Original Article

Integrin β1 mediates 5-fluorouracil chemoresistance under translational control of eIF4E in colorectal cancer

Zhengchuan Niu*, Pingping Xu*, Dexiang Zhu*, Wentao Tang, Meiling Ji, Qi Lin, Tianyu Liu, Li Ren, Ye Wei, Jianmin Xu

Department of General Surgery, Zhongshan Hospital of Fudan University, Shanghai 200032, China. *Equal contributors.

Received April 22, 2018; Accepted May 23, 2018; Epub October 1, 2018; Published October 15, 2018

Abstract: Purpose: In recent years, aberrant mRNA translational control has gained much attention as a critical player in the malignant process of tumors. Eukaryotic initiation factor 4E (eIF4E), by binding to the mRNA cap, can regulate specific protein synthesis, contributing to malignancy in human tumors. However, integrin β1 mediated chemoresistance under translational control remains unknown in colorectal cancer. Patients and methods: The expression relationship between eIF4E and Integrin β1, along with their clinical significance was investigated in colorectal cancerous tissues of 118 cases using immunohistochemistry. Cell transfection techniques of small interfering RNA (siRNA) and cDNA expression plasmid were applied to investigate the molecular relationship of integrin β1 and eIF4E and their biological effects on 5FU resistance in SW480 and LoVo cell lines. Results: The expression of eIF4E and integrin β1 was positively correlated in colorectal cancer, and patients with high expressions of both markers tended to have a worse prognosis according to a Kaplan-Meier survival analysis. Integrin β1 could contribute to 5-fluorouracil (5FU) resistance in colorectal cancer cell lines. Moreover, the protein expression of β1 could be regulated by eIF4E, interestingly, without any change of mRNA expression level. Significantly, Hoechst/PI double staining and an MTT assay proved integrin β1 could contribute to cellular survival and 5FU resistance under translational control of eIF4E in these cells. Conclusion: We conclude that integrin β1 mediated 5FU chemoresistance in colorectal cancer could be translationally regulated by eIF4E. Promisingly, targeting key molecules of this translational apparatus may provide an innovative therapeutic strategy for colorectal cancer.

Keywords: Integrin β1, eukaryotic initiation factor-4E, chemo resistance, colorectal cancer

Introduction

Colorectal cancer (CRC) is one of the most common malignancies globally [1, 2]. Although fluorouracil-based systemic chemotherapy has become an integral option in treating advanced colorectal cancer, a considerable number of patients still have disease recurrence and progression after surgery due to 5FU resistance.

Recently, translational control has been proved to participate in cancer progression and cancer response to stresses [3], which can cause adaptive changes in cancer cells that stimulate the protein expressions in stress-response genes [4]. Within the eukaryotic initiation factor 4F (eIF4F) complex, eIF4E is considered the least common rate-limiting component [5], functioning as a central regulator in translation-al control [6]. It is capable of selectively binding weak mRNAs, which have a long structure [7-9]. Subsequently, eIF4E can enhance the translation of these mRNAs into proteins associated with tumor malignancy, including fibroblast growth factor (FGF-2), vascular endothelial growth factor (VEGF), c-myc, cyclin D1, matrix metalloprotease 9 (MMP-9) and others [10, 11]. When cancer cells are exposed to the 5FU chemotherapeutic drug, translational control of relevant gene expression is required for cellular survival to respond to 5FU induced damage.

Recently, a possible connection between translational control and integrins was proposed [12]. Studies proved integrin-dependent translational control could play a critical role in the regulation of tumor malignancy [13]. Among all the integrins, it has been widely proven that β1
integriing signaling can participate in promoting cellular survival and tumor progression [14-17]. In addition, both β1 integrin and eIF4E have been demonstrated to be involved in chemotherapy in different tumors [18-23]. However, the clinical and biological relationship between eIF4E and integrin β1 remains obscure. In this study, we observed the expressions of eIF4E and integrin β1 in colorectal cancer and further investigated their implications in cellular apoptosis and chemo resistance, which may promisingly provide new therapeutic strategies for improving patients' prognosis.

