Expression of the Translation Initiation Factor eIF4E in the Polyp-Cancer Sequence in the Colon[1]

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Abstract

The eukaryotic translation initiation factor 4E (eIF4E) has been shown to play a key role in cell growth, and several studies have documented an increased expression of eIF4E in a number of solid tumors, including breast, bladder, cervical, and head and neck cancers. This study was done to evaluate the potential role of eIF4E in the polyp-cancer sequence in the colorectum. Eighty-seven cases with lesions in the colorectum with a variety of histopathological diagnoses were randomly selected from the archives of the Pathology Department at Louisiana State University Health Sciences Center-Shreveport. Appropriate sections were selected for immunostaining with eIF4E. The medical records of the patients were reviewed, and demographic information was collected. All statistical analyses were performed using SAS software. A statistically significant relationship was found between the level of eIF4E expression and histological type of lesion: the lowest level of eIF4E expression was found in normal colon tissue, whereas the highest level of eIF4E expression was found in colorectal adenocarcinomas. Carcinomatous lesions were found to have a 43 times higher chance of having a high level of eIF4E expression compared with normal tissue (95% confidence interval, 8.0–213.6, $P < 0.0001$). In a multivariate analysis, histological type was the only variable that showed a significant relationship with eIF4E expression; no effect was found due to age, gender, race, history of polyps, and family history. The results from this study are consistent with other data from the literature and support the suggestion that eIF4E is strongly involved in colon tumorigenesis. eIF4E might be a useful intermediate biomarker for use in chemoprevention intervention studies in patients with colorectal polyps.

Introduction

An increase in protein synthesis is necessary to facilitate cell proliferation. The up-regulation of protein synthesis is directly related to increased expression and function of translation factors. eIF4E[3] plays a key role in cell growth, and a number of observations suggest that increased expression and activity of eIF4E might be one of the key effects on oncogene expression, resulting in neoplastic transformation (1, 2).

eIF4E functions as the cap-binding subunit of the eIF4E complex, an ATP-dependent helicase that unwinds excess secondary structure in the 5′-UTR of mRNAs. A low level of eIF4E/F is limiting for the translation of some mRNAs, particularly those with long, G/C-rich 5′-UTRs (3). eIF4E overexpression results in a specific increase in the translation of these weakly competitive mRNAs, many of which encode products that stimulate cell growth and angiogenesis, *e.g.*, fibroblast growth factor 2 and vascular endothelial growth factor (4–6), cyclin D1 (7), and ribonucleotide reductase (8). Several lines of evidence support the key role that eIF4E plays in cancer development and/or progression. First, overexpression of eIF4E can cause neoplastic transformation of cells or accentuate neoplastic features (9, 10). Second, reducing eIF4E with antisense RNA or reducing its function by overexpression of the inhibitory 4E-BP proteins can suppress the oncogenic properties of many cell lines (11–13). Third, increased expression of eIF4E is ubiquitously found in the majority of solid tumors. These include bladder (14), breast (15, 16), cervical (17), head and neck (18), and prostate tumors (19). Finally, both the expression and activity of eIF4E are regulated at multiple levels by growth factors and oncogenes (20), suggesting a central role in converging signaling pathways leading to transformation. Establishing a greater protein synthesis output may be a necessary step for cancer cells to sustain their rapid proliferation in addition to the preferential increase in synthesis of growth-promoting proteins (21).

Recently, Rosenwald *et al.* (2) suggested that eIF4E was also strongly involved in colon tumorigenesis. In this study, we have further examined this question in a large sample of patients with hyperplastic polyps, adenomatous polyps, and colon adenocarcinomas.

Materials and Methods

Cases were randomly selected from the records of the Department of Pathology at Louisiana State University Health Sciences Center-Shreveport. A total of 87 cases were included in the study [19 with no colon pathology (“normal”), 25 with hyperplastic polyps, 20 with tubular adenomas (*i.e.*, adenomatous polyps), and 23 cases with adenocarcinoma of the colon]. The material for some of the normal controls was taken from

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[3] The abbreviations used are: eIF4E, eukaryotic translation initiation factor 4E; UTR, untranslated region.

the resection margins of colon resection specimens, but the majority were histologically normal colon biopsies.

