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Expression Profile of Eukaryotic Translation Factors in Human Cancer Tissues and Cell Lines

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Several studies have demonstrated the overexpression of certain eukaryotic translation factors in human cancer cell lines and in malignant tissues. In this study, with human cancer cell lines derived from lungs, breast, prostate, and skin, we have examined the expression profile of 36 translation factors consisting of 27 initiation factors, 8 elongation factors, and 1 termination factor. Translation initiation factors 2C2 and 4E1 and translation elongation factors 1A2 and 1δ were found overexpressed (2- to 2000-fold) in many of the cancer cell lines compared to their corresponding normal cell lines. Among the translation factors analyzed, translation elongation factor 1A2 exhibited the most significant alteration in expression: 10- to 2000-fold overexpression was noticed in nine out of ten cancer cell lines analyzed. Whether the overexpression of translation elongation factor 1A2 can be used as a potential tumor marker was tested with the cancer profiling array (BD Biosciences, Palo Alto, CA) consisting of 241 paired cDNA samples generated from 13 different cancer/noncancer tissue types. Overexpression of translation elongation factor 1A2 was noticed in several tumor tissue samples, most notably in the human colon cancer samples which exhibited at least a twofold overexpression among 35% of the samples analyzed. Besides colon, tumor samples derived from lungs, kidney, rectum, and ovary also exhibited more than a twofold overexpression of translation elongation factor 1A2 in at least 20% of the samples analyzed. These results indicate that human carcinogenesis is often associated with alterations in the expression of various translation factors especially the overexpression of eukaryotic translation elongation factor 1A2.

Key words: translation factors; expression profile; molecular marker

INTRODUCTION

Alteration in the expression of genes, in particular those regulating the growth and division of cells, may result in pathological conditions, most notably in the development of malignancy. Expression of genes, including those involved in carcinogenesis, is regulated at many different levels: transcription, RNA polyadenylation and splicing, export of mRNA from the nucleus to the cytoplasm, mRNA degradation, and translation of mRNA to synthesize the protein it is encoded for. In the last several years, the regulation of gene expression at the level of translation has attracted much attention particularly in relationship to cancer.

The process of translation of mRNA to synthesize proteins is very complex. Several dozen translation factors (genes) controlling the three distinct steps of translation, viz: initiation, elongation, and termination, have been identified and characterized [1,2]. The involvement of some of the translation factors, particularly that of eukaryotic translation initiation factor 4E (eIF4E), in the process of carcinogenesis is implicated based on the observations that: (i) several different cancer cell lines and tumor samples exhibit elevated expression of these factors [3–5]; (ii) ectopic expression of certain translation factors resulted in cell transformation and tumorigenesis [6–8]; and (iii) antisense mRNA-mediated reduction in the cellular expression level of certain translation factors resulted in a reversal of the oncogenic properties of the cancer cells [9,10]. Recently, we have demonstrated, for the first time, the potential involvement of alterations in the expression of specific translation factors in carcinogenesis because of exposure to cadmium—an occupational carcinogen. Transformation of Balb/c-3T3 cells in vitro by exposure to cadmium was found to be associated with the overexpression of translation initiation factor-3 and translation elongation factor-1δ [11,12]. In addition, transfection-mediated overexpression of both

Abbreviations: eIF, eukaryotic translation initiation factor; eEF, eukaryotic translation elongation factor; eTF, eukaryotic translation termination factor.

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translation initiation factor 3 and translation elongation factor-1δ genes resulted in the transformation of mouse embryonic fibroblasts (NIH3T3 cells) and the tumorigenic potential of the cadmium transformed Balb/c-3T3 cells was significantly reversed by the antisense mRNA for these genes. In the present investigation, we have further extended our studies regarding the role of translation factors in human carcinogenesis. With RNA isolated from several different human cancer cell lines, we have studied the expression profile of 36 translation factors consisting of 27 initiation factors (eIFs), 8 elongation factors (eEFs), and 1 termination factor (eTF). Furthermore, we have investigated whether the overexpression of one of the translation factors, translation elongation factor 1A2 (eEF1A2), can potentially be used as a marker for malignancy with a Cancer Profiling Array (BD Biosciences) consisting of 241 paired cDNA samples generated from 13 different cancer/noncancer tissue types.

