Expression of the Eukaryotic Translation Initiation Factors 4E and 2 α in Non-Hodgkin's Lymphomas

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Transition of cells from quiescence to proliferation requires an increase in the rate of protein synthesis, which is regulated in part by two key translation initiation factors, 4E and 2 α . The expression and activity of both factors are increased transiently when normal resting cells are stimulated to proliferate. They are constitutively elevated in oncogene transformed cultured cells, and overexpression of either initiation factor in rodent cells makes them tumorigenic. In this study we investigate an association between the expression of translation initiation factors and lymphomagenesis. We have analyzed the expression of the protein synthesis initiation factors 4E and 2 α by immunohistochemistry in reactive lymph nodes and several types of non-Hodgkin's lymphoma representing a wide range of clinical behaviors based on the Revised European-American Lymphoma behavioral classification. The study included 7 benign lymph nodes with follicular hyperplasia, 26 indolent lymphomas (6 marginal zone lymphomas, 7 small lymphocytic lymphomas, and 13 follicular lymphomas, grades 1 and 2), 16 moderately aggressive lymphomas (8 mantle cell lymphomas and 8 follicular lymphomas, grade 3), 24 aggressive lymphomas (14 large-B-cell lymphomas and 10 anaplastic large-cell lymphomas), and 15 highly aggressive lymphomas (7 lymphoblastic lymphomas and 8 Burkitt's lymphomas). Strong expression of initiation factors 4E and 2 α was demonstrated in the germinal centers of reactive follicles. Minimal or no expression was seen in the mantle zones and surrounding paracortices, indicating that high expression of initiation factors 4E and 2 α is associated with the active proliferation of lymphocytes. Most cases of aggressive and highly aggressive lymphomas showed strong expression of initiation factors 4E and 2 α , in contrast to the cases of indolent and moderately aggressive lymphoma, in which their expression was intermediate between the germinal centers and the mantles of reactive follicles.

A positive correlation was found between the expression of both initiation factors 4E and 2 α and the Revised European-American Lymphoma behavior classification ($P < 0.05$). Thus, constitutively increased expression of initiation factors 4E and 2 α may play an important role in the development of lymphomas and is correlated with their biological aggressiveness. (*Am J Pathol* 1999, 155:247-255)

Neoplastic transformation is a multistep process. A defining characteristic of the malignant phenotype is the rate of cell growth and proliferation. Normal cells proliferate transiently in response to appropriate extracellular growth factors or mitogens, and they readily withdraw into quiescence on cessation of growth stimuli. In contrast, transformed cells often become independent of extracellular growth factors and proliferate continuously. Cell growth and proliferation rates depend critically on the rate of protein synthesis. It has been shown that a 50% inhibition of protein synthesis is sufficient to completely arrest cell replication.¹⁻³ The up-regulation of net protein synthesis after mitogenic stimulation is due to increased expression and function of translation initiation factors.^{4,5} Experiments with cultured cells have demonstrated that expression of the eukaryotic initiation factors 4E and 2 α (eIF-4E and eIF-2 α) increases after resting lymphocytes or fibroblasts are treated with mitogens or growth factors, respectively.⁶⁻¹⁰ The least abundant component of the eIF-4F complex is eIF-4E, which is responsible for binding the 5' cap structure present on virtually all eukaryotic mRNAs and transferring mRNAs to the ribosomes. The two other subunits of the eIF-4F complex are eIF-4A, a helicase that is responsible for unwinding mRNA secondary structures, and eIF-4G, which holds the complex together and is responsible for ribosome binding.⁴ The eIF-2 initiation factor complex, which transfers initiator methionine tRNA to the 40 S ribosomal subunit, is composed of eIF-2 α and two other proteins, eIF-2 β and eIF-2 γ . The eIF-2 α subunit is the rate-limiting component of the protein synthesis initiation factor 2 (eIF-2), and the phosphorylation of eIF-2 α by the interferon-inducible kinase (PKR) inactivates the whole eIF-2 complex.¹¹ The expression of both eIF-4E and eIF-2 α is transiently increased in normal cells when they leave the

