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Clinical Significance of Eukaryotic Initiation Factor 4E (*eIF4E*) Level among Cases Suffering Basal Cell Carcinoma of Skin

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Eukaryotic initiation factor 4E (*eIF4E*) has been reported to act as a prognostic biomarker in various cancers, but its actual effect on basal cell cancer (BCC) of the skin is rarely reported. Our research measured *eIF4E* levels and discussed its consequence in BCC of the skin.

Material/Methods: Semi-quantitative real-time polymerase chain reaction (RT-PCR) and western blotting analysis were used to detect relative expression level of *eIF4E* in specimens at both mRNA and protein levels. The relationship of *eIF4E* level with clinical profiles was analyzed via chi-square test. Additionally, prognostic value of *eIF4E* was analyzed via Kaplan-Meier and cox regression analysis.

Results: We found that *eIF4E* was over-expressed in tumor tissues, in comparison to bordering cancer-free tissue samples. Besides, elevated *eIF4E* level exhibited a strong relation to metastasis, TNM stage, and differentiation. Kaplan-Meier analysis revealed cases harboring high *eIF4E* levels faced shortened overall survival compared to cases of low levels (log rank test, *P*=0.018). Moreover, *eIF4E* could act as an independent biomarker for the prognosis of BCC of the skin, according to Cox regression analysis.

Conclusions: The level of *eIF4E* was upregulated and significantly correlated with the development of BCC of the skin. Thus, it might be a promising prognostic biomarker and therapy target for BCC of the skin.

MeSH Keywords: Carcinoma, Basal Cell • Eukaryotic Initiation Factor-4E • Prognosis

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Background

Basal cell cancer (BCC) of the skin is the most common cutaneous cancer in the world, especially among white populations [1]. Solar radiation is generally considered to be a major environmental risk factor for this cancer [2]. BCC of the skin is characterized by slow growth, rare metastasis, and local spread [3]. Treatments for BCC of the skin include surgical resection, radiotherapy, cryotherapy, phototherapy, and creams. Although these treatments are effective for primary BCC of the skin, locally advanced cases only have limited options in the treatment [4]. When facing difficulties in histopathological analysis, androgen receptor (AR) and cluster of differentiation 10 (CD10) staining of total resection biopsy can more accurately differentiate between BCC and trichoepithelioma (TE) [5]. In the identification of morpheaform BCC, cytokeratin 20 (CK20) and AR combined staining showed the greatest specificity and sensitivity. Targeting, the specificity of CK20-AR+immunophenotype has been reported to be 95.24%, and its sensitivity 83.33% [6]. In addition, prognostic biomarkers which are associated with the mechanism of the cancer may guide cancer treatments. In previous studies, few markers were correlated with BCC of the skin prognosis. Therefore, it is necessary to find some effective and accurate biomarkers for the patients with BBC of the skin.

Eukaryotic initiation factor 4E (*eIF4E*) stands for a central component in the initiation and regulation of translation in eukaryotic cells [7]. Its protein is a limiting component of eukaryotic protein synthesis initiation complex and abnormal expression level of this protein is proved to be correlated with carcinogenesis [8,9]. Aberrant expression of *eIF4E* was reported to be significantly associated with various types of cancers, such as melanoma, oral squamous cell carcinoma, breast cancer, nasopharyngeal carcinoma, and lung cancer [10–14]. However, the level and clinical significance of *eIF4E* in BCC of the skin are rarely reported.

In our research, we detected *eIF4E* levels for cancer tissues and matched cancer-free tissues, and we analyzed the relationship between *eIF4E* levels and clinical characteristics for patients suffering BCC of the skin. In addition, overall survival rate was analyzed, and prognostic value of the gene was evaluated.

Material and Methods

Cases and specimens

We included 106 patients with BCC of the skin who were pathologically diagnosed in Ordos City Center Hospital in our research. No patients had undergone chemotherapy or radiotherapy ahead of sampling. Our research conformed to the rule of the Research Ethics Committee of the hospital. Informed consents were signed by all participants before specimen collection. Cancer tissues and neighboring regular tissue samples were extracted and frozen in liquid nitrogen immediately. Then the samples were stored at –80°C ahead of application. All patients participated in a 5-year follow-up investigation. Clinical information and survival status were collected in a database. Cases dying from sudden accidents or other illnesses were removed from this research.