MATERIALS AND METHODS

Cell Culture and Isolation of RNA

Human cancer cell lines, viz: lung adenocarcinoma (catalogue number CRL-5868), small lung cancer (catalogue number CRL-5929), primary ductal carcinoma (catalogue number CRL-2324), and malignant melanoma (catalogue number CRL-1974) were purchased from American Type Cell Culture (ATCC; Manassas, VA). Epstein–Barr virus transformed lymphoblastoid cell lines developed from the respective patients were also purchased from ATCC and were used as the controls. Normal human prostate cell line—RWPE-1 (catalogue number CRL-11609), was also purchased from ATCC. Recipes for the cell culture medium and the culturing conditions are available with ATCC (http://www.atcc.org). Normal human primary breast cell lines used in this study were developed in the laboratory of Dr. Ainsley Weston (National Institute for Occupational Safety and Health, Morgantown, WV) and their culturing was performed as previously reported [13,14]. Total RNA, free of contaminating DNA, was isolated from the cells with the RNeasy Mini Kit (Qiagen, Inc., Valencia, CA).

Total RNA isolated from human prostate adenocarcinoma cells (PC-3) and breast adenocarcinoma cells (MCF-7, MDA-MB-361, T-47, MDA-MB-453, and DU 4475) were purchased from Ambion, Inc. (Austin, TX). The purity and integrity of all RNA samples were determined by UV-spectrophotometry and by agarose gel electrophoresis analysis, respectively.

Gene Expression Analysis by Quantitative Real-time PCR

Maloney murine leukemia virus (MMLV) reverse transcriptase-catalyzed synthesis of cDNA with total RNA isolated from the normal and cancer cells was performed with the Advantage RT-for-PCR kit (BD Biosciences) according to the procedure provided by the manufacturer. Nucleotide sequences for the primers used to amplify the various translation factors and the house-keeping genes (*β-actin* and *GAPDH*) are listed in Table 1. The PCR amplification, detection of the amplified gene product, and their quantitation were performed with the SYBR green PCR kit (PE Applied Biosystems, Foster City, CA) and the ABI-PRISM 5700 sequence detection system (PE Applied Biosystems) following the instructions provided by the manufacturer. The PCR amplified gene products were analyzed by agarose gel electrophoresis to ensure that only the intended product was amplified in each case.

The expression levels of all translation factors in each of the cell lines was normalized with the expression level of the housekeeping genes in the corresponding samples. The normalized value obtained for each cancer cell line was divided by that obtained for the corresponding control cell line and was represented as the fold overexpression. In the case of the normal breast epithelial cell lines, a control expression level was determined by taking mean of the expression values from six individual normal cell lines. This normalized mean expression level was used as the control value to calculate the fold overexpression in each of the cancer cell lines used in the study.

cDNA Hybridization to Confirm the Overexpression of Translation Elongation Factor 1A2 (eEF1A2) in Cancer Cells

The overexpression of eEF1A2 noticed in all but one of the cancer cell lines compared with the corresponding normal cells as evidenced from the results of real-time PCR analysis (see the ''Results'' for more details) was confirmed by cDNA hybridization. For this purpose, 1 μg total RNA from each of the cancer and normal cell lines was reverse transcribed to synthesize cDNA with the Advantage RT-for-PCR kit (BD Biosciences). The total cDNA synthesized was applied on a positively charged nylon membrane (Amersham Biosciences, Piscataway, NJ) with a vacuum manifold (Schleicher and Schuell Biosciences, Keene, NH). A DNA fragment consisting of 307 nucleotides upstream of the poly A tail of the eEF1A2 cDNA was PCR amplified with the primers 5′-TGC CAC ACA GCC CAC ATC G-3′ (forward) and 5′-CTT GCC CGC CTT CTG CGC C-3′ (reverse). The PCR amplified DNA fragment was analyzed by agarose gel electrophoresis and purified with the microcon spin columns (Millipore Corporation, Bedford, MA) according to the procedure provided by the supplier. The purified DNA fragment was labeled with digoxigenin (Roche Molecular Biochemicals, Indianapolis, IN) by random prime labeling and was used as the probe for hybridization