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resting G₀ period and proliferate in response to extracellular growth stimuli. The expression of these factors is constitutively high in oncogene-transformed and tumor cells.¹²⁻¹⁴ Importantly, overexpression of either eIF-4E or eIF-2 α is sufficient to transform cells to a malignant phenotype.^{4,14-17}

Non-Hodgkin's lymphomas (NHLs) are common neoplasms of the lymphoreticular system, accounting for 60% to 70% of all lymphomas.¹⁸ Previous findings demonstrate that increased transcription of both eIF-4E and eIF-2 α is induced by *c-myc*^{8,19} and suggests that they may be important in the genesis of Burkitt's lymphoma and other lymphoid tumors with increased *c-myc* expression. The expression of these initiation factors in lymphomas, however, has not been previously investigated. NHLs may be categorized as indolent, moderately aggressive, aggressive, or highly aggressive lymphomas based on the Revised European-American Lymphoma (REAL) classification.²⁰ We hypothesized that the increased expression of eIF-4E and eIF-2 α might correlate with the biological activity of NHLs. In this study, we evaluated eIF-4E and eIF-2 α in reactive lymph nodes and several types of lymphoma to determine their level of expression and correlation with tumor grade.

Materials and Methods

Tissue Specimens Studied

We studied 7 cases of follicular hyperplasia; 26 cases of indolent lymphomas, including 6 marginal zone lymphomas, 7 small lymphocytic lymphomas, and 13 follicular lymphomas (grade 1 and 2); 16 cases of moderately aggressive lymphomas, including 8 mantle cell lymphomas and 8 follicular lymphomas (grade 3); 24 cases of aggressive lymphomas, including 14 large-B-cell lymphomas and 10 anaplastic large-cell lymphomas; and 15 cases of highly aggressive lymphomas, including 7 lymphoblastic lymphomas and 8 Burkitt's lymphomas accessioned between 1990 and 1997 at the University of Massachusetts Medical Center. All cases were reviewed by at least two pathologists (S. Wang and G.A. Pihan or S. Wang and B.A. Woda) to confirm the diagnoses. The lymphomas were grouped into indolent, moderately aggressive, aggressive, and highly aggressive lymphomas based on the REAL behavioral classification.²⁰

Western Blot Protein Analysis

Western blot analysis was performed as described previously,^{9,10,21} except that protein lysates were obtained from lymph nodes that had been stored at -70°C . Protein lysates were obtained by pulverizing portions of lymph node cooled in liquid nitrogen. The frozen tissue powder was extracted with lysis buffer (0.5% Nonidet P-40, 420 mmol/L NaCl, 20 mmol/L Tris, pH 7.5, 2 mmol/L phenylmethylsulfonyl fluoride, and 0.02 mmol/L leupeptin). The suspension was forcefully passed 10 times through an 18-gauge needle to disrupt cellular material, after which the lysates were kept on ice for 15 minutes, aliquoted,

and frozen at -20°C . Before gel electrophoresis, each lysate was centrifuged at 10×10^3 rpm at 4°C for 15 minutes. Supernatants were collected, and the protein content was analyzed by the Bradford assay (BioRad, Hercules, CA). Forty micrograms of total protein from each lysate was run on 8% SDS-polyacrylamide gel and blotted for 18 hours at 20 V at 40°C onto Immobilon polyvinylidene difluoride (PVDF) membrane (Amersham, Arlington Heights, IL) using a liquid transfer solution containing 25 mmol/L Tris, pH 7.5, 192 mmol/L glycine, 10% methanol, and 0.01% SDS. The monoclonal mouse anti-eIF-4E antibody (1:1000 dilution; Transduction Laboratories, Lexington KY), monoclonal mouse anti-eIF-2 α antibody (from E. Henshaw, Rochester University; 1:4000 dilution)²² and monoclonal mouse anti-actin antibody (Amersham, N-350; 1:5000 dilution) were used sequentially, followed by horseradish-peroxidase-conjugated anti-mouse IgG (1:3000 dilution; Promega, Madison, WI) to detect the corresponding proteins using the ECL developing system (Amersham).

