World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2540

World J Gastroenterol 2012 May 28; 18(20): 2540-2544
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Detection of eukaryotic translation initiation factor 4E and its clinical significance in hepatocellular carcinoma

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Received: November 6, 2011 Revised: February 20, 2012
Accepted: February 26, 2012
Published online: May 28, 2012

Abstract

AIM: To study the expression of eukaryotic translation initiation factor 4E (eIF4E), which is closely correlated with malignant tumors, and its relationship to prognosis in hepatocellular carcinoma.

METHODS: Western blotting was performed to quantify the elF4E protein expression in the normal human liver cell line L02 and the hepatoma cell lines Hep3B, HepG2, and Huh7. Forty-six hepatocellular carcinoma samples with complete clinical data were obtained from Changzheng Hospital during the period of December 2008 to July 2009. The expression of eIF4E in the tumor samples and their adjacent tissues were detected by immunohistochemistry. The relationship between the test results and hepatocellular carcinoma (HCC) prognosis was statistically analysed by using a COX proportional hazard model.

RESULTS: Western blotting analysis showed that there were distinct eIF4E protein bands in all three of the hepatoma cell lines. In particular, the HepG2 cell line had the highest level of eIF4E protein expression. The L02 cell group had a low eIF4E expression. Immunohistochemical assay showed that there were 32 cases in which the tumour tissue expression was higher than their adjacent tissues, accounting for 69.57%. There were also 14 cases in which the tumour tissue expression was lower or no significant difference was found, accounting for 30.43%. COX proportional hazards model analysis showed that HCC prognosis was related to the depth of invasion, the overexpression of eIF4E and p53, possibly as independent HCC prognostic predictors.

CONCLUSION: In summary, eIF4E expression is associated with liver cancer, and patients with high eIF4E expression levels have a higher risk of recurrence.

Key words: Hepatocellular carcinoma; Eukaryotic translation initiation factor 4E; Western blotting; Immunohistochemistry; Prognosis

Peer reviewer: Ezio Laconi, Professor, University of Cagliari, Via Porcell 4, 09125 Cagliari, Italy

Wang XL, Cai HP, Ge JH, Su XF. Detection of eukaryotic translation initiation factor 4E and its clinical significance in hepatocellular carcinoma. *World J Gastroenterol* 2012; 18(20): 2540-2544 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2540.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2540

INTRODUCTION

Eukaryotic translation initiation factor 4E (eIF4E) is a member of the eIF family. It can specifically bind to the cap structure located at the 5' end of mRNAs named the "m7GpppN cap", which is necessary for mRNA translation initiation, and affects mRNA metabolism, processing, transportation and translation[1]. It plays an important role

in regulating the initial stage protein synthesis[2,3]. eIF4E is highly expressed in a variety of human malignancies[4-7], which has been confirmed to be relevant to the occurrence, invasion and metastasis of carcinomas such as head and neck squamous cell carcinoma[8], laryngeal cancer, non-small cell lung cancer[9-11], breast cancer[12-18], thyroid cancer, oesophageal cancer, stomach cancer, cholangiocarcinoma, colon cancer[19], non-Hodgkin's lymphoma, acute or chronic myeloid leukaemia[20], and lymphoma[21,22]. Experiments have also confirmed that eIF4E is closely related to the prognosis of many carcinomas. However, eIF4E-related studies in the context of hepatocellular carcinoma (HCC) are still rare.

In this study, we separately compared the eIF4E expression levels in normal liver cells with liver cancer cell lines and liver cancer tissues with precancerous tissues. Additionally, we investigated the influence of eIF4E expression on the prognosis of liver cancer. This research may provide an experimental basis for exploring new ways to treat liver cancer.

MATERIALS AND METHODS

Study objects

We selected 46 patients with pathological evidence of HCC and complete clinical data from Shanghai Changzheng Hospital who had liver surgery from January 2007 to January 2009. In these 46 cases, there were 40 males and 6 females who ranged in age from 31 years to 77 years (median age: 52.26 years). With regards to histological grade, there were 42 cases of moderately differentiated HCC, and 4 cases were poorly differentiated. A total of 33 patients had a cancer embolus in the intrahepatic bile duct or vein or had an infiltrated peplos, and 13 patients had no cancer tissue in cutting edge and gallbladder and no infiltrated peplos. p53 pathological testing was positive in 39 patients and negative in 7 cases. None of the patients received preoperative radiotherapy or chemotherapy. The follow-up time was 24 mo, and no case was lost.

