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ORIGINAL ARTICLE

Eukaryotic Elongation Factor 2 (eEF2) is a Potential Biomarker of Prostate Cancer

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Abstract Eukaryotic elongation factor 2 (eEF2), a key regulator of protein synthesis, is involved in the progression of several types of cancer. This first study was to investigate the relationships between eEF2 protein and prostate cancer (PCa). Immunohistochemical staining was used to verify eEF2 protein in a set of 97 formalin-fixed, paraffin-embedded primary PCa tissues. Expression of eEF2 protein in positive cells was characterized by cytoplasmic staining. Correlations with clinicopathological factors were evaluated by Chi-square or Fisher's exact probability tests. eEF2 protein was found in 74 out of 97 (76.29%) patients. eEF2-positive had higher PSA and Gleason score than negative in all patients. In addition, the positive expression of eEF2 protein was significantly associated with PSA and Gleason score (P = 0.007 and 0.002). However, no significant correlations occurred between expression of eEF2 protein and TNM stage (P = 0.292). In those eEF2 protein-positive patients, we have found staining intensity of eEF2 protein was not only associated with PSA and Gleason score, but also associated with TNM stage (P = 0, 0.014 and 0.001, respectively). To conclude, our study indicates that expression of eEF2 protein is a potential biomarker for evaluating PCa.

Keywords Prostate cancer · Eukaryotic elongation factor 2 · Immunohistochemistry

Introduction

Prostate cancer (PCa) is a significant burden in the health of older man. PCa is the most commonly diagnosed non-skin cancer in men, accounting for 21% of newly diagnosed non-skin cancers in 2016; this amounts to 180,890 new cases of PCa, including 26,120 deaths in 2016 [1]. Over the last decade, because of the changing in dietary pattern and westernized lifestyle, the morbidity of PCa was steadily increased in China [2]. A central aim in oncology research is to develop valid biomarkers that are associated with the natural history of the disease and with response to specific therapies. So that patients and physicians can make decisions on optimal frontline, adjuvant and salvage treatments based on unique biomarkers [3].

High level protein biosynthesis is one of characteristics of cancer cell metabolism [4]. Protein translation consists of three general steps, initiation, elongation and termination. Although the initiation step appears to be the most commonly targeted by signal transduction pathways deregulated in cancer, the deregulation of elongation step in translation is also involved in tumorigenesis. Overexpression of eukaryotic elongation factor 1A was reported in ovarian cancer [5] and breast cancer [6]. Recently, it was reported that eEF2 protein is not only highly expressed in human breast, lung, gastric and colorectal carcinoma tissues, but not in normal tissues, indicating eEF2 being an effective TAA target for immunotherapy [7, 8]; but also associated with higher incident of early lung adenocarcinoma recurrence [9] and expression of vascular endothelial growth factor (VEGF) in hepatocellular carcinoma [10]. However, there's no researcher claimed the clinical significance of eEF2 protein in PCa patients.

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The present study aims to investigate the expression of eEF2 protein in PCa tissue through immunohistochemistry, and explore the relationships between expression of eEF2 protein with clinicopathological parameters of PCa patients.

Materials and Methods

Patients

Paraffin embedded samples from 97 PCa patients were enrolled in this study. All the patients were admitted to the First Affiliated Hospital of Suzhou University from November 2013 to December 2015. Inclusion criteria: 1, not received surgical resection or radio−/chemo−/hormonal treatment before tissue collection; 2, with full information of TNM staging; 3, with confirmed diagnosis with PCa by postoperative pathological examination. All procedures performed in studies involving human participants were in accordance with the ethical standards of First Affiliated Hospital of Suzhou University's research committee. Informed consent was obtained from all individual participants included in the study.

Immunohistochemistry (IHC)

For eEF2 protein,4 μm sections of tissue were mounted on super-frost slides. Paraffin sections were deparaffinized. Microwaving antigen retrial was performed in citrate buffer (pH 6.0) for 15 min then returned to room temperature and washed in PBS. The sections were incubated with eEF2 antibody (San Cruz, California, USA) at 4 °C overnight diluted 1:100 in PBS. The sections were then stained with 3, 39-diaminobenzidine tetra-hydrochloride (DAB) for 10 min and counterstained with hematoxylin, dehydrated and mounted. Negative controls were treated with PBS without primary antibody under the same conditions. All immune-stained slides were evaluated independently by two pathologists who were blinded to the outcomes of patients. According to semi-quantitative counting method, the degree of antibody expression was evaluated by measuring both intensity and extent of staining: negative (−), 0–5%; weakly positive (+), 6–25%; moderately positive (++), 26–50%; and strongly positive (+++), >50%.

