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Inhibition of eukaryotic translation initiation factor 4E is effective against chemo-resistance in colon and cervical cancer

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ABSTRACT

Although cancer patients initially respond well to chemotherapy, they eventually develop resistance and relapse. In this work, we demonstrate that eIF4E-targeting therapy is a potential sensitizing strategy for overcoming chemoresistance and progression in cancer. We show that ribavirin, an anti-viral drug and pharmacological eIF4E inhibitor, effectively inhibits proliferation and decreases viability of paclitaxel-resistant cervical cancer and 5-FU-resistant colon cancer cells while is less toxic to human fibroblast cells. Importantly, oral administration of ribavirin significantly inhibits paclitaxel-resistant colon and 5-FU-resistant cervical cancer growth in xenograft mouse cancer model without causing significant toxicity in mice. Consistently, combination of ribavirin with paclitaxel or 5-FU sensitizes colon and cervical cancer cells to chemotherapeutic agents treatment *in vitro* and *in vivo*. We further confirm that the mechanism of the action of ribavirin in chemoresistant cancer cells is through suppressing eIF4E function. In addition, specific eIF4E knockdown via two independent siRNA mimics the effects of ribavirin in chemoresistant colon and cervical cancer cells. Cell cycle analysis indicate that ribavirin enhances the anti-proliferative effect of 5-FU by additionally arresting cells at G2/M phase via increasing cyclin B1, p-histone H3(Ser10) and Mad2 levels. Our work demonstrates that eIF4E inhibition using inhibitor or siRNA, either as single agent or in combination, could sensitize chemoresistant cancer cells to paclitaxel or 5-FU treatment and thereby improving the efficacy of chemodrug. Our findings demonstrate the therapeutic value of inhibiting eIF4E, particularly in chemoresistant cancers. Our findings also suggest ribavirin as a promising sensitizing drug for cancer treatment.

1. Introduction

The success of chemotherapy is limited by the gradual development of chemoresistance in especially those patients with tumors developed from epithelial tissues such as colon and cervical. Chemoresistance, either intrinsic or acquired, is caused and sustained via a variety of distinct mechanisms and pathways. These include changes in the activity of membrane transporters (such as ATP-binding cassette transporters), increased drug metabolism, increased repair of drug-induced DAN damage, changes of the target enzyme (such as thymidylate synthase and topoisomerases) and deregulation of programmed cell death [1,2]. Recent studies suggest that signalling transduction molecules, such as eukaryotic translation initiation factor 4E (eIF4E), might be involved in chemoresistance [3,4].

The eIF4E is essential for cap-dependent mRNA and its function represents a key regulatory node in the control of mRNA translation and protein expression [5]. In particular, eIF4E preferentially enhances the translation of c-Myc, cyclin D1 and vascular endothelial growth factor (VEGF) which are those genes that play critical roles in tumor transformation and progression [6]. Indeed, eIF4E overexpression has been observed in a wide variety of human tumors and directly contributes to tumor development [7–9]. Prognostically, eIF4E overexpression often correlates with poor clinical outcome in human cancers [10]. Although the majority of the research on the role of eIF4E in tumor are focused on carcinogenesis and tumor development, few studies recently demonstrated that eIF4E is upregulated in tumor cells in response to chemoresistance [3,4]. Whether eIF4E function critically contributes to tumor chemoresistance is not fully explored.

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In this study, we firstly generated chemodrug-resistant colon and cervical cancer cell lines and investigated the effects of eIF4E inhibition using pharmacological inhibitor ribavirin or genetic siRNA approach in these cell lines *in vitro* and *in vivo*. Our findings highlight the therapeutic value of eIF4E inhibition in those cancers that are resistant to chemotherapy and suggest ribavirin as a promising drug candidate to overcome chemoresistance.

2. Materials and methods

2.1. Cell culture and drugs

Human colon cancer cell lines HCT-116 and SW620, human cervical cancer cell lines CaSki and SiHa, and normal human fibroblast BJ-5ta were purchased from the American Type Culture Collection and cultured according to manufactures' recommendations. Briefly, complete medium containing Minimal Essential Media, fetal bovine serum (final to 10%, Hyclone, UK), L-glutamine (2 mM, Invitrogen, US) and penicillin/streptomycin (Invitrogen, US) was used. Fluorouracil (5-FU), ribavirin and paclitaxel were obtained from Sigma and dissolved in DMSO, and kept in −20 °C.

2.2. Generation of resistant cell lines

SiHa-r, CaSki-r, HCT-116-r and SW-620-r cells were established by culture SiHa, CaSki, HCT-116 and SW-620 parental cells in the medium containing paclitaxel and 5-FU, respectively. The dose of paclitaxel and 5-FU started with 0.5 nM and 1 μM, and it was increased by a dose gradient that was 50%–75% of the previous dose. The next dose was given until the cells were stable in proliferation without significant death. SiHa-r and CaSki-r were maintained in culturing medium containing 100 nM of paclitaxel. HCT-116-r and SW-620-r were maintained in culturing medium containing 50 μM of 5-FU.