Immunohistochemistry

All biopsies in this study were fixed in 10% buffered formalin and paraffin embedded by routinely processing with a VIP Tissue Tek processor (Miles Scientific, Naperville, IL). Sections were cut at a thickness of 4 μm , heated at 60°C for 30 minutes, and then deparaffinized and hydrated through a series of xylene and alcohol baths before staining. The slides were microwaved in a proprietary citrate-buffered antigen retrieval solution (BioTek Solutions, Santa Barbara, CA) for 5 minutes in an 800-W microwave oven. After replenishment of this solution, the slides were microwaved again for an additional 5 minutes and then allowed to cool for 20 minutes. Immunohistochemical staining was performed with a monoclonal mouse antibody to eIF-4E (1:100 dilution; Transduction Laboratories) and a monoclonal mouse antibody to eIF-2 α (1:2000; obtained from E. Henshaw, University of Rochester), using a standard avidin/biotin complex (ABC) method as implemented on a Techmate 1000 (BioTek) automated immunostainer. Antibody diluent buffer was used as a negative control. The staining procedure consisted of a 30-minute incubation in the primary antibody followed by brief buffer washes and then incubation in a cocktail of biotinylated anti-mouse IgG/IgM (BioTek) for 30 minutes. The slides were then washed, incubated in avidin/biotin complex (BioTek) for 30 minutes, rewashed, and then reacted with diaminobenzidine and hydrogen peroxide to visualize the end product. The sections were counterstained with hematoxylin.

Evaluation of Immunostaining

The immunostained sections were examined on an Olympus microscope (Tokyo, Japan) at $\times 400$. The mantle zone and scattered small lymphocytes, both representing the population of resting cells, displayed barely detectable staining or no staining at all with antibodies to either eIF-4E or eIF-2 α . Thus, these cells were used as an

Figure 1. Western blot analysis of eIF-4E and eIF-2 α in quiescent, reactive, and neoplastic lymph nodes. NL, quiescent lymph node; FH, follicular hyperplasia; FL, follicular lymphoma; SLL, small lymphocytic lymphoma; BL, Burkitt's lymphoma; LB, large-B-cell lymphoma.

internal negative control. The cytoplasmic staining was assessed as follows: 0, no or barely detectable staining in the mantle zone lymphocytes or in scattered small lymphocytes in the paracortex; 1+, cells stained more intensely than small lymphocytes in the mantle areas but weaker than in germinal centers of reactive follicles; 2+, cells stained as strong or stronger than centroblasts in the germinal centers. The blood vessels, which are strongly positive for eIF-2 α , served as internal positive control for eIF-2 α . Scattered plasma cells, demonstrating strong staining for eIF-4E, served as internal positive control for eIF-4E.

Data Analysis

The nonparametric Wilcoxon-Mann-Whitney rank sum test was used to assess the correspondences between eIF-2 α expression and biological behavior (indolent < moderately aggressive < aggressive < highly aggressive) based on the REAL classification. The Spearman Rank order correlation test was used to assess the relationship between eIF-4E expression and biological behavior.²³ Statistical significance was set at the level of $P < 0.05$ (two sided).

Results

Characterization of eIF-4E and eIF-2 α Expression in Normal Lymph Nodes and Lymphoma-Affected Nodes by Western Blotting

We obtained frozen tissue from one nonreactive lymph node, two lymph nodes with follicular hyperplasia, two cases of follicular lymphoma, one small lymphocytic lymphoma, one Burkitt's lymphoma, and one large-B-cell lymphoma. Equal amounts of total protein were separated by gel electrophoresis, transferred onto PVDF membrane, and analyzed with anti-eIF-4E and anti-eIF-2 α antibody. The anti-actin antibody was used to confirm even loading of total protein extracted from lymph nodes.