**Materials and methods**

**Antibody and reagent**

Primary antibodies against eIF4E (Epitomics, Burlingame, USA) and Integrin β1 (Proteintech, Chicago, USA) were used for both immunohistochemistry staining and Western blotting.

### Table 1. Association between eIF4E expression, Integrin β1 expression and clinicopathologic variables in colorectal cancer cases

<table>
<thead>
<tr>
<th>Clinicopathologic Factor</th>
<th>β1 expression</th>
<th>P Value</th>
<th>eIF4E expression</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High n=37</td>
<td>Low n=62</td>
<td>Negative n=19</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>P Value</td>
<td></td>
<td>P Value</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
<td>34</td>
<td>11</td>
<td>30</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>28</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.268</td>
<td></td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>25</td>
<td>33</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td>≥65</td>
<td>12</td>
<td>29</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Tumor Diameter (cm)</td>
<td>0.039</td>
<td></td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>&gt;5</td>
<td>21</td>
<td>27</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>≤5</td>
<td>16</td>
<td>35</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Location</td>
<td>0.19</td>
<td></td>
<td>0.302</td>
<td></td>
</tr>
<tr>
<td>Right Colon</td>
<td>5</td>
<td>14</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Left Colon</td>
<td>15</td>
<td>14</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Rectum</td>
<td>17</td>
<td>34</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>T stage</td>
<td>0.647</td>
<td></td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>T2</td>
<td>3</td>
<td>10</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>T3</td>
<td>8</td>
<td>16</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>T4</td>
<td>25</td>
<td>33</td>
<td>11</td>
<td>32</td>
</tr>
<tr>
<td>Lymph node Invasion</td>
<td>0.001</td>
<td></td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>41</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Positive</td>
<td>22</td>
<td>21</td>
<td>2</td>
<td>26</td>
</tr>
<tr>
<td>M stage</td>
<td>0.001</td>
<td></td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>26</td>
<td>58</td>
<td>19</td>
<td>35</td>
</tr>
<tr>
<td>M1</td>
<td>11</td>
<td>4</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>TNM stage</td>
<td>&lt;0.001</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>7</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>31</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>III</td>
<td>19</td>
<td>20</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>IV</td>
<td>11</td>
<td>4</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Differentiation</td>
<td>0.289</td>
<td></td>
<td>0.251</td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Moderate</td>
<td>22</td>
<td>49</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>Poor/undifferentiated</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>
Besides, antibodies used for the detection of β-actin (4E8H3), αv (P2W7), β3 (N20) and β6 (C-19) were also used in Western blotting, and they were all purchased from Santa Cruz Biotechnology (Santa Cruz, USA). 5-Fluorouracil was obtained from Millipore Sigma (Darmstadt, Germany).

**Clinical sample and immunohistochemistry**

One hundred eighteen patients who were pathologically diagnosed with colorectal cancer were enrolled in our study between May 2008 and November 2010 at Zhongshan Hospital of Fudan University. All the patients received successful, radical surgery as their initial treatment by the same operating team. Meanwhile, adequate tissue samples and complete clinicopathologic data were acquired from the patients and archived. Routine postoperative follow-up for the patients was conducted via phones or the outpatient clinic. In all, we collected tissues from 118 patients with an average age of 61.7 years and ranging from 23 to 88 years. The detailed characteristics are shown in Table 1. All patients or relatives involved in this study have signed an informed consent approved by the Ethics Committee of our institution.

For the immunohistochemistry staining of eIF4E and integrin β1, cancerous tissues from these patients were used in our study, as described in our previous study [24]. Following incubation and deparaffinization, sections were subject to microwave antigen retrieval. The endogenous peroxidase of tissues was inactivated using 3% H2O2. Then sections were blocked with goat serum and incubated with a primary antibody overnight. The next day, they were incubated with a secondary antibody for 30 min. Following DAB and hematoxylin application, sections were sealed and then observed using a lighted microscope (Olympus, Japan). The semi-quantitative method was used to evaluate staining results by three individuals [24].