Statistical Analysis

SPSS 19.0 software (SPSS Inc., Chicago, IL, United Stated) was used for data analyzed. Chi-square and Fisher's exact tests were used to analyze associations between expression of eEF2 protein and clinicopathological features. For all the analyses, associations were considered to be significant if the *P* value was smaller than 0.05.

Results

Clinical and Pathological Features

The clinical and pathologic features for the 97 patients included in the analyses are detailed in Table 1. The ages rank from 52 to 83 years old (average age = 72 years), and PSA levels rank from 6 to 70 ng/ml (average PSA = 23.06 ng/ml). According to median age, all patients was divided into two groups. A further TNM staging following the American Joint Committee on Cancer (AJCC, 2002) standard identified 2 stage I patients, 86 stage II, 7 stage III and 2 stage IV. A further classification based on Gleason score showed 35 low (<7), 17 moderate (7) and 45 (>7) high grade tumors, and PSA levels showed 18 low (<10 ng/ml), 42 moderate (10–20 ng/ml), 37 (>20 ng/ml) high grade tumors.

Expression of eEF2 Protein and Clinicopathological Characteristics

Immunohistochemical reactivity of eEF2 protein was confined to cytoplasm of cells. Typical diffused cytoplasmic staining of eEF2 protein is shown in Fig. 1. Out of 97 PCa patients, 74 (76.29%) were positive in expression of eEF2 protein. Expression of eEF2 protein presented significant differences between age groups ($P = 0.039$), among PSA groups ($P = 0.007$) and Gleason score groups ($P = 0.002$). In addition, we found the positive expression of eEF2 protein was significantly associated with aforementioned parameters. However, no significant correlations occurred between eEF2 expression and TNM stage ($P = 0.292$) (Table 2).

Table 1 Clinical and pathologic characteristic for 97 patients with prostate cancer

Variable	n (%)
Age at surgery(y)	
≤ 72	41(42.27)
> 72	56(57.73)
PSA(ng/ml)	
< 10	18(18.57)
10–20	42(43.29)
> 20	37(38.14)
Gleason score	
< 7	35(36.08)
7	17(17.53)
> 7	45(46.39)
2002 TNM stage	
I	2(2.06)
II	86(88.66)
III	7(7.22)
IV	2(2.06)

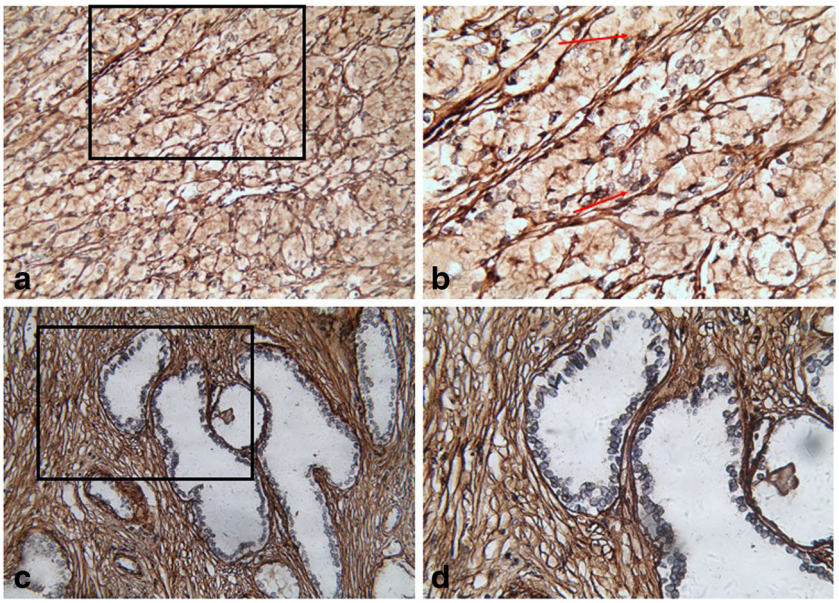

Fig. 1 Immunohistochemical staining of eEF2 protein in prostate cancer samples and adjacent normal samples. The typical diffuse cytoplasmic staining of the protein can be found in prostate cancer. **a, b:** eEF2 positivity was observed in the cytoplasm of prostate cancer cells; **c, d:** eEF2 negativity was observed in the cytoplasm of adjacent normal cells. The black frame shows where the images in the right panel come from. The red arrows points to the tumor cells with positive expression of eEF2 protein. Magnification in the left panel: 200×; Magnification in the right panel: 400 ×

The Staining Intensity of eEF2 Protein and Clinic Pathological Characteristics

Comparisons of clinical and pathologic features by the staining intensity of eEF2 protein were executed in this study. In eEF2 protein-positive PCa tissues, weak staining was 38/74 (51.35%), and moderate to strong staining was 36/74 (48.65%) (Fig. 2). The staining intensity of eEF2 protein was not only significant differences between age groups (P = 0.046), among PSA groups (P = 0) and Gleason score groups (P = 0.014), but also associated with these parameters. In addition, it was also significantly associated with TNM stage (P = 0.001) (Table 3).