Table 1. Oligonucleotide Primers Used for Real-Time PCR Amplification to Determine the Expression of Translation Factors

Translation factor	GenBank accession no.	Nucleotide sequence
1. eIF1A	XM_010716	F 5′-ACGTTGCCTGGCCTCCAGC-3′ R 5′-GACCTCGCGGCGTCTCTGAC-3′
2. eIF2α	XM_031051	F 5′-ACTGAACTTGCGAGGCAGATGG-3′ R 5′-CAGCTTTGGCTTCCATTTCTTCTG-3′
3. eIF2β	XM_047088	F 5′-TCCACAAGTCGTCCGAGTAGGAAC-3′ R 5′-GGAGATGTTTGGGCTGACGATG-3′
4. eIF2γ	XM_018474	F 5′-CCACAAGTCGTCCGAGTAGGAAC-3′ R 5′-GGAGATGTTTGGGCTGACGATG-3′
5. eIF2B(β)	XM_007427	F 5′-AGGTGATCATTGGCACGAAGACC-3′ R 5′-TGCCAGTGCCAGAGTGTGAGTTC-3′
6. eIF2B(γ)	XM_001905	F 5′-ACTGAACCTGGCTCCCTATGATGC-3′ R 5′-CGCACCTGTGATCTGGACAAGTC-3′
7. eIF2B(ε)	XM_029136	F 5′-CACTCATTCCCGGCACAACATC-3′ R 5′-GCTGCCAATGACAGTGCCAGAG-3′
8. eIF2C1	NM_012199	F 5′-GAAGACGCCGGTGTATGCTGAG-3′ R 5′-TTCTTCACCTGCACACACTGCG-3′
9. eIF2C2	XM_050335	F 5′-CAGCACCGGCAGGAGATCATAC-3′ R 5′-GCTTGAAGCGCGTGGACTTG-3′
10. eIF3α	XM_032385	F 5′-AAGCCACCATGAAAGATGATCTGG-3′ R 5′-CAAGGGATTGTGGGCAACATAAAG-3′
11. eIF3γ	XM_030370	F 5′-TGCCACCTCTTCCAGCTCCAC-3′ R 5′-GCACTTGCTTCACGGCTGAATC-3′
12. eIF3δ	XM_049497	F 5′-CAACCGCAGAGCCGACGAC-3′ R 5′-GAAAGGCCGGAAGAGCTCCTG-3′
13. eIF3ε	XM_010886	F 5′-CAGACCCTGCGGCAGCATC-3′ R 5′-TGGAAGAGCAGGACCAGGCAG-3′
14. eIF3(6)	XM_005235	F 5′-TTGGTGGCTTGTCTTGAGGATTTC-3′ R 5′-CTGATACACTGGTGGATGCGACAG-3′
15. eIF3(7)	XM_038646	F 5′-CCTCTGTTGCGTACCGTTACCG-3′ R 5′-ATGACGCCATCGTGCTCACAAC-3′
16. eIF3(8)	XM_051114	F 5′-AGTCGAGTGGCCGAGCCAAG-3′ R 5′-ACCTTCTCCTGCTCCTGGTTGC-3′
17. eIF3(9)	XM_042082	F 5′-TTCGGACTGCACGGTCATGAAC-3′ R 5′-CGACGTAGCGCCCAGTAGGATC-3′
18. eIF3(10)	XM_049795	F 5′-CAGAGGACCAAGACGTGGGATG-3′ R 5′-TACGCCAGGATGATCGCTCATC-3′
19. eIF4β	XM_006675	F 5′-GCATTTGGCAGTGGGTATCGC-3′ R 5′-CACGACCGATCATCCCGTCTG-3′
20. eIF4γ1	XM_029132	F 5′-TGCACCTGGAGGGCGACTG-3′ R 5′-CTGGGCGAGCAGCTTCTGATG-3′
21. eIF4γ2	XM_006326	F 5′-AGGAATTGCCAGCAAGGATTCG-3′ R 5′-AGCCTTGCGAGGAACCCAATG-3′
22. eIF4γ3	XM_001846	F 5′-CTGCAACAGCTCGGCCAAATAC-3′ R 5′-TCCTGTGAGCTGCTTCACGGTC-3′
23. eIF4E	XM_017925	F 5′-GCTACTAAGAGCGGCTCCACCAC-3′ R 5′-TCGATTGCTTGACGCAGTCTCC-3′
24. eIF4H(2)	XM_040853	F 5′-ACGACGATCAGGCCTACAGCAG-3′ R 5′-CCTTCTGGCTACGGGAACCATG-3′
25. eIF5	XM_040909	F 5′-CAATGTCAACCGCAGCGTGTC-3′ R 5′-TTGCCTTTGCCCTCAACCTTG-3′
26. eIF5A	XM_036657	F 5′-CTTCCAGCTGATTGGCATCCAG-3′ R 5′-TCCTTGCCAAGGTCTCCCTCAG-3′
27. eIF5A2	XM_039788	F 5′-GGCTTCGTGGTGCTCAAAGGAC-3′ R 5′-TTGGCATGACCATGCTTTCCAG-3′
28. eEF-1α1	NM_001402	F 5′-ACAACATGCTGGAGCCAAGTGC-3′ R 5′-GGTTCCACTGGCATTGCCATC-3′
29. eEF-1A2	NM_001958	F 5′-TGCCACACAGCCCACATCG-3′ R 5′-TCCTCCAGCTTCTTGCCAGAGC-3′