Major materials and reagents

The cell lines used for Western blotting were the human liver cancer cell lines Hep3B, HepG2, Huh7, and the normal human liver cell line L02, which was provided by Shanghai Cell Biology Institution of Academia Sinica.

eIF4E (P-2) is a mouse anti-human monoclonal antibody raised against full-length eIF4E (Santa Cruz Biotechnology, Inc.). It is recommended for the detection of eIF4E by Western blotting (dilution: 1:200; dilution range: 1:100-1:1000) and immunohistochemistry (including paraffin-embedded sections; dilution: 1:50; dilution range: 1:50-1:500). The streptavidin-perosidase (SP) kit was provided by Fuzhou Maixin Biotechnology, Inc.

Detection methods

Western blotting analysis: We tested the *eIF4E* gene expression level in normal liver cells and different liver cancer cell lines. The four of cell lines (i.e., HepG2, Hep3B, Huh7, and L02) were incubated with high glucose Dulbecco's modified Eagle's medium containing 10% fetal bovine serum at 37.0 ℃ with 5% CO_2 until the cell concentration reached 5×10^6 cells/mL. Then, we sequentially performed the protein extraction, bicinchoninic acid protein quantification, sodium dodecyl sulfate-polyacrylamide gel electrophoresis electrophoresis, protein transfer, membrane closure, antibody incubation, and Bio-Rad chemiluminescence.

Immunohistochemistry: We detected the eIF4E protein expression levels in HCC and their adjacent tissues (SP, a particular type of immunohistochemistry). The tumour and adjacent tissues from the same patient were fixed, dehydrated, sectioned, and made into paraffin biopsies. We made 46 paraffin sections. The steps of the SP kit included heating on a baking sheet, incubation, washing, sealing, staining, drying, dehydration, and mounting. To analyse the results, we used two scoring methods. The samples were placed in an electron microscope and were scored for staining intensity as follows. 0: No colour; 1: A yellow colour; 2: A claybank colour; and 3: A brown colour. We then graded the samples for the positivity rate as follows. 0: No positive tumour cell staining; 1: ⩽ 10% positive cells; 2: 11% to 50% positive cells; 3: 51% to 75% positive cells; and 4: > 75% positive cells. Finally, we added the two scores together, and the sum represented the immunohistochemical score as follows. -: 0; +: 1 to 4; ++: 5 to 8; and + + +: 9 to 12. Each cancer tissue section was compared with its adjacent tissue.

Follow up: We analysed the number of cases that had HCC recurrence and metastasis during the post-operative 24 mo. The liver cancer recurrence risk was measured using COX proportional hazards model for statistical analysis. The patient age, gender, histological grade, depth of invasion, eIF4E, p53 status and other prognostic indicators were used for the COX proportional hazards model analysis.

Statistical analysis

We used the SPSS 17.0 statistical software for the statistical analysis. A COX proportional hazards model testing level of $\alpha = 0.05$ and a $P < 0.05$ was considered statistically significant.

RESULTS

eIF4E protein expression in liver cancer cell lines by western blotting

We tested the eIF4E protein expression level in the liver cell line L02 and the liver cancer cell lines Huh7, HepG2, Hep3B. The eIF4E protein bands are shown in Figure 1. The bands were detected by Bio-Rad chemiluminescence to obtain the data shown in Table 1.

The liver cancer cell lines HepG2, Huh7, Hep3B significantly expressed the eIF4E protein, and in particular, the HepG2 cell line had the highest level of eIF4E protein expression. The normal liver cell L02 also expressed

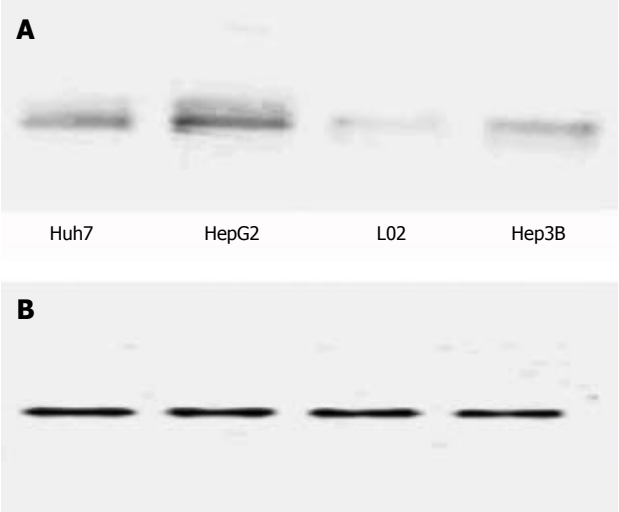

Figure 1 Eukaryotic translation initiation factor 4E protein and glyceraldehyde-3-phosphate dehydrogenase protein bands in a normal human liver cell line and three hepatoma carcinoma cell lines. A: Eukaryotic translation initiation factor 4E protein bands; B: Glyceraldehyde-3-phosphate dehydrogenase protein bands.

