miR-141 regulation of EIF4E expression affects docetaxel chemoresistance of non-small cell lung cancer

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Abstract. The present study investigated the role of miR-141 regulation of eukaryotic initiation factor-4E (EIF4E) expression in docetaxel chemoresistance of human non-small cell lung cancer (NSCLC). The expression of miR-141 in docetaxel chemoresistant patients with NSCLCs was markedly higher than those of non-docetaxel chemoresistant patients with NSCLCs. The expression of EIF4E in docetaxel chemoresistant patients with NSCLCs was markedly lower than those of non-docetaxel chemoresistant patients with NSCLCs. Downregulation of miR-141 suppressed cell proliferation, induced cell death and increased caspase-3 activity in H1299 or H2009/docetaxel cells. Downregulation of miR-141 also increased the protein expression of EIF4E, VEGF, c-Myc and Bax in H1299 or H2009/docetaxel cells. Conversely, upregulation of miR-141 promoted cell proliferation, inhibited cell death and caspase-3 activity in H1299 or H2009/docetaxel cell. Upregulation of miR-141 suppressed EIF4E, VEGF, c-Myc protein expression and inhibited Bax in H1299 or H2009/docetaxel cells. Thus, the present study is the first to show the induction of miR-141/EIF4E expression in an acquired model of docetaxel chemoresistant patients with NSCLCs. This serves as a mechanism of acquired docetaxel chemoresistant patients with NSCLCs, possibly through direct interactions with VEGF, c-Myc, and Bax, therefore presenting a potential therapeutic target for the treatment of docetaxel chemoresistant patients with NSCLCs.

Introduction

Lung cancer is one of the most common malignant tumors. According to the statistics data (GLOBOCAN) issued by the World Health Organization, lung cancer is the most common cancer with the highest fatality rate for male in 2008; while for female, it is the fourth common one with the fatality rate ranked the second (1). In 2008, lung cancer accounted for 13% (1.6 million) of new onset cancer cases, and 18% of deaths (1.4 million) (2). Lung cancer is the malignant tumor with the highest morbidity in China as well. Its morbidity in 2003-2007 was 48.90/100,000, accounting for 18.39% of all new onset cancer cases; lung cancer was ranked in the top position among regions with cancer registry in China in 2003-2007 (3). The mortality rate was 43.48/100,000, accounting for 25.30% of all new onset cancer cases (4).

Similar to other chemotherapeutics, drug resistance to Taxanes has always been a great obstacle for further enhancing therapeutic effect of chemotherapy, prolonging patient lifetime and improving QOL. A variety of factors may lead to drug resistance to Taxanes, including gene mutation of α-tubulin and p-tubulin, diversified constitutions of p-tubulin hypotype, overexpression of P-gp and increasing of microtubule activity related to the changed microtubule-associated protein. In addition, among a variety of molecular pathways associated with cell cycle and cell apoptosis, some changes on protein expression may be associated with chemotherapy resistance.

Eukaryotic initiation factor-4E (EIF4E) is a protooncogene detected recently. It plays significant role at the initial period of protein synthesis (5). EIF4E overexpression can change expression quantity of malignant tumor related genes. It is the determinant of tumor malignant phenotype (6). It may make cells break through the limitation of normal growth thus leading to carcinogenesis by adjusting expression translation amount of specific malignant related molecules (such as VEGF, cylinD1 and C-myc) (7). Generally, a small amount of expression of elf4E can maintain mRNA translation required by normal life activities of cells. In case of overexpression, it may lead to increasing of mRNA expression translation of a variety of protein coding carcinoma and tumor promoters thus to promote genesis and development of tumor. Research has shown that overexpression of elf4E exists in many malignant tumors (such as: bladder cancer, cervical cancer, leukemia, head and neck malignant tumor, laryngeal cancer, breast cancer, esophagus cancer, colon cancer, prostatic cancer and bile duct carcinoma) (7,8).