(*Continued*)

Table 1. (*Continued*)

Translation factor	GenBank accession no.	Nucleotide sequence
30. eEF-1β1	XM_017660	F 5′-CCTGCCGACTTGTGTCATGCTC-3′ R 5′-TTTCTTCACTCCTGGCAGGCTG-3′
31. eEF-1β2	XM_027740	F 5′-CAACGATTACCTGGCGGACAAG-3′ R 5′-CGTAGGGCATGACACAAGTCGG-3′
32. eEF-1γ	XM_017896	F 5′-TGCCTTCGCCAGTGTCATCC-3′ R 5′-AAGCTCCTGGCCTCGGAAGAC-3′
33. eEF-1δ	BC012819	F 5′-CAAGCCTTGGGATGATGAGACG-3′ R 5′-CCCAGACCAGCCCGTCCAG-3′
34. eEF-1ε1	XM_015710	F 5′-CTCAGGGCGAGCGACAGATTC-3′ R 5′-GGCTTGCTTGACTAGATGAGCTGC-3′
35. eEF-2	XM_009189	F 5′-GCATTGGTGGTGGTGGACTGC-3′ R 5′-GATGCGCTCGGCAATGGC-3′
36. eTF1	XM_038642	F 5′-AAGGCTTTGGAAATGGGAGCTG-3′ R 5′-CCTCTTCTGTGCCTTGGCAATG-3′
37. β-Actin	M10278	F 5′-CTG GAA CGG TGA AGG TGA CA-3′ R 5′-AAG GGA CTT CCT GTA ACA ATG CA-3′
38. GAPDH	XM_006959	F 5′-GCC TTC TGC ACC ACC AAC TGC-3′ R 5′-GGC AGT GAT GGC GTG GAC TAT G-3′

eIF, eukaryotic initiation factor; eEF, eukaryotic elongation factor; eTF, eukaryotic termination factor; F, forward primer, and R, reverse primer.

with the reverse transcribed cDNA samples applied on the nylon membrane. The hybridized eEF1A2 cDNA was detected with the Dig-easy detection system (Roche Molecular Biochemicals) according to the procedure provided by the manufacturer. Subsequently, the blot was stripped of the hybridized eEF1A2 cDNA probe and rehybridized with the digoxigenin labeled cDNA for β-actin, the housekeeping gene used to normalize the results.

Analysis of eEF1A2 Expression With Cancer Profiling Array

The cancer profiling array (BD Biosciences) consists of 241 paired cDNA samples representing 13 different tissue types. Each pair consisted of a tumor sample and a corresponding normal tissue sample obtained from the same individual. Tissues represented on the array included breast (50 pairs), uterus (42 pairs), colon (34 pairs), stomach (28 pairs), ovary (14 pairs), lungs (21 pairs), kidney (20 pairs), rectum (18 pairs), thyroid gland (6 pairs), prostate (4 pairs), small intestine (2 pairs), pancreas (1 pair), and cervix (1 pair). In addition, the array also included positive (human ubiquitin cDNA, human cot-1 DNA and human genomic DNA) and negative (yeast total RNA, yeast tRNA and *E. coli* DNA) controls for hybridization.