RNA isolation and qRT-PCR analysis

Total RNA was extracted from collected tissue specimens using TRIzol regent (Invitrogen, Carlsbad, CA, USA) according to the instruction of the manufacturer. Residual DNA in extracted RNA samples was dealt with using DNase I. Ultraviolet (UV) absorbance was used to detect the concentration of total RNA (A260/A280) and the quality of the samples was tested through 1% agarose gel electrophoresis. Prime Scrip RT reagent kit (Takara Biotechnology Co., Ltd.) was used to compound cDNA from RNA samples. First strand cDNAs were synthesized and analyzed via polymerase chain reaction (PCR) to detect the expressions of *eIF4E* and β-*actin*. The sequences of primers were as follows [14]:
eIF4E: forward-5'-ATGGCGACTGTCGAACCGG-3';
reverse-5'-GCTATCTTATCACCTTTAGC-3',
β-actin: forward-5'-ATGGATGATGATGATATCGCC-3';
reverse-5'-GTGATGACCTGGCCGTCAGG-3'.
PCR products were visualized through ethidium bromide staining with 1.5% agarose gel and results were analyzed applying Alphaease software.

Western blotting

Proteins were extracted from collected specimens and separated via 12% sodium dodecyl sulfate polyacrylamide-based discontinuous gel electrophoresis (SDS-PAGE). Then proteins brands were transferred onto polyvinylidene difluoride (PVDF) membrane blocked and incubated with rabbit monoclonal anti-*eIF4E* and anti-β-actin primary antibody for 3 hours at room temperature. After being washed 3 times with western washing buffer, the membrane was incubated with secondary antibody for 40 minutes at room temperature. Enhanced chemiluminescence kit (Pierce Chemical) was used for chemiluminescent assay.

Statistical analysis

Statistical analyses were performed with SPSS 18.0 software, and Sigma Plot 12.5 was used for drawing figures. Dissimilarity between 2 groups was compared through Students' *t* test and all data were shown as mean ± standard deviation (SD). In addition, the relationship of *eIF4E* level with clinical profiles among

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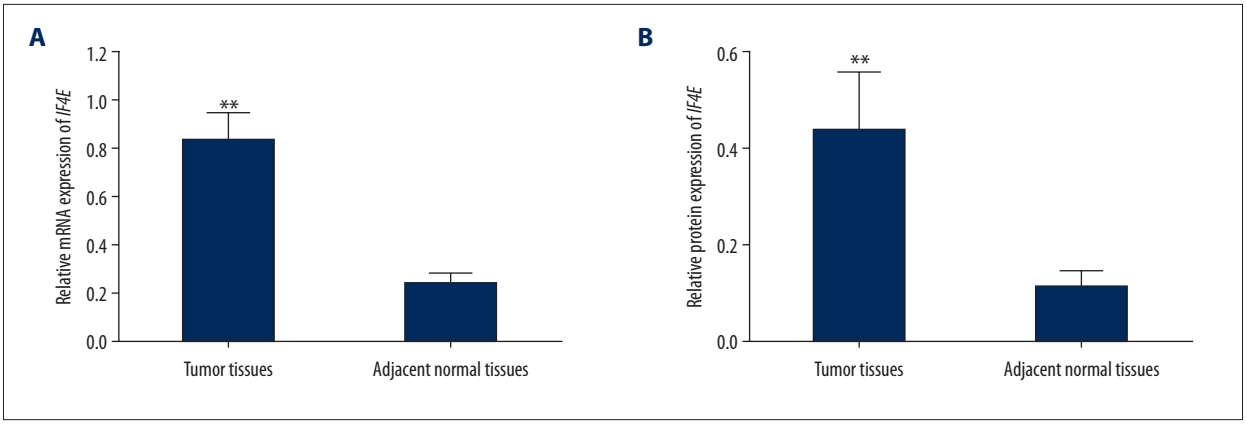

Figure 1. Relative levels for *eIF4E* in basal cell cancer of skin tissues and bordering cancer-free tissue samples. Relative mRNA (**A**) and protein (**B**) expressions of *eIF4E* were both heightened among cancer samples compared to control ones ($P<0.001$).

cases suffering BCC of the skin was analyzed employing chi-square test. Besides, Kaplan-Meier analysis with log rank test assessed overall survival while Cox regression analysis estimated outcome consequence for the gene. $P<0.05$ was considered as statistical significance.