**Cell line and culture condition**

The human colorectal cancer LoVo, SW620, HCT116, SW480, HT29, and Colo320 cell lines were provided by American Type Culture Collection (ATCC, Rockville, MD, USA). All the cells were maintained as monolayers at 37°C, with 5% CO2 and saturated humidity. The culture medium is comprised of DMEM (Gibco, USA) with 20 mM HEPES and 10% fetal bovine serum (Gibco, USA). In addition, 100 IU/ml penicillin and 100 μg/ml streptomycin (Merck, Germany) were also added into medium. The 5FU resistance colorectal cancer cell line LoVo-R was established successfully from the parent LoVo cell line by exposure to gradient concentrations of 5FU for 12 months [25]. Eventually, the maintenance concentration of 5FU for LoVo-R cells was 15 μg/ml.

**Synthesis and transfection of siRNA and plasmid DNA**

To knockdown or upregulate expression of eIF4E or integrin β1, transfection techniques of small interfering RNA (siRNA) and plasmid were used in our study. GenePharma (Biosune, Shanghai China) synthesized the siRNA sequences against eIF4E and integrin β1 as well as the negative control siRNA. The plasmid pMD-eIF4E, containing the human eIF4E Gene ORF cDNA clone in a vector, was maintained in our lab. The plasmid DNA or siRNA mixed with Lipofectamine™ 2000 reagent (Invitrogen, Thermo Fisher Scientific Inc., USA) was diluted in a serum-free medium and then transferred to 6-well plates coated with fibronectin. The siRNA sequences against eIF4E were 5'-GCA-AACCUGGGCUGAUCCUTT-3' and 5'-GGAUGGU-AUGAGCCUAUGTT-3', while the two siRNA sequences against integrin β1 were 5'-GACUAUU-GAAUAUGCUAAUGGA-3' and 5'-AGUGAAG-AUGUGAGCUACUGCA-3' respectively.

**Western blot analyses**

Cells from the culture dishes were lysed on ice for 30 min. Then proteins were separated by SDS-PAGE and transferred onto PVDF membranes. After blocking for 2 hours, the primary antibodies against eIF4E and Integrin β1 were used for incubation overnight at 4°C, followed by the secondary antibody incubation next day. Protein bands were visualized using an enhanced chemiluminescence detection system. β-actin was used as the internal control. ImageJ software was used for Western blot band intensity analysis.

**Flow cytometry for apoptosis analysis**

Cells with 80% confluence were transfected with small interfering RNAs. Subsequently, these cells were exposed to 5FU for 48 h. According to the instructions provided by the
EIF4E regulates β1 mediated 5FU resistance in CRC

manufacturer, the Annexin V-FITC and PI apoptosis detection kit (BestBio, Shanghai, China) was used for analyzing the apoptosis rate of cells with different treatments. After addition and mixture of the detection solution in a dark environment, the cells in each tube were measured by flow cytometry.

Cell viability assay

Cell growth and viability was measured using the Vybrant® MTT Cell Proliferation Assay Kit. Briefly, harvested cells were seeded in a 96-well plate, and then treated with varying concentrations of 5FU for 48 hours. Subsequently, an

Figure 1. elf4E and Integrin β1 expressions and patients’ survival in human colorectal cancer. A-C. Were obtained from colorectal cancerous tissues with negative, low and high elf4E expression respectively (Scale bar 100 μm). D-F. Were obtained from colorectal cancerous tissues with negative, low and high Integrin β1 expression respectively (Scale bar 100 μm). G and H. Represent patients’ 3-Year relapse free survival and 5-Year overall survival according to elf4E with integrin β1 expression.
MTT solution was added into each well for measurement. The wavelength of 540 nm was used for absorbance measurement using a Microplate Reader.

**RNA preparation and RT-PCR**

First, total RNA was isolated from all the cells using TRIzol, obtained from Invitrogen. Next, the PrimeScript™ RT-PCR Kit (Takara, Japan) was used to perform a reverse transcription reaction and then quantitative PCR on ABI Prism 7900HT (Applied Biosystems, Foster City), based on the manufacturer's instruction. The 2-ΔΔCT method was adopted to analyze relative fold change of mRNA. Primer sequences used in the present study were listed as follows: for human β1 integrin, Forward 5'-CCTACTTCTGACAGTGTGATG-3' and Reverse 5'-CCCTTGCTACGGTTGGTTACATT-3'; and for eIF4E, Forward 5'-AAACCTGCGGCTGATCTC-3' and Reverse 5'-CCACATAGGCTCAATACCATCC-3'. In addition, the GAPDH was chosen as internal control in present study.