The full-length cDNA for eEF1A2 was PCR amplified with the primers 5′-CAC CAT GGG CAA GGA GAA GAC C-3′ (forward) and 5′-CTT GCC CGC CTT CTG CGC C-3′ (reverse). The cDNA was then labeled with α-^{32}P-dCTP by random prime labeling with the Prime-it II kit (Stratagene, La Jolla, CA). Finally, the labeled cDNA was hybridized to the cancer profiling array membrane. Hybridization was performed at 68°C with QuikHyb solution (Stratagene) overnight. Membrane washing and stripping were performed according to conventional procedures. Ubiquitin cDNA was used as a house-keeping control gene for normalization of the array hybridization results. Quantitative data analysis was performed with the ImageQuant version 5.0 (Molecular Dynamics, Sunnyvale, CA).

RESULTS

Expression Profile of Translation Factors in Human Cancer Cell Lines

Of the 36 translation factors whose expression was analyzed in 10 different human cancer cell lines and the corresponding controls, 13 translation factors were found to exhibit at least a twofold overexpression in 9 of the cell lines (Table 2). Overexpression of both initiation and elongation factors was noticed in the different cell lines analyzed while the termination factor that was analyzed did not exhibit change in expression in any of the cell lines. Of the various translation factors that were analyzed, the expression of eEF1A2 was the most significantly different with respect to the number of cell lines that exhibited its overexpression as well as the fold change in expression—90% (nine out of ten) of the cell lines analyzed exhibited overexpression of eEF1A2; and the overexpression was as high as approximately 2000-fold as noticed in the lung adenocarcinoma cells (Figure 1 and Table 2). With the exception of the

Table 2. Expression Profile of Various Translation Factors in Human Cancer Cells

	Cell lines									
Gene	1	2	3	4	5	6	7	8	9	10
eIF2B(β)			+			+				
eIF2B(ε)			+			+				
eIF2C2			+		+	+	+	+		
eIF3γ			+			+	+			
eIF3(7)					+				+	
eIF3(8)							+	+		
eIF3(9)			+			+				
eIF4β						+	+			
eIF4γ2			+				+			
eIF4E	+		+				++	+	+	+
eEF1A2	++++	++	+++		++	++	+++	++	++	++
eEF1γ						+	+			
eEF1δ					+	+	+	+	+	

Expression of all the translation factors listed in Table 1 was determined by real-time PCR as described in the ''Materials and Methods.'' Only those genes that exhibited at least a twofold overexpression in two or more of the cell lines analyzed are listed in Table 2.
1, lung adenocarcinoma cells; 2, small lung cancer cells; 3, prostate adenocarcinoma cells (PC-3); 4, malignant melanoma cells; 5, primary ductal carcinoma cells; 6, breast carcinoma cells (T-47); 7, breast adenocarcinoma cells (MCF-7); 8, breast adenocarcinoma cells (MDA-MB-361); 9, breast carcinoma cells (MDA-MB-453); and 10, breast carcinoma cells (DU 4475).
+, 2- to 10-fold overexpression; ++, 10- to 50-fold overexpression; +++, 50- to 100-fold overexpression; ++++, >100-fold overexpression.

malignant melanoma cells, all other cell lines exhibited overexpression of at least one of the translation factors that was analyzed. Besides eEF1A2, significant overexpression was noticed in the case of eIF4E which exhibited at least a twofold overexpression in six out of ten cancer cell lines compared to the corresponding normal cell lines (Table 2).