Table 1 Eukaryotic translation initiation factor 4E protein expression in the liver cells at different levels

	Huh7	HepG2	L02	Hep3B
GAPDH	827.165	884.682	885.437	848.552
eIF4E	3161.861	5651.885	775.440	4496.191

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; eIF4E: Eukaryotic translation initiation factor 4E.

eIF4E; however, its expression level was low. Glyceraldehyde-3-phosphate dehydrogenase, which was used as the internal reference had bands in each cell line, and no obvious differences were observed with this protein.

eIF4E protein expression in liver cancer and adjacent tissues by immunohistochemistry

We next detected the eIF4E protein expression level in HCC and adjacent tissues. There were 46 pathological tissue paraffin blocks in which 32 HCC tissue cases had higher eIF4E protein expression than their adjacent tissues, accounting for 69.57%. A total of 14 HCC tissue cases had lower expression or no significant difference compared with their adjacent tissues, accounting for 30.43%. The scores were weighted 154:97 = 1.59:1, meaning that, in general, HCC tissues had a higher eIF4E protein expression level than the adjacent tissues.

Figure 2 show that HCC tissues stained significantly stronger than adjacent tissues, indicating that tumour tissues had a higher eIF4E protein expression level. The lightly stained central area in Figure 2A represents necrotic tissue.

eIF4E may be an independent risk factor for liver cancer prognosis

Follow-up statistics showed recurrence in 33 cases and death in 12 cases. The patient age, gender, histological grade, depth of invasion, eIF4E overexpression, p53 positive status and other prognostic indicators were used for COX proportional hazards model for screening analysis. Ultimately, the depth of invasion, eIF4E, and p53 were included in the model with a Sig < 0.05 as shown in Table 2. The statistical significance suggests that these three factors are independent risk factors for liver cancer prognosis.

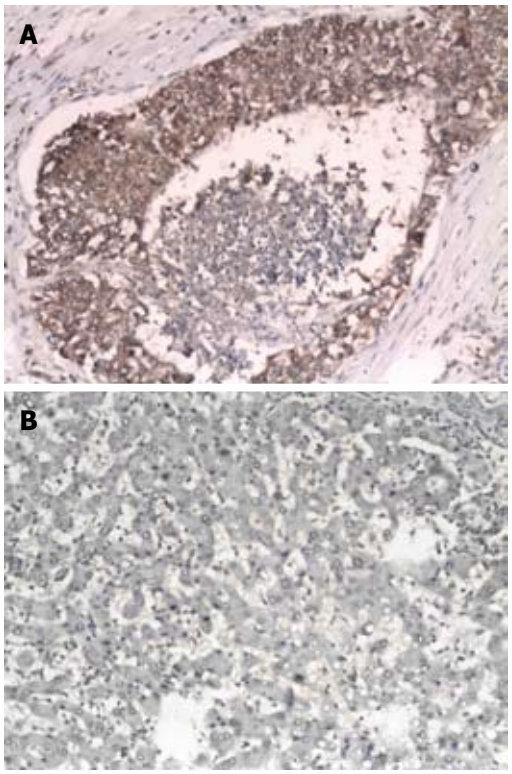

Figure 2 Photo of hepatocellular carcinoma tissue and adjacent tissue by electron microscopy. A: Hepatocellular carcinoma tissue and adjacent interstitial tissue; B: Adjacent tissue. (Hematoxylin and eosin stain, ×100).

DISCUSSION

In eukaryotic cells, translational regulation plays an important role in gene expression. eIF4E is involved in the regulation of the mRNA translation process. It can enhance the translation of some important growth factors and cell growth regulators and affect protein synthesis, the cell cycle, cancer gene activation, and apoptosis; it also plays an important role in malignant transformation and metastasis.

eIF4E regulates the translation of cancer-related mRNAs (i.e., it is involved in the activation of proto-oncogenes, angiogenesis, apoptosis, invasion and metastasis) that are involved in tumour occurrence and development. Normal tissues have a low eIF4E expression level. eIF4E was overexpressed, in a variety of malignant tumours including head and neck squamous cell carcinoma, laryngeal cancer, lung cancer, breast cancer, thyroid cancer and other cancer tissues[4,23]. Its high expression was correlated with tumour invasion and metastasis. However, studies of eIF4E in liver cancer are rare. At present, there are studies