Research has verified that resistance mechanism involves a variety of factors, including drug accumulation reduction, drug...
detoxification increase, changes of drug-resistance-related genes, inhibition of tumor cell apoptosis and enhancement of DNA rehabilitation capacity, and involvement of multiple signal pathways and key cytokines (9). In the last two years, it has been detected that some miRNAs are related to sensitivity and drug resistance of chemotherapy. In case of expression upregulation or downregulation, it will directly lead to expression level abnormity of target gene protein in related pathways of drug resistance (10). Eventually, drug sensitivity of tumor cells is changed through signal pathway of the cell. Such target genes include those related to cell apoptosis, drug transporter, drug target, cell repairing and cell cycle regulation (11). At present, it has been detected that some miRNAs is associated with sensitivity of chemical drug treatment of tumors (12). The present study investigated the role of miR-141 regulation of EIF4E expression in docetaxel chemoresistance of NSCLCs.

Materials and methods

Patients. This study was conducted in accordance with the ethical principles of The Affiliated Hospital of Qingdao University. NSCLC patients had a histological diagnosis of NSCLC from The Affiliated Hospital of Qingdao University. All patients provided written informed consent before undergoing any study procedure. In total, docetaxel chemoresistant patients with NSCLCs was 16 and non-docetaxel chemoresistant patients with NSCLCs was 8.

Real-time quantitative reverse transcription-PCR. Total RNA of tumor samples or cancer cells was isolated using the TRIzol reagent (Invitrogen). Reverse transcription (RT)-PCR was accomplished by the PrimeScript RT Reagent kit (Takara Biotechnology Co., Ltd., Dalian, China) using 1-2 µg total RNA. Real-time quantitative reverse transcription-PCR was performed using SYBR Premix Ex Taq™ II (Takara Biotechnology Co., Ltd.). Expression mRNA levels of miR-141 and EIF4E were defined as positive controls for the $2^{-\Delta\Delta Ct}$ calculation. The designed PCR primers of miR-141 and EIF4E are shown in Table I.

<table>
<thead>
<tr>
<th>Gene</th>
<th>PCR primers</th>
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<tr>
<td>miR-141</td>
<td>F: 5'-CACATCCACCTCCTCCACATC-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-AATGCGGCCGCAACTCAACATCACCAT-3'</td>
</tr>
<tr>
<td>U6</td>
<td>F: 5'-CTCCTTCGCGACAGCACATACT-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-ACGGTCTCAAGATTTGCCGTC-3'</td>
</tr>
<tr>
<td>EIF4E</td>
<td>F: 5'-ATGGCCGACTGTGCGCAGCG-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-ATTAGATTCCGTTTTCTCCCTCTCTG-3'</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: 5'-AAGGGAAGGTTGCTGGATAGG-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-CACATCCACCTCCTCCACATC-3'</td>
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Forward, F; reverse, R.

Statistical analysis. All statistical analyses were performed using SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA). The results are presented as mean ± standard error. The difference between means was analyzed with ANOVA and then Student's t-test.
A p-value of <0.05 was considered to indicate a statistically significant difference.

**Results**

**miR-141 expression in docetaxel chemoresistance of human NSCLC patients.** As shown in Fig. 1A, the miRNA expression of miR-141 in docetaxel chemoresistant patients with NSCLCs was markedly higher than those of non-docetaxel chemoresistant patients with NSCLCs. The miRNA expression of miR-141 in H1299 and H2009 cells treated by docetaxel was also higher than that of H1299 and H2009 cells treated by non-docetaxel group (Fig. 1B and C).

**EIF4E expression in docetaxel chemoresistance of human NSCLC patients.** Then, we found that the miRNA expression of EIF4E in docetaxel chemoresistance of human NSCLC patients (A), H1299 cells treated by docetaxel (B) and H2009 cell treated by docetaxel (C) was significantly higher than those of non-docetaxel chemoresistant patients with NSCLCs. The expression of EIF4E in H1299 cells+DTX, H1299 cells treated by docetaxel; H2009 cell+DTX, H2009 cells treated by docetaxel was also higher than that of H1299 and H2009 cells treated by non-docetaxel group (Fig. 2B and C).