The overexpression of eEF1A2 as evidenced from the results of real-time PCR analysis (Table 2) was further confirmed by the results obtained with the eEF1A2 cDNA hybridization experiment (Figure 2). With the exception of the malignant melanoma cells, all other cancer cell lines analyzed exhibited

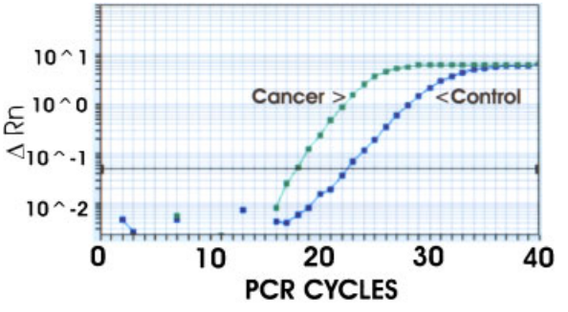

Figure 1. Real-time PCR analysis of translation elongation factor 1A2 (eEF1A2) in human cancer cell lines. Expression of eEF1A2 in the cancer cell lines and corresponding controls was determined by real-time PCR as described in the text. Expression of eEF1A2 in the human lung adenocarcinoma cells and the corresponding control cell line is presented as a representative one. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

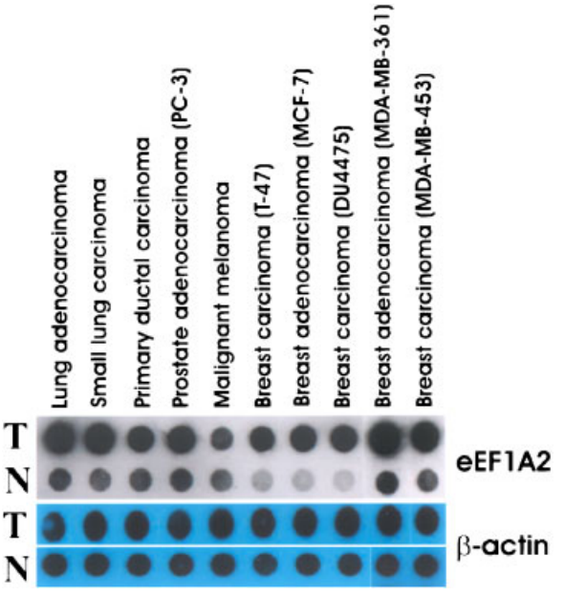

Figure 2. Expression profile of translation elongation factor 1A2 (*eEF1A2*) gene in normal and tumor cell lines. Total cDNA synthesized with RNA isolated from the control and the tumor cells were applied on nylon membranes and hybridized with eEF1A2 cDNA (probe) labeled with digoxigenin as described in the ''Materials and Methods.'' The hybridization signal was detected as described in the text. Subsequently, the blot was stripped to remove the hybridized eEF1A2 cDNA probe and rehybridized with labeled cDNA for *β-actin*—the house-keeping gene used. N, normal cells; T, tumor cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Table 3. Overexpression of Eukaryotic Translation Elongation Factor 1A2 (eEF1A2) in Cancer Tissue Samples as Evidenced From Results of the Cancer Profiling Array Analysis

Tissue	No. of samples	Overexpression (>twofold) (%)
Breast	50	0 (0)
Uterus	42	3 (7)
Colon	34	12 (35)
Stomach	28	2 (7)
Ovary	14	3 (21)
Cervix	1	0 (0)
Lungs	21	5 (24)
Kidney	20	4 (20)
Rectum	18	4 (22)
Intestine	2	0 (0)
Thyroid	6	0 (0)
Prostate	4	0 (0)
Pancreas	1	0 (0)

significant overexpression of eEF1A2 compared to the corresponding control cells.

Expression Profile of eEF1A2 in Human Cancer Samples

Results of the eEF1A2 cDNA hybridization to the cancer profiling array revealed significant overexpression of the transcript for eEF1A2 among several different cancer samples that were represented on the array (Table 3). Among the tissues represented on the array, colon exhibited eEF1A2 overexpression in 76% of the cancer samples, and the overexpression was at least twofold higher compared to the corresponding controls for 35% of the colon cancer samples (Table 3 and Figure 3). Even though the array consisted of few samples from the same patients at an early and late stage of tumorigenesis, there was no noticeable association between the expression of eEF1A2 and the cancer stage. Besides colon, significant (at least a twofold) overexpression of eEF1A2 was noticed among cancer samples derived from lungs (24%), rectum (22%), kidney (20%), and ovary (21%). It may be noteworthy that none of the 50 breast cancer samples represented on the array exhibited any significant overexpression of eEF1A2.