Table 2 Relationship between positive expression of eEF2 protein and clinicopathological features of prostate cancer

Variable	Negative n (%)	Positive n (%)	$\chi 2$	P value
Age at surgery(y)			4.275	0.039[b]
≤ 72	14(34.15%)	27(65.85%)		
> 72	9(16.07)	47(83.93%)		
PSA(ng/ml)			–	0.007[a]
< 10	8(44.44%)	10(55.56%)		
10–20	12(28.57%)	30(71.43%)		
> 20	3(8.11%)	34(91.89%)		
Gleason score			–	0.002[a]
< 7	15(42.86%)	20(57.14%)		
7	4(23.53%)	13(76.47%)		
> 7	4(8.89%)	41(91.11%)		
2002 TNM stage			–	0.292[a]
I	1(50%)	1(50%)		
II	22(25.58%)	64(74.42%)		
III	0	7(100%)		
IV	0	2(100%)		

[a] Fisher exact test

[b] Chi-square test

Discussion

The rapid growth of cancer cells is associated with an overall increase of protein synthesis. Translation is regulated at the initiation and elongation step and deregulated in cancer through a variety of mechanism [4]. Protein translation factors, such as eukaryotic initiation factor 4E (eIF4E) and eukaryotic elongation factor 1A2 (eEF1A2) have been shown to be associated with oncogenesis [11, 12]. Their expression correlates with tumor cell growth, invasion, metastasis and hence a poor prognosis in lung, breast, ovarian caner and acute myeloid leukemia [13–15].

eEF2 is a gene that plays an important role in the polypeptide chain elongation step. Overexpression and hyperactivity of eEF2 in cancer are associated with cancer cell progression and early tumor recurrence [7, 16], and high level of protein synthesis is vital to maintain cancer cell metabolism [17]. Treatment with huanglian-jiedu decoction has been reported to suppress human hepatocellular carcinoma through inhibition of eEF2 [18]. These results indicated that aberrant up-regulation of eEF2 played an important role in the tumorigenesis. To our knowledge, compared with Oji's study that over-expression of eEF2 protein was detected in 75% (3 of 4) of PCa [8], we present the first study with respect to the value of eEF2 protein in a larger sample of human PCa. A total of 97 PCa samples was applied to immunostain for eEF2 protein in the current study.

Accuracy staging is useful for surgical planning and for deciding whether to proceed with sparing the neurovascular bundle, which carries the intrinsic dangers of positive surgical