As is illustrated in Figure 1, the antibodies that we used are specific for their protein targets. The eIF-4E antibody recognizes a 25-kd protein,¹⁵ and the eIF-2 α antibody recognizes a 36-kd protein.²² It is of note that the levels of both eIF-4E and eIF-2 α is increased in reactive lymph

nodes and in the lymph nodes affected by lymphomas. These data are consistent with previous data illustrating the specific recognition of eIF-2 α in cultured lymphocytes^{6,7} and colonic tissue²¹ as assessed by Western blotting using these antibodies.

Expression of eIF-4E and eIF-2 α in Reactive Lymph Nodes and NHLs

We studied seven lymph nodes exhibiting follicular hyperplasia; each strongly expressed eIF-4E and eIF-2 α within the germinal centers (Figure 2, A and B). Scattered activated lymphocytes in the paracortical zones expressed high levels of eIF-4E and eIF-2 α as well.

All indolent follicular lymphomas (grades 1 and 2), both small-cell type (six cases) and mixed-cell type (seven cases) (Figure 2C) showed weaker eIF-4E expression (2+) than the follicles of a reactive lymph node. High level (2+) eIF-2 α expression was seen in three of six and two of seven cases (Figure 2D), respectively, with intermediate (1+) expression in the remainder of cases.

Marginal zone lymphomas (MZL) showed weak (1+) expression of eIF-4E in five of six cases and of eIF-2 α in four of six cases. The remaining cases of MZL showed strong (2+) expression of eIF-4E or eIF-2 α . We noted that the tumor cells with plasmacytoid differentiation demonstrated stronger expression of both eIF-4E and eIF-2 α than the monocytoid tumor cells (data not shown).

Small lymphocytic lymphomas (SLLs) demonstrated 2+ eIF-4E staining in a geographical pattern in three of seven cases. The scattered immunoblasts and paraimmunoblasts and the pseudofollicular proliferation centers of SLLs demonstrated stronger expression of eIF-4E than the small lymphocytic component (Figure 2E). Three of seven cases of SLL were diffusely but strongly (2+) eIF-2 α positive (Figure 2F), and the remainder were diffusely and weakly (1+) positive.

In mantle cell lymphomas, most of the cases displayed 1+ positivity for both eIF-4E and eIF-2 α . The tumor cells express less eIF-4E (Figure 2 G) and eIF-2 α (Figure 2H) than the residual germinal centers in most of these cases. However, they usually showed stronger staining than normal mantle zone lymphocytes of non-neoplastic lymph nodes (Figure 2A for eIF-4E and 2B for eIF-2 α ; Table 1). Similarly, in the moderately aggressive follicular lymphomas (grade 3, large-cell type), one of eight cases expressed both eIF-4E and eIF-2 α strongly (2+), two cases expressed either eIF-4E or eIF-2 α strongly (2+) with intermediate (1+) expression of the other, and the remaining five cases displayed intermediate expression of both.

The expression of eIF-4E and eIF-2 α in several classes of aggressive lymphomas was studied. eIF-2 α was highly expressed (2+) in all cases of large-B-cell lymphoma, whereas eIF-4E was strongly expressed in 10 of 14 cases (Figure 3, A and B). In our 10 cases of anaplastic large-cell lymphomas, 6 were strongly eIF-4E and 8 were strongly eIF-2 α positive (Figure 3, C and D). The majority of lymphoblastic lymphomas strongly expressed eIF-4E (six of seven) and eIF-2 α (five of seven) (Figure 3, E and F). All of the cases of Burkitt's lymphomas were strongly