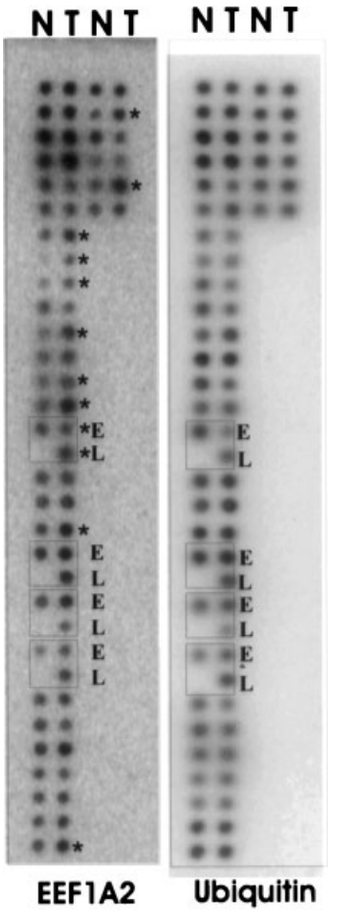

Figure 3. Expression profile of translation elongation factor 1A2 (*eEF1A2*) gene in normal and tumor tissue samples. The cancer profiling array (BD Biosciences) was hybridized with human eEF1A2 cDNA or ubiquitin cDNA (house-keeping gene) labeled with α^{32}P-dCTP. As described in the ''Materials and Methods,'' the array consisted of 241 paired cDNA samples representing 13 different tissue types as well as controls for hybridization experiment. Hybridization results obtained with the colon samples (34 pairs) alone are shown in the figure as a representative one and summary of the hybridization experiment is presented in Table 3. The array was washed and the hybridization signal was detected as described in the ''Materials and Methods.'' In certain cases, the tumor samples were obtained from the same patient at an early as well as at a later stage of tumor development and such samples along with the corresponding controls are represented within the square boxes (E, Early Stage; L, Late Stage). Further details such as age, race, and disease history, etc. about the cancer patients from whom the tumor samples were derived to isolate RNA that was subsequently reverse transcribed to synthesize cDNA and applied on the cancer profiling array can be found at www.clontech.com. N, Normal samples; T, Tumor samples; *, Two fold difference compared with the control.

DISCUSSION

It has been previously reported that certain translation factors are overexpressed in solid tumors as well as in cancer cell lines compared to their corresponding counterparts [4,5,8,15]. In the present, rather more comprehensive study, we have analyzed the expression profile of about three dozen translation factors with a variety of human cancer cell lines and their corresponding controls. Our results further support the hypothesis that an alteration in expression, notably overexpression of the translation factors, is often found associated with human carcinogenesis. The overexpression of eIF4E, eEF1A2 and eEF1δ, as noticed in the present study, has previously been reported in human cancer cell lines and/or in solid tumors [4,8,16] while the overexpression of eIF2C2 as noticed by us has not been reported in the literature. Among the translation factors analyzed, eEF1A2 exhibited the highest alteration in expression among the cancer cell lines compared to the controls. Nine out of ten (90%) of the cancer

cell lines analyzed exhibited at least a tenfold overexpression of eEF1A2 and the highest overexpression of approximately 2000-fold was noticed in the case of the lung adenocarcinoma cells compared to the corresponding control cells (Table 2). Taken together, our data seem to indicate that the overexpression of eEF1A2 may be associated with human carcinogenesis.

Whether or not the overexpression of eEF1A2 can potentially be used as a molecular marker for tumorigenesis was further investigated with a cancer profiling array consisting of 241 paired cDNA samples generated from 13 different cancer/noncancer tissue types. Results of the eEF1A2 cDNA hybridization to the array revealed that the transcript for eEF1A2 was overexpressed in several tumor samples compared to their corresponding controls. It is to be mentioned that for reasons that are not known at present, the fold overexpression of eEF1A2 among the tumor samples represented on the cancer profiling array was much lower compared to the cancer cell lines used in this study. Among the tumor samples represented on the array, those originating from colon exhibited at least a twofold overexpression of eEF1A2 transcript in 35% of the samples analyzed. Besides colon, tumors originating from lung, kidney, rectum, and ovary exhibited at least a twofold overexpression in 20% or more of the samples analyzed. These results further support the potential for using eEF1A2 overexpression as a molecular marker for human cancer. However, further validation of these findings with large number of human tumor samples is required prior to the use of eEF1A2 as a potential molecular marker for human cancer.

