (58.7)	1.6	0.001
Non-responders	69 (64.5)	43 (41.3)	0.6	