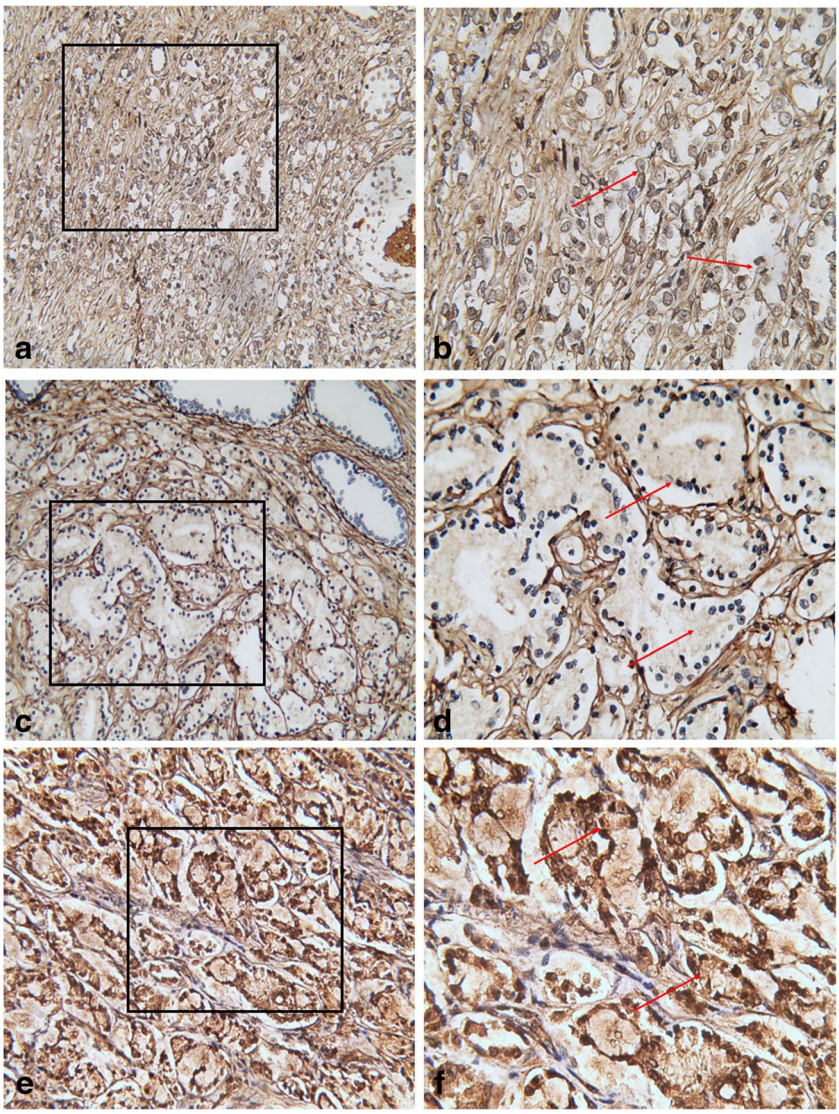

Fig. 2 Immunohistochemical staining of eEF2 in prostate cancer samples. **a, b:** eEF2 weak positivity was observed in the cytoplasm of prostate cancer cells; **c, d:** eEF2 moderate positivity was observed in the cytoplasm of prostate cancer cells; **e, f:** eEF2 strong positivity was observed in the cytoplasm of prostate cancer cells. The black frame shows where the images in the right panel come from. The red arrows points to the tumor cells with positive expression of eEF2 protein. Magnification in the left panel: 200×; Magnification in the right panel: 400 ×

Table 3 Relationship between expression level of eEF2 protein and clinicopathological features of prostate cancer

Variable	Weak (n = 38)	Moderate to strong(n = 36)	$\chi 2$	P value
Age at surgery(y)			3.991	0.046[b]
≤ 72	18	9		
> 72	20	27		
PSA(ng/ml)			–	0[a]
< 10	9	1		
10–20	22	8		
> 20	7	27		
Gleason score			8.596	0.014[b]
< 7	15	5		
7	8	5		
> 7	15	26		
2002 TNM stage			–	0.001[a]
I- II	38	27		
III- IV	0	9		

[a] Fisher exact test

[b] Chi-square test

margins or incomplete tumor removal. Therefore, different clinical and pathological preoperative factors, such as PSA, PSA related parameters and Gleason scores, are of utmost importance for predicting tumor stage and providing benefits for patients [19]. Except for association with age in this study, we found not only that positive expression of eEF2 protein was significantly associated with a higher PSA, but also increasing expression of eEF2 protein was significantly associated with higher PSA. However, positive expression of eEF2 protein wasn't associated with TNM stage.

The Gleason grading system developed by Dr. Donald F. Gleason between 1966 and 1974 and recently reviewed and improved by the 2014 International Society of Urological Pathology remains one of the most powerful predictors for the prognostic outcome of PCa [20]. In this study, Positive expression of eEF2 protein is markedly higher in higher Gleason score group. Furthermore, expression levels of eEF2 protein in PCa tissue were significantly associated with Gleason score and TNM stage by staining intensity of eEF2 protein. Our results showed that expression of eEF2 protein is strongly associated with PCa and a useful biomarker for PCa.

Despite the clinical implications, our study has several limitations that need to be considered for interpretation. First, this study was a retrospective study, conducted at a single hospital and included a relatively small patient population. However, this is the largest study to analyze the IHC-measured eEF2 protein in PCa tissues. Secondly, we only used intensity and did not apply other methods to interpret IHC results. Because there are no objective guidelines for interpretation, there may be a discrepancy in the results. Finally, PCa progression is quite tardy. Survival analysis were not estimated using the Kaplan-Meier method due to the shorter time of follow-up from the confirmed date of diagnosis.

In conclusion, our study first indicated that IHC-detected eEF2 expression is significantly associated with higher PSA, Gleason score and TNM stages. Therefore, it could be used as a potential biomarker for evaluating PCa. Meanwhile we are looking forward larger and prospective clinical studies to confirm our results, and well-designed trials to elucidate the role of eEF2 in the tumorigenesis of PCa.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of First Affiliated Hospital of Suzhou University's research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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