All pathological material was reviewed by one of the investigators (E. A. T-H.) for verification of the original diagnosis. Appropriate sections were selected for immunostaining with eIF4E. Paraffin sections (5 μm) were cut, floated in a bath of distilled water at 45°C, placed on positively charged slides (Fisher Scientific, Pittsburgh, PA), and dried at 60°C for 1 h before deparaffinizing. The staining procedure was performed as suggested in the manufacturer's protocol (BioGenex Laboratories, San Ramon, CA). Brief antigen retrieval was performed with citrate buffer. The slides were incubated for 30 min with 100 μl of a 1:200 dilution of an anti-eIF4E polyclonal antibody produced in the laboratory of one of us (A. d. B.). All sections were washed twice with PBS, and a biotin-labeled secondary antibody, *i.e.*, goat antirabbit (BioGenex Laboratories), was added for another 30-min incubation. The sections were rinsed, and enzyme-conjugated streptavidin was applied. Color development occurred over a 5-min period with the addition of 3-amino-9-ethylcarbazole substrate. Finally, slides were rinsed, counterstained with Richard Allen hematoxylin, and mounted with glycerol gelatin mounting medium. Control sections were incubated in PBS substituted for primary antibody. The protein of interest stains red.

Staining intensity of the epithelium for expression of eIF4E was graded manually by light microscopy in a blinded fashion on two separate occasions [the first time by the pathologist (E. A. T-H.) alone; the second time by two independent observers who were unaware of the clinical details working in tandem]. All samples were then scored. Positive staining was defined as granular cytoplasmic staining, which was graded as 0–3+ and assigned corresponding numerical counts, *e.g.*, 1 for 1+, and so forth. Gradation of staining depended on the intensity of staining and the percentage of cells stained. Any differences found in the first and second evaluations were resolved between the observers before a final score was assigned to each case. Additional details on the staining and scoring method used have been described previously (22, 23).

The medical records from all cases were reviewed to obtain basic demographic information (race, sex, and age at diagnosis). Pearson χ^2 or Mantel-Haenszel χ^2 tests were used to determine whether there was an association between the expression of eIF4E and sex, race, or histological group. The *t* test was used to compare mean age at diagnosis between males and females. Cochran-Armitage's test for trend was used to evaluate whether a trend existed in the level of expression of eIF4E with increasing degree of malignancy. For ordinal levels of eIF4E, we performed an ordered logistic regression using the proportional odds model (24) as a function of sex, race, histological group, and previous history of polyps or colorectal carcinoma. All statistical analyses were performed using the statistical software package SAS (25).

Results

The descriptive characteristics of the study population are provided in Table 1. The majority of the patients (67%) were males; approximately half of the patients were African American, and the median age of the study population was 59.9 years.

A characteristic photographic image of the various levels of expression of eIF4E in the different histological groups is shown in Fig. 1. Hyperplastic polyps showed a different pattern of staining, *i.e.*, a more apical staining pattern, which could represent tripping of the stain by the abundantly present mucin in the cytoplasm of the epithelial cells.

Table 1 Descriptive characteristics of patient population

	Male	Female	Total
Age			
<50 yrs	11	10	21
≥50 yrs	39	14	53
Mean age (yrs)	60	52	57.8
Race			
African American	39	6	45
Caucasian	19	23	42
Histology			
Normal	15	5	20
Hyperplastic polyp	19	6	25
Adenomatous polyp	11	8	19
Adenocarcinoma	13	10	23

No association existed between level of eIF4E expression and sex or race ($P > 0.2$; data not shown). The results of the immunostaining with eIF4E by histological group are provided in Table 2. The majority of the cases (71.4%) in which eIF4E could not be detected were normal mucosa, whereas 68.8% of the cases with the highest level of expression were adenocarcinoma cases. No patients with histologically normal mucosa showed the highest level of expression, whereas, on the other hand, 48% of the patients who were diagnosed with adenocarcinoma of the colon expressed the highest level of eIF4E (Table 2). When the level of expression in normal mucosa was compared to expression in all other histologies combined, a significant difference ($P < 0.0001$) was found, with the lowest levels of expression in normal mucosa. Similarly, when adenocarcinomas were compared to all other samples combined, a significant difference in level of expression also existed ($P = 0.0006$), with the highest levels of expression in patients with adenocarcinomas. Using the Mantel-Haenszel χ^2 test, a highly statistically significant relationship was found between degree of malignancy of the mucosa and level of eIF4E expression (Mantel-Haenszel $\chi^2 = 25.1$; $df = 1$; $P < 0.0001$).