**Propidium Iodide (PI) and nuclear staining assay**

Propidium iodide (PI) is a popular red-fluorescent nuclear and chromosome counterstain. Since PI does not permeate living cells, it is commonly used to detect dead or late apoptotic cells. Hoechst stains are part of a family of blue fluorescent dyes used to stain DNA, and they are less toxic than DAPI and can ensure a higher viability of stained cells. In our experiments, cells were transfected with small interfering RNA followed by exposure to 5FU for 48 h. Then, they were treated with PI and Hoechst 33258 working solution (from Thermo Fisher, USA) for observing cellular death or late apoptosis induced by 5FU. Fluorescence images were taken under an Olympus inverted fluorescence microscope and analyzed using Image-Pro Plus 6.0 software.

**Statistical analysis**

SPSS 24.0 software was used to perform all the statistical analyses. The Kaplan-Meier method and log-rank test were applied in survival analyses. Statistical differences were compared using a one-way ANOVA test, a Chi-square test, and Fisher's exact test or post-hoc in our study. \( P<0.05 \) was considered statistically significant.

**Results**

**EIF4E and Integrin β1 expression and clinical significance in colorectal cancer tissues**

All the sections from the 118 cases were assessable based on their immunohistochemistry analysis. For the elf4E cohort, 85.6% (101/118) cases exhibited a positive expression (Figure 1A-C; Table 1). Among them, 45 patients were categorized into the high elf4E expression group; and 56 were defined as low expression. In addition, the remaining 17 patients' tissue samples were stained negatively. For integrin β1, 99 patients showed positive staining with positive rate of 83.9%, of which 37 cases were stained with a high β1 expression (Figure 1D-F; Table 1). In all, 19 patients were negatively stained for integrin β1.

Next, the association between elf4E expression and clinicopathologic variables was analyzed (Table 1). A significant association was found between high elf4E and tumor size, T stage, N stage, M stage, or TNM stage (\( P=0.027, 0.024, 0.002, 0.032 \) and <0.001 respectively). Moreover, there was no relationship between elf4E expression and patients' gender, age, tumor location or differentiation. Similarly, high β1 expression was related to tumor size, N stage, M stage, and TNM stage (\( P=0.039, 0.001, 0.001 \) and <0.001 respectively), but not T stage (\( P=0.647 \)). In addition, we didn't find any association between β1 expression and tumorlocation and differentiation. Meaningfully, according to a Spearman correlation analysis, it was revealed that expression integrin β1 and elf4E were moderately positively correlated (\( r=0.515, P<0.001 \), Supplementary Table 1).

For the patients with both β1 and elf4E positive expression, 92 of 118 cases were stratified into 4 groups: Group 1, low elf4E/low integrin β1 (n=37); Group 2, low high elf4E/integrin β1 (n=18); Group 3, low elf4E/high integrin β1 (n=12); and Group 4, high elf4E/high integrin β1 (n=25). A Kaplan-Meier survival analysis shown that patients in Group 4 had significantly shorter 5-year overall survival (OS) and 3-year disease free survival (DFS) than the other groups (\( P=0.001 \) and 0.002, respectively, log-rank test) (Figure 1G, 1H). In addition, patients with high elf4E or integrin β1 expression only also had a worse DFS and OS (Both \( P=0.001 \).
EIF4E regulates β1 mediated 5FU resistance in CRC

The effect of Integrin β1 on 5FU resistance in colorectal cancer cell lines

Firstly, an MTT assay was performed to observe the antitumor potential of 5FU in a panel of colorectal cancer cell lines. As shown in Figure 2A, the growth of cells exposed to 5FU for 48 hours could be inhibited in a dose-dependent manner. LoVo cells were the most sensitive cell line, while the least sensitive one was SW480. For LoVo and SW480 cells, the IC50 was 27.9 and 70.1 ug/ml respectively (Figure 2B).

