Effect of eIF3a on Response of Lung Cancer Patients to Platinum-Based Chemotherapy by Regulating DNA Repair

Ji-Ye Yin1,2, Jie Shen1, Zi-Zheng Dong2, Qiong Huang1,5, Mei-Zuo Zhong3, De-Yun Feng4, Hong-Hao Zhou1, Jian-Ting Zhang2, and Zhao-Qian Liu1

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 overexpression and RNA interference knockdown of eIF3a were carried out 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 by using MTT assay. The DNA repair capacity of these cells was evaluated by using host-cell reactivation assay. Real-time reverse transcriptase PCR and Western Blot analyses were carried out to determine the effect of eIF3a on the DNA repair genes by using cells with altered eIF3a expression.

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

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. Clin Cancer Res; 17(13); 4599–609. ©2011 AACR.

Introduction

Lung cancer is the most prevalent cancer worldwide and the 5-year survival rate is approximately 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). Although 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 xeroderm a pimentosum group proteins (e.g., XPA and XPC; ref. 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 to eIF3m (16, 17). eIF3 plays a critical role in all translation initiation steps. First, it is involved in dissociating the posttermination 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 reassociation of free 40S and 60S subunits. Second, eIF3 participates in the 43S preinitiation complex formation by binding to the 40S ribosome and...
facilitates recruiting GTP-eIF2-tRNA-methionine ternary complex. Third, eIF3 stimulates mRNA binding with 43S preinitiation complex. It is also involved in the scanning and recognizing start codon. In addition to this 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).

**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 the increased expression of DNA repair proteins although the mechanism of upregulation of DNA repair is not yet understood. In this study, 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.

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 overexpression, respectively, increased and decreased the cellular resistance to cisplatin and anthocycline 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. Antibodies against proliferating cell nuclear antigen (PCNA), XPA, XPC, RPA32, and RPA72 were from Santa Cruz Biotechnology. Antibody against RPA14 was from Abcam. Cell culture media and reagents were obtained from Invitrogen. 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: (a) histologically or cytopologically confirmed lung cancer; (b) receiving no radiotherapy and biological therapy before chemotherapy; (c) having been treated with more than 2 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 by using the Response Evaluation Criteria In Solid Tumor (RECIST) guidelines (28).

For the purpose of our analysis, patients with complete response (CR) and partial response (PR) were considered as responders whereas patients with stable disease (SD) and progressive disease (PD) were identified as nonresponders. 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 by the Eastern Cooperative Oncology Group (ECOG) criteria (29). All other demographic and clinical information were obtained from the 2 hospitals mentioned earlier.

#### Patient characteristics

A total of 211 lung cancer patients—165 males (78.2%) and 46 females (21.8%)—with median age of 56 years (range, 22–77) were collected in this retrospective study; their major demographic and clinical characteristics are shown in Supplementary 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) whereas 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, 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 bronchoscopes 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 minutes followed by incubation in xylene for 2 × 10 minutes and rehydration through graded ethanol to distilled water. Antigen retrieval was done by heating samples in 1 mmol/L EDTA (pH 8.0) for 20 minutes. Nonspecific staining was blocked by 10% goat serum in PBS buffer for 20 minutes at room temperature and endogenous peroxidase activity was quenched by incubation in 3% H2O2 for 10 minutes. 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) and peroxidase-conjugated streptavidin. The staining was visualized by using 3,3'-diaminobenzidine tetrahydrochloride substrate kit (Zhongshan Goldenbridge) according to the manufacturer's instructions and all samples were counterstained with hematoxylin and eosin before viewing with a Leica DMi 4000B inverted microscope.

All IHC staining were evaluated independently by 2 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 semiquantitatively as previously described (30). Briefly, a numeric intensity score was set from 1 to 4 (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 to 400 was obtained by multiplying the intensity score and the fraction score. Scores of 0 to 200 were considered as low and scores of 201 to 400 were considered as high levels of expression.