**Downregulation of miR-141 reverses docetaxel chemoresistance of human NSCLC growth.** Downregulation of miR-141 cells inhibited miR-141 expression (A and B) and cell viability (C and D) of H1299 and H2009 cells treated by docetaxel. The downregulation of miR-141 in H1299 and H2009 cells treated by docetaxel was also higher than that of H1299 and H2009 cells treated by non-docetaxel group (Fig. 3B and C).
of EIF4E in docetaxel chemoresistant patients with NSCLCs was markedly lower than those of non-docetaxel chemoresistant patients with NSCLCs (Fig. 2A). Moreover, the miRNA expression of EIF4E in H1299 and H2009 cells treated by docetaxel was also lower than that of H1299 and H2009 cells treated by non-docetaxel group (Fig. 2B and C).

**Downregulation of miR-141 reverses docetaxel chemoresistance of human NSCLC.** Therefore, negative control and miR-141 inhibitor plasmids were transfected into H1299 and H2009 cells. MiR-141 inhibitor plasmids significantly inhibited the miR-141 expression in H1299 and H2009 cells treated by docetaxel, compared with negative control group (Fig. 3A and B). Downregulation of miR-141 significantly inhibited cell viability of H1299 and H2009 cells treated by docetaxel, compared with negative control group (Fig. 3C and D).

**Downregulation of miR-141 reverses docetaxel chemoresistance of human NSCLC death and caspase-3 activity.** Then, we detected the downregulation of miR-141 affect on docetaxel chemoresistance of human NSCLC death and caspase-3 activity. As shown in Fig. 4A-D, the downregulation of miR-141 significantly increased cell apoptosis and caspase-3 activity of H1299 and H2009 cells treated by docetaxel, compared with negative control group.

**Downregulation of miR-141 reverses EIF4E protein expression in docetaxel chemoresistance of human NSCLC.** We evaluated whether the downregulation of miR-141 reverses EIF4E protein expression in docetaxel chemoresistance of human NSCLC. Downregulation of miR-141 significantly induced the protein expression of EIF4E in H1299 and H2009 cells treated by docetaxel, compared with negative control group (Fig. 5A-C).

**Downregulation of miR-141 reverses VEGF protein expression in docetaxel chemoresistance of human NSCLC.** To assess the mechanism of anticancer effect of miR-141 on docetaxel chemoresistance of human NSCLC, VEGF protein expression was measured using western blotting. The results from Western blot showed that the downregulation of miR-141 significantly activated the protein expression of VEGF in H1299 and H2009 cells treated by docetaxel, compared with negative control group (Fig. 6A-C).

**Downregulation of miR-141 reverses c-Myc protein expression in docetaxel chemoresistance of human NSCLC.** We further assessed the mechanism of anticancer effect of miR-141 on docetaxel chemoresistance of human NSCLC, c-Myc protein expression was also measured using western blotting. Western blot showed that the downregulation of miR-141 significantly
promoted the protein expression of c-Myc in H1299 and H2009 cells treated by docetaxel, compared with negative control group (Fig. 7A-C).

**Downregulation of miR-141 reverses Bax protein expression in docetaxel chemoresistance of human NSCLC.** We explored the downregulation of miR-141 affect on Bax protein expression in docetaxel chemoresistance of human NSCLC. As shown in Fig. 8A-C, downregulation of miR-141 significantly activated Bax protein expression in H1299 and H2009 cells treated by docetaxel, compared with negative control group.