Results

Relative level for *eIF4E* among patients suffering BCC of the skin

Semi-quantitative RT-PCR and western blotting analysis measured relative level for *eIF4E* among malignant tissues as well as paired cancer-free tissue samples at mRNA and protein levels, respectively. As shown in Figure 1, *eIF4E* expression showed rising tendency among malignant samples when compared to matched regular ones at both 2 levels ($P<0.000$).

Clinical characteristics of patients with BCC of the skin and their correlations with *eIF4E* expression

The 106 patients with BCC of the skin in this study included 51 males and 55 females, and their average age was 53.4 years old. Table 1 listed clinical characteristics of the cases. Chi-square test examined potential connection for clinical characteristics with *eIF4E* expression. The results suggested that high *eIF4E* level held strong relation to TNM stage ($P=0.011$), differentiation ($P=0.047$) and metastasis ($P=0.023$), but not to gender, age, cancer dimensions or recurrence ($P>0.05$).

Relationship of *eIF4E* levels with overall survival among cases suffering BCC of the skin

To investigate possible link for *eIF4E* expression with overall survival of patients with BCC of the skin, a 5-year' follow-up was conducted. Based on the data from the follow-up, Kaplan-Meier analysis with log rank test showed that patients with high expression of *eIF4E* had shorter overall survival than those with low expression (35.11±2.35 versus 46.38±3.70 months, log rank test, $P=0.018$) (Figure 2). This revealed *eIF4E* expression was related to the prognosis of patients with BBC of the skin. Then unvaried and multivariate analyses using Cox regression analysis were carried out, demonstrating that high *eIF4E* expression (hazard ratio [HR]=2.283, 95% confidence interval [CI]=1.108–4.701, $P=0.025$) could act as an independent biomarker for the prognosis of BCC of the skin (Table 2).

Discussion

EIF4E is located at chromosome 4q21-q25 and encodes a 24 KD protein which binds to mRNA 5'-cap structure at 5'terminus [15,16]. In normal cells, *eIF4E* shows low expression level to maintain normal cellular functions; however. once activated, it selectively enhances the translations of oncogenes and growth factors [13]. Reportedly, *eIF4E* can significantly enhance the translations of basic fibroblast growth factor (FGF-2), cyclin D1 (cell-cycle regulatory protein), pro-oncogene protein (c-Myc), and endothelial growth factor (VEGF) which promote tumor growth, angiogenesis and proliferation [17–20]. In our study, we detected *eIF4E* levels for malignant tissues and bordering cancer-free tissue from cases suffering BCC of the skin. The results suggested that *eIF4E* was upregulated in tumor samples compared with the controls, and that *eIF4E* silencing might be helpful in treating advanced BCC of the skin.

In previous studies, *eIF4E* over-expression was detected in different cancers. For instance, Zhang et al., and Thumma et al., found the upregulation of *eIF4E* in non-small cell lung cancer, and this expression pattern might be related to the cancer advancement and therapy [21,22]. Diab et al., claimed that *eIF4E* was increased and closely related to the grade of colorectal carcinoma [16]. Jiang et al., proved the over-expression of *eIF4E*

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Table 1. The relationship between *eIF4E* expression and clinical characteristics of patients with basal cell cancer of the skin.

Characteristics	Case (n=106)	eIF4E expression		χ^2	*P*
		High (n=66)	Low (n=40)		
Gender				0.495	0.482
Male	51	30	21		
Female	55	36	19		
Age (years)				0.062	0.803
≥55	54	33	21		
<55	52	33	19		
Size				0.602	0.803
≥2 cm	54	33	21		
<2 cm	52	33	19		
Metastasis				5.174	0.023
Yes	23	19	4		
No	83	47	36		
TNM stage				6.534	0.011
T1+T2	52	26	26		
T3+T4	54	40	14		
Differentiation				3.946	0.047
Well	64	35	29		
Poor	42	31	11		
Recurrence				0.621	0.431
Yes	40	23	17		
No	66	43	23		