No effect of a positive family history of colon cancer ($P = 0.85$) or of having had a colon cancer previously ($P = 0.57$) was found on level of eIF4E expression (data not shown). A marginally statistically significant relationship ($P = 0.05$) existed between having had an adenomatous polyp previously and level of eIF4E expression.

In an analysis of maximum likelihood estimates (with sex, race, family history, personal previous history of colon cancer and/or of polyps, and histological type included in the model), the only variable that showed a significant relationship with level of eIF4E expression was the histological type of the lesion. Carcinomatous lesions were found to have a 43 times higher chance of showing a high level of eIF4E expression, compared with the group with normal histology (95% confidence interval, 8.0–213.6; $P < 0.0001$). Similarly, the patients with adenomatous polyps had a 13 times greater chance of showing high levels of eIF4E expression compared with the normal group (95% confidence interval, 2.4–71.6; $P < 0.003$).

Discussion

Malignant transformation is a multistep process that involves the initiation of unregulated cell growth and division. The dysregulation of protein synthesis has profound consequences for normal cellular functions and may be a critical component of the process of malignant transformation.

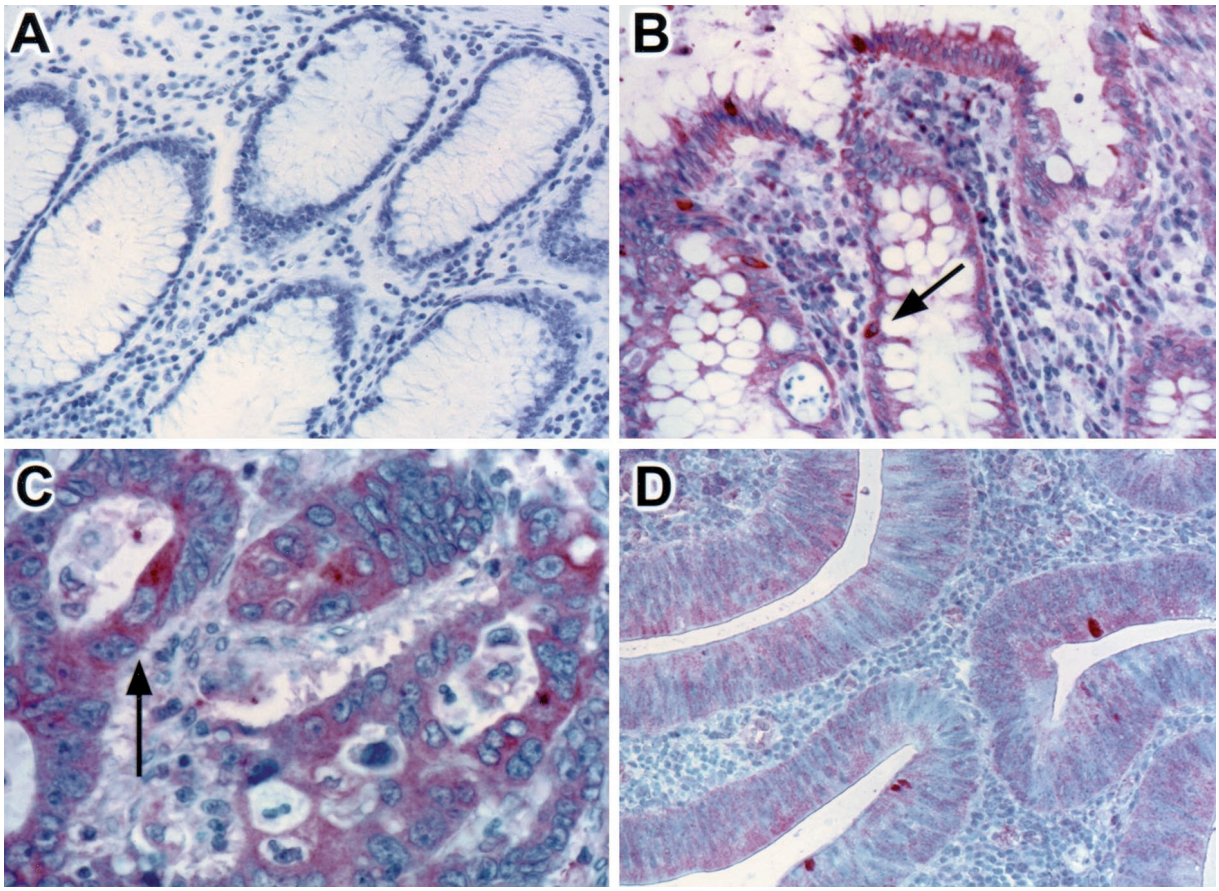

Fig. 1. Characteristic pattern of eIF4E expression. *A*, normal mucosa, with no positive cells. *B*, hyperplastic polyp, with a few cells stained red. *C*, adenocarcinoma, with many cells stained positively. *D*, adenomatous polyp with some positive cells and a diffuse reddish staining of the mucosa. *Arrows* indicate positively stained cells.