Cell culture and generation of stable cell lines
NIH3T3 and H1299 cells were maintained in Dulbecco's modified Eagle's medium and RPMI-1640 media, respectively, supplemented with 10% FBS, 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 nmol/L eIF3a or scrambled control siRNA by using Lipofectamine 2000 as previously described (19, 20), and incubated for 48 hours followed by cell lysis for further analysis. To establish stable clones with eIF3a overexpression, NIH3T3 cells in 12-well plate were transfected with eIF3a cDNA in pC3A (19, 20) or vector control by using Lipofectamine 2000 according to the manufacturer's instructions. Forty-eight hours following transfection, cells were collected and replated in 100-mm dishes followed by selection with 600 µg/mL G418 (Invitrogen) for 2 weeks. The G418 resistant clones were selected and tested for eIF3a expression by using Western blot. The positive stable clones were maintained in the presence of 350 µg/mL G418.

Survival assay
Chemosensitivity was determined by using MTT assay as we described previously (31). Briefly, cells were seeded in 96-well plates and allowed to grow for 24 hours followed by incubation with anticancer drugs for 96 hours. Culture medium was then removed and cells were incubated with 1 mg/mL thiazolyl blue tetrazolium bromide (Sigma) for 4 hours at 37°C. The formazan was solubilized in dimethyl sulfoxide and OD570nm was measured by a Dynex MRX-TC Revelation microplate reader. The IC50 was obtained from the dose–response curves by using GraphPad Prism 5.0 program (GraphPad Software, Inc.).

Real-time reverse transcriptase PCR
Real-time reverse transcriptase (RT)-PCR was carried out as previously described (32). Briefly, total RNAs were isolated by using RNeasy Mini Kit (Qiagen) and 1 µg of total RNAs were used for reverse transcription by using iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's instructions. Protein sample preparation and Western blot analyses

Protein sample preparation and Western blot analysis
Protein sample preparation and Western blot analyses were carried out as previously described (19, 20, 31). Briefly, cells were lysed in radioimmunoprecipitation assay (RIPA) lysis buffer [50 mmol/L Tris (pH 7.4), 150 mmol/L NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1% NP-40, 1 mmol/L sodium orthovanadate, 1 mmol/L EDTA, 1 mmol/L sodium fluoride, 100 µg/mL phenylmethylsulfonylfluoride (PMSF), 100 µg/mL dithiothreitol (DTT)] at 4°C for 30 minutes. The lysates were clarified by centrifugation at 10,000 × g for 10 minutes at 4°C. To prepare nuclear extracts, cells were first lysed in buffer A [10 mmol/L HEPES (pH 7.9), 10 mmol/L KCl, 0.1 mmol/L EDTA, 0.1 mmol/L EGTA, 1 mmol/L DTT, 1 mmol/L PMSF, 1 mmol/L sodium orthovanadate, 6% NP-40] and
centrifuged for 5 minutes at 1,500 × g. The nuclear pellets were then extracted by using RIPA buffer. Protein concentration of the lysate samples was determined by using the Bio-Rad Protein Assay kit.

For Western blot analysis, protein samples were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Bio-Rad). The membranes were then blocked with 5% nonfat milk and incubated with primary antibodies overnight followed by washing and reaction with horseradish peroxidase–conjugated secondary antibody. The reaction was detected by using ECL reagents (GE Healthcare) and the signals were captured by X-ray films.

**Host-cell reactivation assay**

The firefly luciferase assay-based host cell reactivation (HCR) assay was carried out as previously described (33). Briefly, the pCMVLuc plasmid (50 μg/mL) in a 24-well plate was UV irradiated on ice by a Stratalinker UV Crosslinker (Stratagene). UV-induced damages were verified by using PCR (34) with a forward primer of 5'-CGGTATCTTATCATGTCTG-3' and a reverse primer of 5'-TGCTTCTGACA-CAACAGT-3'. The UV-damaged or unirradiated control plasmids (0.4 μg) were then used to transfect 4 × 10⁴ cells in a 24-well plate by using Lipofectamine (Invitrogen). A pRL-TK (Promega) 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 by using a Dual-Luciferase Reporter Assay System (Promega) on a luminometer (Berthold).