positive for both eIF-4E and eIF-2 α (Figure 3, G and H). The residual non-neoplastic lymphocytes in all lymphoma cases studied demonstrated very weak or nondetectable expression of both eIF-4E and eIF-2 α , however, the plasma cells were strongly positive for eIF-4E, and blood vessels were strongly positive for eIF-2 α . Statistical analysis demonstrated a strong correlation between the expression of both eIF-4E ($P < 0.05$) and eIF-2 α ($P < 0.05$) and the REAL behavior classification, with weak expression of eIF-4E and eIF-2 α corresponding with less aggressive lymphomas and strong expression corresponding with more aggressive lymphomas. The correspondence between eIF-4E and eIF-2 α expression was strong ($P < 0.05$), which neither indicates nor precludes independence of expression (Table 1).

Discussion

Non-Hodgkin's lymphomas (NHLs) display a broad spectrum of clinical behavior, and it has been shown that the biological grade of NHLs may be correlated with their proliferation index.²⁴ In this study we examined the expression of the translation initiation factors eIF-4E and eIF-2 α in reactive lymph nodes and a spectrum of NHLs. In all cases, the expression of either eIF-4E or eIF-2 α is higher in lymphomas than in the mantle zones of reactive follicle centers, which are composed of small virgin B cells (Table 1). Our data demonstrate a strong correlation between the expression of both eIF-4E ($P < 0.05$) and eIF-2 α ($P < 0.05$) and the REAL behavior classification, with increased expression of these factors as lymphomas progress from indolent to aggressive clinical behavior.

Some lymphomas histologically and phenotypically resemble components of the normal lymph node. The marginal zone lymphomas are indolent but express higher levels of eIF-4E and eIF-2 α than their quiescent, non-neoplastic counterparts. Follicular lymphomas of all grades frequently express eIF-4E and eIF-2 α at a level intermediate to the germinal centers and the mantle zones of reactive lymph nodes. The constitutive expression of eIF-4E and eIF-2 α at a moderately increased level, as compared with resting lymphocytes in the mantles, may reflect the slow, but continuous proliferation of the indolent and moderately aggressive lymphomas.

A well known feature of small lymphocytic lymphoma (SLL) is that it can convert from its indolent state into an aggressive diffuse large-B-cell lymphoma (Richter's syndrome).²⁵ In this study, more cases of SLL demonstrated strong (2+) eIF-4E and eIF-2 α expression than other indolent lymphomas. Furthermore, some cases of SLL showed strong eIF-4E staining in a geographical pattern with a stronger expression in the pseudofollicle prolifer-

ation centers than in the small lymphocytic component. As we have only limited clinical follow-up on these patients, we have not determined whether the expression of these initiation factors is associated with the aggressive transformation of SLL.

Mantle cell lymphoma is a moderately aggressive lymphoma.²⁰ Our study demonstrates that the expression of eIF-4E and eIF-2 α in mantle cell lymphoma is similar to other indolent lymphomas (Figure 2 and Table 1), suggesting that additional mechanisms (eg, transcriptional activation of cyclin D1 gene resulting from t(11;14) chromosomal translocation)²⁶ contribute to the more aggressive course of mantle cell lymphomas as compared with indolent lymphomas.

The aggressive and highly aggressive lymphomas, characterized by high proliferation rates, including large-B-cell (Figure 3, A and B), anaplastic large-cell (Figure 3, C and D), lymphoblastic (Figure 3, E and F), and Burkitt's (Figure 3, G and H) lymphomas strongly express eIF-4E and eIF-2 α . The expression of eIF-4E and eIF-2 α in these lymphomas is comparable or stronger than that expressed by reactive follicle center lymphocytes (Figure 3, A-H, and Figure 2, A and B). Our data show that strong expression of eIF-4E and eIF-2 α correlates with higher tumor grades. These results indicate that constitutively increased expression of eIF-4E and eIF-2 α and consequentially increased protein synthesis, in part, account for high growth and division rates and aggressive biological behavior. The data also suggest that there might be cooperativity between eIF-4E and eIF-2 α in the process of neoplastic transformation; however, this mechanism as of yet has not been formally proven.