Table 2 Results of eIF4E immunostaining by histology

	eIF4E level				
	None	+	++	+++	Total
Histology					
Normal mucosa	10	7	2		19
Hyperplastic polyp	1	13	9	2	25
Adenomatous polyp	1	7	9	3	20
Adenocarcinoma	2	4	6	11	23
Total	14	31	26	16	87

Protein synthesis requires the unwinding of the 5′-UTRs of mRNAs by the RNA helicase complex. The binding of eIF4E to mRNAs with long 5′-UTRs increases the likelihood of binding by the RNA helicase and subsequent translation initiation; eIF4E is the rate-limiting step in this binding reaction. Thus, an abundance or overexpression of eIF4E results in an increased probability of translation and expression of the ordinarily "translationally repressed" mRNAs with long 5′-UTRs, many of which encode oncoproteins and growth factors. This can in turn lead to changes in the phenotype of the cell. It has been shown that overexpression of eIF4E can result in malignant transformation in cell lines, whereas several studies have found an overexpression of eIF4E in human tumors such as breast cancer, head and neck cancers, prostate cancer, and cervical cancer.

It is generally accepted that the majority of colorectal adenocarcinomas develop via the polyp-cancer-sequence. This means that most malignant tumors develop in/from a (benign) polyp. In this study, we have correlated the level of expression of eIF4E with various stages in the carcinogenic process in the colorectum, varying from normal mucosa to invasive carcinoma. A highly statistically significant relationship was found between malignant potential of the lesion and the level of eIF4E expression, with the highest levels found in adenocarcinomas, and the lowest levels found in histologically normal mucosa. These findings are consistent with the findings of Rosenwald *et al.* (2) and suggest that eIF4E is indeed involved in colon tumorigenesis. Rosenwald *et al.* (2) also suggest that eIF4E is a downstream target of the APC/β-catenin/Tcf-4 pathway. It has been suggested that eIF4E plays a role in carcinogenesis by increasing general protein synthesis and by preferentially upregulating a subset of putative growth-promoting proteins. When the expression of eIF4E was evaluated in a panel of normal and breast cancer cell lines, the elevation of eIF4E was found not to be a reflection of increased proliferation rates. In fact, the rate of cell division was independent of the level of eIF4E, *e.g.*, breast cancer cell line MCF7 grew more slowly than normal cell line MCF10A, yet the level of eIF4E was increased in the breast cancer cell line. This indicates that eIF4E is indeed in general a marker of malignancy and not of proliferation (26).

Several chemoprevention studies with a variety of agents

in patients with adenomatous polyps in the colorectum are currently underway to determine whether pharmacological intervention can prevent the recurrence of polyps and thereby (at least theoretically) prevent the development of a carcinoma. The ultimate goal of these chemoprevention studies is to reduce colon cancer incidence and mortality. However, this requires long term follow-up of a large number of patients. Therefore, many investigators have looked for intermediate markers (biomarkers) that could provide an indication of the ultimate effect on the incidence of cancer and are related to tumor development and change in value under the influence of intervention. The results of our study suggest that eIF4E might be a useful intermediate marker in chemoprevention studies of colon neoplasms: eIF4E definitely seems to play a role in the polyp-cancer sequence vital to colon carcinogenesis. However, to our knowledge, no data are available to show that levels of eIF4E expression change with the administration of an effective chemopreventive agent. We are currently evaluating this in our ongoing chemoprevention study of patients with adenomatous polyps.

In summary, in our study, we found a highly significant positive correlation between the level of expression of eIF4E and histological lesions in the colorectum, with normal mucosa showing the lowest level of expression, and adenocarcinomas showing the highest level of expression. This suggests that eIF4E might be a useful intermediate marker (biomarker). Additional experiments need to be carried out to evaluate the effect of pharmacological intervention on eIF4E expression.

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