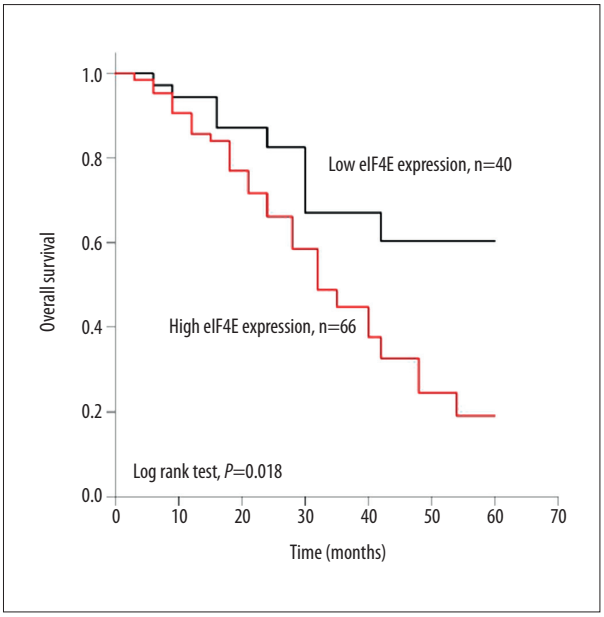

Figure 2. Overall survival among cases suffering basal cell cancer of the skin according to *eIF4E* levels. Cases possessing low expression of *eIF4E* had prolonged overall survival compared to high ones, according to Kaplan-Meier analysis with log rank test (*P*=0.018).

and its effects on the remission of acute myeloid leukemia [23]. The activation of *eIF4E* participated in the progression of cervical neoplasia and was a marker of malignant transformation [24]. The activation of Akt/mammalian target of rapamycin (mTOR) pathway could help increase *eIF4E* level among cancer margins in head and neck cancer [25]. Wang et al. detected the expression of *eIF4E* and confirmed that its over-expression could lead to the recurrence of hepatocellular carcinoma [26]. *eIF4E* might be a therapy target in varied cancers. Thumma et al. reported that *eIF4E*-silencing could reduce the growth of cells in non-small cell lung cancer and enhance its chemo-sensitivity [22]. In a research by Sun and colleagues, *eIF4E* downregulation was proved to suppress the multiplication, emigration, and intrusion of colorectal carcinoma cells [27]. In this study, we used semi-quantitative RT-PCR and western

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Table 2. Cox regression analysis adjusted for clinical factors for estimating the prognostic value of *eIF4E* in patients with basal cell cancer of the skin.

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P*	HR	95%CI	*P*
eIF4E (high *vs.* low)	2.283	1.108–4.701	0.025	2.283	1.108–4.701	0.025
Gender (Male *vs.* Female)	1.015	0.581–1.771	0.959	–	–	–
Age (years) (≥55 *vs.* <55)	1.054	0.602–1.847	0.854	–	–	–
Size (≥2 cm *vs.* <2 cm)	0.801	0.459–1.397	0.434	–	–	–
Metastasis (yes *vs.* no)	1.351	0.737–2.478	0.331	–	–	–
TNM stage (T3+T4 *vs.* T1+T2)	1.292	0.732–2.281	0.377	–	–	–
Differentiation (poor *vs.* well)	1.238	0.702–2.184	0.460	–	–	–
Recurrence (yes *vs.* no)	1.182	0.676–2.068	0.557	–	–	–

blotting to detect *eIF4E* expression in malignant samples, and we found an upward tendency.

In a previous study, *eIF4E* was proven to participate in the origination and advancement of malignancies like lung adenocarcinoma [14]. To explore the role of *eIF4E* in the development of BCC of the skin, the relationship between its expression and clinical characteristics was analyzed in this study. *eIF4E* overexpression had close relation to metastasis, higher TNM stage, and poor tumor differentiation. These results indicated that *eIF4E* might be involved in the progression of BCC of the skin.

Growing evidences has shown that *eIF4E* might be a promising biomarker for cancer prognosis. According to Khosravi et al., high *eIF4E* levels were adverse for melanoma cases' survival and its level could be a promisingly prognostic marker and therapy target for this cancer [10]. In the study by Liang et al., diminished *eIF4E* level could predict better tumor-specific survival among cervical cancer cases, being a prognostic marker and treatment target for this cancer [28]. In our research, we also analyzed overall survival among the cases suffering BCC of the skin according to *eIF4E* levels. Survival analysis unveiled high *eIF4E* levels were connected to cancer-related death among the cases. Sufferers possessing high *eIF4E* levels had shortened overall survival compared to low ones. In addition, Cox regression analysis confirmed *eIF4E* alone could predict outcomes for BCC of the skin.

Conclusions

Our finding shows that *eIF4E* is upregulated in BCC of the skin tissues, compared with normal skin tissues. And high expression of *eIF4E* is significantly correlated with metastasis, high TNM stage, and poor tumor differentiation. *eIF4E* expression might be a promising prognostic marker and a therapy target for BCC of the skin. However, considering limited samples scales and other unfavorable factors during our experiment, some further studies are still needed.

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