**Upregulation of miR-141 reverses docetaxel chemoresistance of human NSCLC growth.** On the contrary, we used miR-141 mimics to increase miR-141 expression in H1299 and H2009 cells treated by docetaxel, which was used to analyze the upregulation of miR-141 affect on docetaxel chemoresistance of human NSCLC growth. As shown in Fig. 9A and B, miR-141 mimics significantly increased the miR-141 expression in H1299 and H2009 cells treated by docetaxel. In addition, the upregulation of miR-141 significantly increased cell viability of H1299 and H2009 cells treated by docetaxel, compared with negative control group (Fig. 9C and D).

**Upregulation of miR-141 reverses EIF4E protein expression in docetaxel chemoresistance of human NSCLC.** To further
confirm that the upregulation of miR-141 reverses EIF4E protein expression in docetaxel chemoresistance of human NSCLC. In contrast, the EIF4E protein expression of H1299 and H2009 cells treated by docetaxel was significantly inhibited by upregulation of miR-141, compared with negative control group (Fig. 11A-C).
Upregulation of miR-141 reverses VEGF protein expression in docetaxel chemoresistance of human NSCLC. To measure VEGF protein expression in docetaxel chemoresistance of human NSCLC following upregulation of miR-141, VEGF protein was measured using western blotting. Upregulation of miR-141 significantly suppressed VEGF protein of H1299 and H2009 cells treated by docetaxel, compared with negative control group (Fig. 12A-C).

Upregulation of miR-141 reverses c-Myc protein expression in docetaxel chemoresistance of human NSCLC. We measured c-Myc protein expression in docetaxel chemoresistance of human NSCLC using western blotting after upregulation of miR-141. The results showed a significant inhibition in c-Myc protein expression of H1299 and H2009 cells treated by docetaxel after upregulation of miR-141 than the control cells (Fig. 13A-C).

Upregulation of miR-141 reverses Bax protein expression in docetaxel chemoresistance of human NSCLC. Next, we measured whether the upregulation of miR-141 reverses Bax protein expression in docetaxel chemoresistance of human NSCLC. As expected, Bax protein expression of H1299 and H2009 cells treated by docetaxel significantly suppressed the protein expression of Bax, compared with negative control group (Fig. 14A-C).

Discussion

Lung cancer is one of the most common malignant tumors. It is a malignant tumor with the highest fatality rate as well. According to histologic classification, 80% of lung cancer is non-small cell lung cancer (NSCLC) (2). Due to the absence of effective early diagnosis method, most of the patients at preliminary diagnosis have lost the opportunity
for surgery (13). In consequence, chemotherapy has become the main treatment method of NSCLC (14). Docetaxel, as a third generation cytotoxic drug, has been applied on first-line therapy for NSCLC at advanced stage (15). Similar to other chemotherapeutics, the increasing drug resistance also affects its clinical application and curative effect of chemotherapy (4). Therefore, to illuminate its resistance mechanism has important realistic significance on selection of therapeutic strategy, preventing or reversing the drug resistance to the maximum extent and improving curative effect of chemotherapy. Our work demonstrates that miR-141 expression in docetaxel chemoresistant patients with NSCLCs was markedly higher than those of non-docetaxel chemoresistant patients with NSCLCs. Downregulation of miR-141 significantly inhibited cell viability, and increased cell apoptosis and caspase-3 activity of H1299 and H2009 cells treated by docetaxel.

Experiments have shown that many malignant tumors in clinic are accompanied by overexpression of eIF4E (5). In addition, its expression level is closely related to differentiation degree of malignant tumors and metastasis. In the past 10 years, the cap binding protein eIF4E of mRNA has obtained increased attention during tumorigenesis and the development process (16). High expression of eIF4E exists on a variety of malignant tumors, such as breast cancer, laryngeal carcinoma, esophagus cancer, thyroid cancer, gastric cancer, lung cancer and leukemia. Furthermore, its expression degree is related to tumor malignant degree (17). High expression of eIF4E can obviously affect normal cell phenotype, induce cell proliferation activity, promote cell conversion and lead to genesis and metastasis of tumor (16). Transfer of eIF4E antisense RNA to the cell or inhibition of its combination with competitiveness of eIF4E binding proteins, will reduce protein translation, inhibit cell transformation, reduce tumorigenesis, alleviate infiltration and metastasis (18). In the present study, we showed that the expression of EIF4E in docetaxel chemoresistant patients with NSCLCs was markedly lower than those of non-docetaxel chemoresistant patients with NSCLCs. Downregulation of miR-141 significantly induced the protein expression of EIF4E in H1299 and H2009 cells treated by docetaxel. Tejero et al showed that miR-141 regulates VEGF in early stage non-small cell lung cancer adenocarcinoma (22).