Then, we investigated expression levels of integrins in SW480 and LoVo cell lines. As was shown in Figure 2C and 2D, Western blot analysis showed a higher β1 expression in SW480 cells compared to LoVo, but no significant differences of β3, β6 and αv expression existed between the two cell lines.

Next, we sought to explore the effect of β1 on 5FU induced cellular apoptosis inhibition in cancer cells. Firstly, as shown in Figure 3A, 3B and 3D, SW480 cells transfected with β1-siRNA showed an obvious downregulation of β1 expression at the mRNA and protein levels, but no changes of elf4E expression were found in these cells. Besides, LoVo-R cells had higher β1 and elf4E expressions compared to the LoVo cells in Figure 3C and 3E. Furthermore, we evaluated the cellular apoptosis effect using the Annexin V and PI detection assays. Then, all the cells were exposed to 50 ug/ml 5FU for 48 hours. As shown in Figure 3F-I and 3M, the apoptotic rates of β1-siRNA transfected SW480 cells were higher than in the cells treated with the negative control or untreated cells. Similarly, LoVo-R cells showed a lower apoptosis rate and <0.001 respectively; Supplementary Figure 1).

Figure 2. Cell Viability assay for different colorectal cancer cell lines exposed to 5FU. A. The growth of cells exposed to 5FU for 48 hours could be inhibited in a dose dependent manner. LoVo cells were the most sensitive cell line, while the least sensitive one was SW480. B. For LoVo and SW480 cells, the IC50 was 27.9 and 70.1 ug/ml respectively. C and D. Western blot analysis showed the integrin expression profiles of in SW480 and LoVo cells. *Represents P<0.05.
EIF4E regulates β1 mediated 5FU resistance in CRC

A

B

C

D

E

F

G

H

I

J

K

L

M

Protein expression (Fold of β-actin)

Protein expression (Fold of β-actin)

Protein expression (Fold of β-actin)

Protein expression (Fold of β-actin)

Protein expression (Fold of β-actin)

Protein expression (Fold of β-actin)

Protein expression (Fold of β-actin)
EIF4E regulates β1 mediated 5FU resistance in CRC

than LoVo cells when exposed to 5FU (Figure 3J-L and 3M). Above all, it was clear that colorectal cancer cells expressing higher β1 tended to have the ability of pro-survival and anti-apoptosis when exposed to 5FU.

Expression relationship of eIF4E and β1 in CRC cells treated with eIF4E siRNA or plasmid

We have known that eIF4E can selectively enhance the translation of “weak” mRNAs, encoding proteins associated with malignancy. In addition, a positive correlation exists between the protein expressions of eIF4E and β1 based on our IHC finding. Therefore, we further investigated whether β1 mRNAs could also be translated into proteins under the control of eIF4E in CRC cells.

Firstly, we treated SW480 cells with eIF4E siRNA (Figure 4A-C), which resulted in a significantly decreased mRNA expression of eIF4E at 48 hours by RT-PCR analysis, and then decreased protein expressions of eIF4E at 72 hours in these cells. Interestingly, the protein expression of β1 decreased accordingly, but there was little change of β1 mRNA expression for these cells.

Then, we treated LoVo cells with plasmids containing eIF4E-cDNA (Figure 4D-F). Correspondingly, eIF4E mRNA expression increased significantly at 48 hours, followed by increased eIF4E protein expression at 72 hours. Similarly, we found a significantly increased protein expression of β1; also, β1 mRNA expression did not change based on our RT-PCR analysis.

To sum up, it can be safely summarized that eIF4E upregulates the β1 protein expression, meaningfully, without affecting the β1 mRNA level.

E4F can translationally mediate Integrin β1 induced 5FU Chemo resistance of cancer cells

In this part, we investigated the β1 induced pro-survival and chemo resistance under trans-
Figure 5. elf4E can translationally regulate integrin β1 mediated anti-apoptosis and chemoresistance to 5FU. (A, B) Cellular fluorescence assay was performed to observe the PI positive rate in LoVo cells treated with elf4E expression plasmid when exposed to 5FU. Cells on the bottom row of (A) were treated with PBS without 5FU as negative control, while all the other cell in (A) were treated with 5FU. For each group, PI (+) cells were quantified using 10 high-power fields under microscope. (C and F) In MTT assay, 5FU inhibited growth of SW480 and LoVo cells in a dose-dependent way, which showed β1-integrin can protect cells from 5FU induced growth inhibition and apoptosis regulated by elf4E. (D and E) Then, On the other hand, Western blot was performed to investigate apoptosis-related molecular change in SW480 cells treated with elf4E siRNA when exposed to 5FU. Shown are the mean and standard deviation of three independent experiments. *P<0.05.