Table 2 COX proportional hazards model analysis

	B	SE	Wald	df	Sig	Exp(B)	95% CI for Exp(B)	
							Lower	Upper
Eukaryotic translation initiation factor 4E	1.971	0.926	4.529	1	0.033	7.179	1.169	44.100
Depth of invasion	3.122	1.211	6.650	1	0.010	22.690	2.115	243.423
Histological grade	0.410	1.156	0.126	1	0.723	1.506	0.156	14.527
Gender	1.671	1.152	2.104	1	0.147	5.319	0.556	50.890
Age	-0.017	0.028	0.354	1	0.552	0.983	0.930	1.040
p53	-3.208	0.825	15.118	1	0.000	0.040	0.008	0.204

B: Coefficient of regression; SE.: Standard error; Wald: The index of regression effect; df: Degrees of freedom; Sig: *P* value; Exp(B): Odds ratio.

that involve the targeting eIF4E in head and neck squamous cell carcinoma[24,25], breast cancer[13-18], non-small cell lung cancer[26], blood malignancies[27-29] and other studies[6,30-32]. However, few studies have focused on targeting eIF4E in HCC.

In this study, we tested the expression of eIF4E protein in a normal human liver cell line and three different liver cancer cell lines. eIF4E protein expression was high in the three liver cancer cell lines and higher than in the normal liver cell L02. The HepG2 cell line had an especially high level of eIF4E protein expression. By comparing 46 cases of human liver cancer and adjacent tissues, we found that eIF4E protein expression was higher in most of the cancer tissues than in the adjacent tissues. COX proportional hazards model analysis showed that the depth of invasion, eIF4E, and p53 status were independent risk factors of liver cancer prognosis.

Based on these studies, we believe that eIF4E protein expression may be closely associated with the occurrence of human liver cancer development and prognosis. It has been confirmed *in vivo* and *in vitro* that sorafenib treatment can inhibit the RAF/MEK/ERK signal transduction pathway, reduce the eIF4E phosphorylation level, reduce Mcl-l protein, and induce hepatoma cell apoptosis[33,34]. Accordingly, we suggest that lower levels of *eIF4E* gene expression may inhibit liver cancer. Targeting and adjusting the eIF4E level and activity may inhibit cancer cell growth[6,30,31,35], which may become a new paradigm in the field of the biological treatment of liver cancer[36].

COMMENTS

Background

Hepatocellular carcinoma (HCC), which has a poor prognosis and a low five-year survival rate, is the most common malignant tumour in our country. At present, there are no effective therapies including radiotherapy, chemotherapy, and surgery. Eukaryotic translation initiation factor 4E (eIF4E) plays an important role in the translation initiation phase of a eukaryotic cell. It has been confirmed that eIF4E can specifically bind to the 5' mRNA cap (m7GpppN) and modulate its translation and expression. Its expression is closely associated with the generation, infiltration, and metastasis of many tumours such as head and neck, larynx, lung, mammary gland, thyroid gland, oesophagus, stomach, bile duct, colon.

Research frontiers

There are many researchers that are targeting eIF4E in head and neck squamous cell carcinoma, breast cancer, non-small cell lung cancer, blood malignancies and other carcinomas; however, studies that involve the targeting eIF4E in HCC are rare.

Innovations and breakthroughs

Research concerning the effects of eIF4E on HCC is limited. The authors tested the expression of the eIF4E protein in liver cancer cell lines and cancer tissues and used COX proportional hazards model analysis to show that eIF4E was an independent risk factor HCC prognosis.

Applications

The targeted regulation of the level and activity of eIF4E may inhibit cancer cell growth, which may become a new treatment paradigm in the liver cancer field.

Terminology

eIF4E is a member of the eIF family. It can specifically bind to the cap structure located at the 5' end of mRNAs named the "m7GpppN cap", which is necessary for mRNA translation initiation, and affects mRNA metabolism, processing, transportation and translation. It plays an important role in regulating the initial stage protein synthesis. eIF4E is highly expressed in a variety of human malignancies, which has been confirmed to be relevant to the occurrence, invasion and metastasis of carcinomas such as head and neck squamous cell carcinoma, laryngeal cancer, non-small cell lung cancer, breast, thyroid cancer, oesophageal cancer, stomach cancer, cholangiocarcinoma, colon cancer, non-Hodgkin's lymphoma, acute or chronic myeloid leukaemia, and lymphoma. Experiments have also confirmed that eIF4E is closely related to the prognosis of many carcinomas.

Peer review

This paper is interesting and worth being published if authors can satisfactorily address the concerns raised regarding immunohistochemical expression of eIF4E.

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