It has been proven by overexpression of eIF4E in breast cancer, that NSCLC and prostatic cancer instead of typical benign lesions overexpression of eIF4E may be an important mark at the malignant transformation process (23). A large number of proteins shall be generated to maintain rapid proliferation of tumor cells. It has been detected in the cells from eIF4E that synthesis of only few proteins has been significantly improved (24). The synthesis of most proteins slightly increased. In vitro experiment has verified that the ‘minority proteins’ of cells with overexpression of eIF4E include: CCND1, c-myc and ODC, which can regulate cell cycle process and promote tumorigenesis, as well as promoters for fibroblast growth factor and vascular endothelial cell growth factor (25). Our work also demonstrates that downregulation of miR-141 significantly promoted the protein expression of c-Myc in H1299 and H2009 cells treated by docetaxel. Zhang et al proposed that microRNA-141 is involved in nasopharyngeal carcinoma-related gene through c-Myc pathways (26).

Bax is the first member of bcl-2 being confirmed with cell apoptosis promotion effect (27). In case of high expression of Bax protein, it can form Bax homodimer and/or Bax-Bcl-2 heterodimer with Bcl-2 thus inhibiting Bcl-2 functions, accelerating apoptosis (28). The expression of Bcl-2 and Bax, as well as the proportion of the two (Bax/Bcl-2) have key effects on the survival of cells after accepting apoptosis signal (29). In case of overexpression of Bcl-2, the cell will survive; and in case of overexpression of Bax, the reaction of cell on death signal will be enhanced and cell death will increase (23). In this study, we found that downregulation of miR-141 significantly activated Bax protein expression in H1299 and H2009 cells treated by docetaxel. Zhang et al reported that miR-141 regulated EIF4E expression in docetaxel chemoresistance of breast cancer cells (19). These data suggest that miR-141 is a potential target for chemosensitizing BCs.

It has been detected that overexpression of dF4E exists in a variety of malignant tumors (8). In addition, the overexpression is positively correlated to infiltration and metastasis ability of tumor. Overexpression of eIF4E can selectively strengthen the expression of a series of malignant proteins. Such malignant proteins include angiogenesis protein (such as FGF and VEGF), proliferation adherence and infiltrating related proteins. VEGF is a upregulated protein after overexpression of eIF4E (18). It is a key effective factor during angiogenesis process. Angiogenesis will increase after overexpression of VEGF which can provide adequate oxygen and nutrients for ectopic endometrium and promote adhesion and plant growth of ectopic cells; new vessels can produce and secrete a variety of cytokines, growth factors and degradation enzymes thus accelerate adherence infiltration of ectopic endometrium; amplification of new blood vessels in endothelial cells provides opportunity for distant metastasis of ectopic endometrium via circulatory system (20-21). Importantly, our present results showed that downregulation of miR-141 significantly activated the protein expression of VEGF in H1299 and H2009 cells treated by docetaxel. Tejero et al showed that miR-141 regulates VEGF in early stage non-small cell lung cancer adenocarcinoma (22).

In conclusion, the results of the present study demonstrated that miR-141 suppressed cell proliferation, induced cell death and increased caspase-3 activity in H1299 or H2009/docetaxel cells via EIF4E, VEGF, c-Myc protein expression and inhibited Bax in H1299 or H2009/docetaxel cells.

References