Hoechst/PI double staining was performed on the LoVo cells after treatment with 5FU for 48 hours.
hours. As is shown in Figure 5A and 5B, LoVo cells transfected with eIF4E plasmid exhibited a lower percentage of Hoechst (+)/PI (+) cells under microscopy. Importantly, when cells were treated with eIF4E plasmid and β1-siRNA simultaneously, the PI positive cell rate of these cells returned to a level similar to that of the untreated cells. For the cell viability assay, LoVo and SW480 cells were exposed to various 5FU concentrations for 48 h. As shown in Figure 5C and 5F, the cellular viability decreased in a dose-dependent manner in these cells. Similarly, cancer cells containing higher eIF4E expression, such as LoVo cells transfected with eIF4E plasmid or SW480 cells untreated with eIF4E siRNA, showed a higher cellular survival index at different dose points.

To further investigate whether the Bcl-2 family and caspases participated in integrin β1 mediated resistance to 5FU, we examined the protein expressions of Bcl-2, Bax and cleaved Caspase 3 in SW480 cells after exposure to 5FU for 48 hours. A Western blot analysis (Figure 5D and 5E) demonstrated the expression of eIF4E and β1 was significantly decreased in SW480 cells treated with eIF4E siRNA, which could subsequently lead to decreased expression of Bcl-2, and increased expressions of Bax and cleaved caspase-3. These data show that the apoptotic activity at the molecular level was weakened by the downregulation of β1-integrin expression under translational control of eIF4E.

Discussion

For advanced colorectal cancer, 5-Fluorouracil (5FU) based adjuvant chemotherapy has been the first line treatment option following surgery [26, 27]. However, many patients are unable to obtain any survival benefit due to chemo resistance. Therefore, an elucidation of the underlying molecular mechanisms is required to improve patients’ prognosis. In the present study, we found that the protein expression of integrin β1 and eIF4E were positively moderately-correlated in colorectal cancer, which could be indicative of poorer prognosis in these patients. Then, we revealed that integrin β1 could be upregulated by eIF4E at the translational level, which could protect colorectal cancer cells from 5-fluorouracil induced apoptosis. The findings provide a new perspective in understanding the mechanism of 5FU chemo resistance, which may lead to innovative therapies for CRC.

Recently, translational control has gained much attention in exerting a critical effect on cancer development and progression among different cancers [11, 28]. It can allow for coordinated and immediate changes in protein levels when responding to environmental stress like irradiation, hypoxia and exposure to chemotherapy drugs [29]. eIF4E, as a rate-limiting element of the eIF4F translation initiation complex, is able to selectively bind “weak” mRNAs containing long 5’UTR with a complex secondary structure [12, 30, 31], and thus promote protein synthesis to adapt to stress stimuli. Interestingly, the 5'untranslated regions of integrin β1 mRNA possess significant secondary structures with more than 75% GC content (GENE ID 3688), which met the criteria of “weak mRNA”. Meanwhile, we found that expressions of eIF4E and integrin β1 were positive correlated in these patients’ tissues. From this point, a reasonable hypothesis may be posed that translation of integrin β1 mRNA could be regulated by eIF4E, thereby contributing to the 5FU induced chemo resistance of colon cells. To substantiate it, we down and upregulated the expression of eIF4E in colorectal cancer cells, revealing that the β1 protein expression changed accordingly with eIF4E variation, while mRNA levels did not change obviously during this process. Additionally, when knocking down the expression of β1 in SW480 cells, there was no any change for eIF4E mRNA and protein expression. Taken together, we can conclude that eIF4E can regulate the expression of integrin β1 at the translational level.