We have previously demonstrated that *c-myc* transcriptionally activates expression of eIF-4E and eIF-2 α in cultured fibroblasts.⁸ Although *c-myc* rearrangement is always present in Burkitt's lymphomas, it is found only in a subset of other lymphomas, including diffuse large-cell and anaplastic large-cell lymphomas arising in AIDS patients,²⁷ and gastric large-cell lymphomas²⁸ as well as in post-transplant lymphoproliferative disorders,²⁹ and it may accompany histological transformation of SLL to a clinically more aggressive large-cell lymphoma.³⁰ Our study shows that eIF-4E and eIF-2 α are almost always elevated in the non-Hodgkin's lymphomas. Consequently, *c-myc* overexpression in Burkitt's lymphomas and in some cases of other lymphomas may explain high levels of eIF-4E and eIF-2 α , although in other types of lymphomas lacking overexpression of *c-myc*, an alternate mechanism for up-regulation of eIF-4E and eIF-2 α should exist.

The role of eIF-4E and eIF-2 α in lymphomagenesis warrants further investigation. It has been demonstrated

Figure 2. eIF-4E (A) and eIF-2 α (B) immunostaining of a lymph node with follicular hyperplasia demonstrated a clear demarcation between the germinal center and its surrounding mantle with strong (2+) cytoplasmic eIF-4E and eIF-2 α staining in the germinal center and negative staining in its mantle. The plasma cells and blood vessels in the paracortex demonstrated 2+ cytoplasmic eIF-4E and eIF-2 α staining, respectively. eIF-4E (C) and eIF-2 α (D) immunostaining of follicular lymphomas demonstrated weak (2+) and strong (2+) cytoplasmic staining in the tumor follicles of follicular lymphoma, respectively, and negative staining in residual mantles. eIF-4E immunostaining of small lymphocytic lymphoma demonstrated the ill-defined pseudofollicles with a 2+ cytoplasmic staining of the paraimmunoblasts and a 1+ staining in the nonproliferating areas (E). eIF-2 α immunostaining of small lymphocytic lymphoma demonstrated diffuse 2+ cytoplasmic staining (F). eIF-4E immunostaining of mantle cell lymphoma demonstrated a 1+ cytoplasmic staining in the tumor cells and a 2+ cytoplasmic staining in the residual germinal center (G). eIF-2 α immunostaining of mantle cell lymphoma demonstrated a 1+ cytoplasmic staining in the tumor cells and a 2+ staining in the blood vessels (H). Magnification, $\times 100$.

Table 1. Expression of eIF-4E and eIF-2 α in Reactive Lymph Nodes and NHLs

Cases	eIF-4E	eIF-2 α
M/FH		
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
FL/SC		
1	1	1
2	1	1
3	1	2
4	1	1
5	1	2
6	1	2
FL/MC		
1	1	2
2	1	1
3	1	2
4	1	1
5	1	1
6	1	1
7	1	1
FL/LC		
1	1	1
2	1	1
3	1	1
4	2	2
5	1	1
6	1	2
7	1	1
8	2	1
SLL		
1	1	1
2	1	2
3	1	2
4	2	2
5	2	1
6	1	1
7	2	1
MCL		
1	0	1
2	1	2
3	2	1
4	1	1
5	1	2
6	1	1
7	1	1
8	1	1
MZL		
1	1	1
2	1	1
3	1	1
4	1	1
5	1	1
6	2	2
GC/FH		
1	2	2
2	2	2
3	2	2
4	2	2
5	2	2
6	2	2
7	2	2

in cell culture models that eIF-4E preferentially increases synthesis of specific growth-promoting proteins, including cyclin D1, *myc*, ornithine decarboxylase, and fibroblast growth factor (FGF) as well as proteins responsible for tumor angiogenesis (vascular permeability factor and FGF) and metastasis (V6 splice variant of CD44 surface glycoprotein and collagenase type IV).^{9,10,31-35} It remains to be established whether eIF-4E and eIF-2 α , in addition to their general role in mRNA translation, may preferentially increase synthesis of growth- and metastasis-facilitating proteins in lymphomas. It should be emphasized that the general increase in protein synthesis due to constitutively elevated activity of eIF-4E and eIF-2 α , by itself, would facilitate accumulation of cellular pro-