2.3. Cell proliferation, cell cycle and viability assays

$5X10^3$ cells per well were seeded in 96-well plates for proliferation assay and $5X10^5$ cells per well were seeded in 12-well plates for viability assay. Cells were treated with agents for 3 days. After treatment, cell proliferation activity were determined using the CellTiter 96 AQueous One Solution Cell Proliferation assay kit (Promega, US). Cell viability was determined by labeling cells with Annexin V/7-AAD (BD Biosciences, US) staining prior to flow cytometry. The quantification of quantification of Annexin V-negative/7-AAD-negative viable cells was performed using flow cytometry software. The cell cycle was assessed by staining cells with Propidium iodide (PI) and followed by flow cytometry. Briefly, cells were fixed in ice-cold 70% ethanol and then stained with PI (Sigma, US). Stained cells were analysed on Beckman Coulter FC500 and the percentage of Go/G1, S and G2/M were quantified by CXP software.

2.4. Western blotting

Proteins were extracted in RIPA buffer (Life Technologies Inc, US) supplemented with 1X protease inhibitor cocktail and phosphatase inhibitor (Roche, US) and quantified by the DC protein assay kit (Bio-Rad, US). Equal amount of proteins was resolved using denaturing SDS–PAGE and then processed for western blot using designated primary and secondary antibodies (see Figure Legends). Targeted proteins were detected using the enhanced chemiluminescence kits (Amersham Biosciences, UK).

2.5. Overexpression and knockdown

Cell lines overexpressing eIF4E S209A or S209D were established by retroviral transduction using the murine stem cell virus (MSCV)-internal ribosome entry site (IRES) constructs as previously described [11]. Plasmids containing cDNA encoding the eIF4E S209D or S209A were obtained from Dr. Zhang's laboratory [12]. Specific knockdown of eIF4E were accomplished by transfecting cells with siRNA. Two independent siRNAs targeting different regions were synthesized by RiboBio (Guangzhou, China). The target sequences of human eIF4E siRNA were previously described [13]. One million cells were transfected with 500 nM siRNAs against eIF4E using 2 μg Dharmafect Transfection Reagent. Control siRNA, which consists of a scrambled seuqnece was used as a negative control. Transfected cells were harvested for cellular assays 24 h post-transfection.

2.6. Real time RT-PCR

Total RNA was prepared from 10^6 cells using RNeasy total RNA kits (Qiagen, Germany) and The concentration and quality of RNA were determined by photometric measurement at 260/280 nm. 1 μg was used for first-strand cDNA synthesis using iScript cDNA Synthesis Kit (Bio-rad, CA). c-Myc, VEGF and β-actin expression were measured using CFX96 RT PCR machine with the Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen, US). The sequence of primers are the same as previously reported [14].

2.7. Transplantation of tumor cells into nude mice

All of the animal experiments strictly followed institutional guidelines and were approved by Institutional Animal Care and Use Committee of Yangtze University. The Six-week-old BALB/c nude mice were subcutaneously injected with 3×10^7 CaSki-r or SW620-r cells. Tumour length and width were measured every three days and tumor volume was estimated using formula: volume = length × $width^2/2$. When tumors reached approximately 100 mm^3, the mice were randomly divided into two groups (n = 10 per group) for control (20%/80% DMSO/saline) and ribavirin (gavage, 300 mg/kg). After 3–4 weeks treatment, mice were euthanized by an overdose of isoflurane when control tumor reached ~1500 mm^3.

2.8. Statistical analyses

All figures except Fig. 2 were obtained from three independent experiments. All data are expressed as mean ± SEM. Student's *t*-test was performed by unpaired Student's *t*-test. A p-value <0.05 was considered statistically significant.

3. Results

3.1. Ribavirin is active against chemo-resistant cervical and colon cancer cells in vitro

We firstly generated paclitaxel-resistance cervical cancer cells (CaSki-r and SiHa-r) and 5-FU resistant colon cancer cells (SW620-r and HCT-116-r) and compared their sensitivity to chemotherapeutic agents with parental cells. We showed that CaSki-r, SiHa-r, SW620-r and HCT-116-r exhibited significantly higher resistance to paclitaxel or 5-FU (Supplementary Fig. S1). The IC50 of paclitaxel or 5-FU in resistant-cancer cells was at least 10-fold higher or 20-fold higher than that in parental cancer cells (Supplementary Fig. S1).

Ribavirin is a known novel type of eIF4E inhibitor [15]. To investigate whether eIF4E is the mechanism of chemoresistance,

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