In humans, the *eEF1A2* gene is mapped to 20q13.3, and this locus has been found to play an important role in tumorigenesis. For example, amplification of the 20q13 locus is considered to be a marker for late stage ovarian [17–19] and breast cancer [20–22] and amplification of 20q13.3 is correlated with poor clinical prognosis and increased tumor aggressiveness [19,23,24]. Results of our cancer profiling array hybridization experiment, demonstrating the overexpression of *eEF1A2* gene in 20–35% of the cancer samples originating from colon, lung, kidney, ovary, and rectum, is in agreement with the overexpression of *eEF1A2* gene noticed in human ovarian cancer samples by Anand et al. [8]. However, the reason(s) for the lack of overexpression of eEF1A2 among the breast cancer samples represented on the array that was used in this study is not understood in spite of the previous publications that demonstrated the amplification of 20q13.3 [20–22].

The molecular mechanism(s) that is responsible for the overexpression of *eEF1A2* gene during carcinogenesis is not fully understood. The overexpression associated with the amplification of the *eEF1A2* gene has previously been reported in some of the ovarian cancer samples analyzed by Anand et al. [8]. However, one of the ovarian cancer samples analyzed by these investigators exhibited overexpression but not amplification of eEF1A2 gene suggesting that eEF1A2 overexpression may occur independent of amplification. The expression of certain translation factors, e.g., the initiation factor 4E (eIF4E), has been found to be influenced by overexpression of the cellular protooncogene, *ras* [25,26]. Whether eEF1A2 overexpression is the downstream effect of cellular protooncogenes or cell growth regulating genes whose expression might have been deregulated during carcinogenesis is yet to be investigated. Furthermore, analysis of the *eEF1A2* gene promoter may provide additional information regarding the mechanisms that regulate the overexpression of *eEF1A2* gene during tumorigenesis.

Translation factors that are traditionally thought to have essential housekeeping roles for cell viability, based on their involvement in protein synthesis, have recently been implicated in playing a causal role in neoplastic development [8,27–32]. Previous studies carried out in our laboratory have identified and characterized translation initiation factor 3 (eIF3) and translation elongation factor-1δ (eEF1δ) as two novel cadmium-responsive proto-oncogenes [11,12]. Similar neoplastic potential has previously been reported for other eukaryotic translation factors such as eIF3E [33], eIF4E [34], eIF4F [35], eIF4G [6], and eEF1A2 [8].

In spite of the evidence implicating the involvement of certain translation factors in neoplastic development, the underlying mechanisms responsible for their oncogenic potential are less well understood. The cellular translational protooncogene whose mechanism of action has been elucidated to some extend is eIF4E. In addition to causing a global increase in protein synthesis, overexpression of eIF4E has been found to result in the translational activation of specific genes such as *ras* [36], *cyclin D1* [34,37], c-*myc* [38], and ornithine decarboxylase [39]—genes that are known to have a significant role in malignant cell transformation. It is not known whether the overexpression of eEF1A2 results in the upregulation of the expression of proteins that activate cell growth and division, resulting in malignant cell transformation; further investigation is warranted. With respect to the mechanisms of oncogenesis by eEF1A2, its presumed role as an antiapoptotic gene may assume major importance. The first line of evidence supporting a protective role of eEF1A2 against apoptosis is derived from Wasted mice (wst/wst) exhibiting a spontaneous recessive lethal mutation leading to loss of eEF1A2 expression [40]. The absence of eEF1A2 expression in Wasted mice resulted in increased apoptosis in their thymic and lymphoid organs [41]. Further supportive evidence for the antiapoptotic role of eEF1A2 is derived from the observation that this gene is capable of protect-

ing muscle cells from caspase-3-induced apoptosis [42]. While it is possible that the oncogenic potential of eEF1A2, at least in part, is because of its ability to inhibit apoptosis, further experimental evidence is required prior to making such a conclusion.

In summary, the data presented in this study demonstrated that alteration in the expression profile of translation factors represent a major molecular event associated with human carcinogenesis. However, further validation studies employing a large number of tumor samples are required to further understand the significance of overexpression of translation factors, especially that of eEF1A2, in human carcinogenesis.

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