**Statistical analysis**

For comparison of differences between groups, Pearson’s χ² 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 by IHC. Figure 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 Supplementary 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 histologic types, possibly because of their small sample size (Table 1; Supplementary Table S2). In general, chemotherapy responsive patients had higher eIF3a expression in their lung cancers. Approximately 52.1%, 65.6%, and 65.0% of chemotherapy responders of SCC, adenocarcinoma, and SCLC patients, respectively, had high eIF3a level. 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 nonresponders 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 chemosensitivity.

**Effect of eIF3a knockdown on cellular response to anticancer drugs**

Because all enrolled patients received platinum-based chemotherapy as their first line of treatments, we next tested whether eIF3a expression affects cisplatin sensitivity by using cell lines. For this purpose, we first conducted an experiment to knockdown eIF3a in human lung cancer cell line H1299 and tested whether these cells become more resistant to cisplatin. As shown in
Figure 2A, eIF3a expression was successfully reduced by siRNA as determined by 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 Figure 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 whether eIF3a knockdown also affects the cellular response to other commonly used anticancer agents including doxorubicin, etoposide, vincristine, and vinblastine. As shown in Figure 2C, knocking down eIF3a expression significantly increased cellular resistance to doxorubicine 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 expression on cellular NER activity

The 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 whether altering eIF3a expression affects NER activity, which is responsible for repair of DNA damages induced by cisplatin, using HCR assay. For this purpose, a plasmid containing luciferase reporter was UV irradiated to generate DNA adducts which were confirmed by using PCR analysis (Fig. 4C). The damaged reporter plasmids were then transfected into H1299 cells with reduced eIF3a knockdown and NIH3T3 cells with eIF3a overexpression followed by analysis of luciferase activity. As shown in Figure 4A and B, 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 overexpression 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

<table>
<thead>
<tr>
<th>Chemotherapy response</th>
<th>eIF3a level</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>Low (%)</td>
<td>High (%)</td>
</tr>
<tr>
<td>SCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>15 (30.6)</td>
<td>25 (52.1)</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>34 (69.4)</td>
<td>23 (47.9)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>15 (40.5)</td>
<td>21 (65.6)</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>22 (59.5)</td>
<td>11 (34.4)</td>
</tr>
<tr>
<td>SCLC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>4 (26.7)</td>
<td>13 (65.0)</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>11 (73.3)</td>
<td>7 (35.0)</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>4 (66.7)</td>
<td>2 (50.0)</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>2 (33.3)</td>
<td>2 (50.0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>38 (35.5)</td>
<td>61 (58.7)</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>69 (64.5)</td>
<td>43 (41.3)</td>
</tr>
</tbody>
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Table 1. Chemotherapy response and eIF3a expression

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including p27 and ribonucleotide reductase M2 (19, 20).

Together with the 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 whether 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 overexpression. As shown in Figure 5A and C, 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 whereas the protein level of all these proteins in NIH3T3 stable clones with eIF3 overexpression decreased compared with the control clone. However, the protein level of PCNA in these cells was not changed.

To examine whether the effect of eIF3a on the expression of NER proteins is at the transcriptional level, we determined the mRNA level of these proteins by using real-time RT-PCR. As shown in Figure 5B and D, the mRNA level of none of these NER proteins was affected by either reducing eIF3a expression in H1299 cells or overexpressing eIF3a in NIH3T3 cells. Thus, we conclude that eIF3a regulates the expression of NER proteins.
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 with the nonresponders of lung cancer patients to platinum-based chemotherapy. We further showed 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. Because cisplatin is also a primary anticancer 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 earlier, 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. On the basis of 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 downregulating 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 Rad23B 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 2 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 upregulation 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). Thus, it is 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 anticancer drugs that destabilize microtubules. This finding is surprising because we previously found that eIF3a upregulates 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 regulation of DNA repair proteins.
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 anticancer 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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