Among all the 24 members of integrins from combinations of 18 α and 8 unique β subunits, the β1 subunit is the most widely expressed type in cells, which can be involved in the modulation of cellular proliferation and survival under various death-inducing conditions [14, 32-34]. In present study, we found colorectal cancer cells with a lower expression of β1 tended to have a higher apoptosis rate and a lower cellular viability under 5FU exposure. Moreover, suppressing eIF4E expression using 4E-siRNA could led to downregulation of β1 protein expression in these cells, resulting in a similar phenomenon. On the other hand, when cells
were transfected with an eIF4E-expressed plasmid, these cells showed a higher β1 protein level with a decreasing apoptotic rate. The pro-apoptosis or death stimuli can trigger the cellular intrinsic apoptosis pathway, which is regulated by the Bcl family and executed by activated caspase-3 [35, 36]. According to our Western blot results, we also found that Bcl2 and Bax and cleaved caspase-3 were involved in this process following 5FU treatment. These results showed that integrin β1 could protect cells from 5FU induced apoptosis, thus enabling cancer cells with a chemo-resistance phenotype.

The biological implications of selective translation are clearly important for cancer cells to adapt to stress stimuli. Although some mechanisms of selective translation such as IRES and miRNA-mediated control of translation initiation are recently getting much attention, the process of how it works remains largely unexplored [4, 37]. To some degree, the regulation of translation may be both the cause and the consequence of 5FU induced cellular apoptosis [38, 39]. When exposed to 5FU, the deregulation of translation initiation in these cells could regulate the expression of integrin β1, leading to chemoresistance through an enhanced resistance to apoptosis. Nevertheless, how integrins transmit signaling and regulate gene expression still remain as an open question [40-42]. Encouragingly and interestingly, recent studies have revealed integrin-dependent translational control could trigger more complicated signaling cascades involving cancer progression than expected [13]. Inspired by these new findings, our future research will reasonably focus on further tumor regulatory mechanisms underlying the eIF4E-integrin β1 loop signaling, which may contribute to the identification of translational machinery and molecular targets for anticancer therapeutics in colorectal cancer.

Taken together, expressions of eIF4E and integrin β1 were moderately positively correlated, and they were associated with patients’ poorer prognosis in colorectal cancer. Moreover, integrin β1 could be regulated by eIF4E at the translational level, which subsequently contributed to cellular survival and anti-apoptosis, leading to 5FU chemo resistance. Significantly, further the mechanical investigation of how translational control determines the cellular response to 5FU may provide a better insight into improving chemo resistance, and ultimately lead to the development of new therapeutic modalities for colorectal cancer.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81402341 and 81602036, 81372315 and 81472228) and the Shanghai Engineering Research Center of Colorectal Cancer Minimally Invasive (No. 17DZ2252600). We thank Prof. Jun Niu from Qilu Hospital of Shandong University and TC Wang from Columbia University for all their assistance with this study.

Disclosure of conflict of interest

None.

Address correspondence to: Jianmin Xu, Department of General Surgery, Zhongshan Hospital of Fudan University, Building 1, Room 1101, 180 Fenglin Road, Xuhui District, Shanghai 200032, China. Tel: +86-21-64041990; Fax: +86-21-64041990-296; E-mail: xujmin@aliyun.com

References

EIF4E regulates β1 mediated 5FU resistance in CRC


EIF4E regulates β1 mediated 5FU resistance in CRC


**Supplementary Table 1.** Correlation between integrin β1 expression and eIF4E expression in human colonic carcinoma tissues ($r=0.515$, $P<0.001$, Correlation Spearman)

<table>
<thead>
<tr>
<th>Integrin β1 Expression</th>
<th>eIF4E Expression</th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative 10</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High 0</td>
<td>12</td>
<td>25</td>
</tr>
</tbody>
</table>

**Supplementary Figure 1.** Kaplan-Meier analysis of patients’ survival according to eIF4E and Integrin β1 expression. A and B. Patients’ 3-Year disease free survival and 5-Year overall survival after surgery according to eIF4E expression through immunohistochemical staining accordingly; C and D. Patients’ 3-Year disease free survival and 5-Year overall survival after surgery according to Integrin β1 expression through immunohistochemical staining. Numbers 0, 1, and 2 in the charts represent negative, low, and high expression respectively.