Table 1. Continued

Cases	eIF-4E	eIF-2 α
LBCL		
1	1	2
2	1	2
3	2	2
4	2	2
5	2	2
6	2	2
7	2	2
8	1	2
9	2	2
10	2	2
11	2	2
12	1	2
13	2	2
14	2	2
ALCL		
1	2	2
2	2	2
3	2	2
4	1	1
5	2	2
6	1	2
7	1	2
8	2	2
9	0	2
10	2	1
LBL		
1	1	1
2	2	2
3	2	1
4	2	2
5	2	2
6	2	2
7	2	2
BL		
1	2	2
2	2	2
3	2	2
4	2	2
5	2	2
6	2	2
7	2	2
8	2	2

M/FH, mantle of follicular hyperplasia; GC/FH, germinal center of follicular hyperplasia; FL/SC, MC, or LC, follicular lymphoma, small-cell type, mixed-cell type, or large-cell type; SLL, small lymphocytic lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; LBCL, large-B-cell lymphoma; ALCL, anaplastic large-cell lymphoma; LBL, lymphoblastic lymphoma; BL, Burkitt's lymphoma.

teins, providing for accelerated growth and division rates. Noteworthy, it has been shown that eIF-4E prevents apoptosis in response to both growth factor withdrawal and *c-myc* activation in cultured fibroblasts.³⁶ It remains to be determined whether the initiation factors eIF-4E and eIF-2 α act to prevent apoptosis in lymphomas, thus contributing to tumor growth.

The role of other proteins involved in the regulation of protein synthesis and their association with hematopoietic malignancies are being elucidated. An inactivator of eIF-2 α , eIF-2 α kinase acts by phosphorylation of eIF-2 α in growth-factor-deprived cells and has been mapped to the 2p21-22 locus, which is nonrandomly involved in chromosome rearrangements in myeloproliferative disorders.³⁷ Furthermore, the gene encoding interferon-regulatory factor-1 (IRF-1), known to induce expression of eIF-2 α kinase in response to interferon, has been mapped to the 5q31.1 region, which is deleted in 5q-associated leukemias.³⁸ Two genes encoding RNA helicases, which, like eIF-4A, unwind mRNA secondary structures, potentially facilitating translation initiation, are involved in chromosomal translocations, *inv(11)(p15q22)* and *11q23*, associated with lymphoid and myeloid malignancies.³⁹⁻⁴¹

Figure 3. eIF-4E and eIF-2 α immunostaining of large-B-cell lymphoma (A and B), anaplastic large-cell lymphoma (C and D), lymphoblastic lymphoma (E and F), and Burkitt's lymphoma (G and H) demonstrated strong (2+) eIF-4E and eIF-2 α cytoplasmic staining in the tumor cells. The residual normal lymphocytes in these lymphomas demonstrated negative eIF-4E and eIF-2 α staining. Magnification, $\times 100$.

It has been suggested that increased expression and function of initiation factors and, consequently, a constitutive increase in protein synthesis is a key tumorigenic event.¹⁴ Analysis of these factors in human neoplasms is still limited; however, it has been found that eIF-4E is elevated in breast and head and neck carcinomas,^{42,43} and both eIF-4E and eIF-2 α are increased in colonic neoplasms.²¹ Our present findings establish NHLs as one of the neoplasms associated with elevated expression of eIF-4E and eIF-2 α . It remains to be established whether these translation initiation factors could serve as markers for lymphoma grading and progression or as targets for therapy.

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