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Original Article

Constitutive Activity of Nuclear Transcription Factor κB is Observed in Follicular Lymphoma

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Constitutive activity of nuclear transcription factor κB (NF-κB) is observed in many pathological types of lymphoma that are associated with a poor clinical course. This suggests that NF-κB and pathways involving NF-κB are possible targets for successfully treating lymphoma. We examined 28 lymph nodes from 28 patients in whom follicular lymphoma was diagnosed from 1996 to 2006 at our institution, which were formalin-fixed and paraffin-embedded. The specimens were stained with an antibody that could recognize activated NF-κB and p65 to determine whether they were positive or negative for NF-κB activation. The clinical courses of the 28 patients were then correlated with the results of the NF-κB staining. The 10 men and 18 women had a mean age of 57.3 years (range, 25-87 years). By follicular lymphoma grade, 10 patients had grade 1, 16 had grade 2, and 2 had grade 3a. Ten patients died due to lymphoma. NF-κB was positive in 6 of the 28 cases. Analysis of the positive and negative staining groups while taking into account the clinical course, sex, age, grade of follicular lymphoma, prognostic index, CD10, CD23, Bcl-2, karyotype t(14;18), and survival showed that no significant differences. Six of the 28 lymph nodes (21.4%) exhibited consistent NF-κB activity. Three of the eleven cases that transformed to aggressive lymphoma were positive for activated NF-κB. Further research to clarify the significance of constitutive NF-κB activity in follicular lymphoma is therefore warranted. 〔*J Clin Exp Hematopathol 50(1) : 45-50, 2010*〕

Keywords: nuclear transcription factor-κB, follicular lymphoma, immunohistochemistry

INTRODUCTION

Follicular lymphoma (FL, previously called follicle center lymphoma) is the most common of the indolent non-Hodgkin lymphomas. It is defined as a lymphoma of follicle center B-cells (centrocytes and centroblasts) that has at least a partially follicular pattern. Follicular lymphoma comprises about 18% of adult non-Hodgkin lymphomas in Japan[1] and 22% worldwide.[2]

Clinically, most patients with FL have widespread disease at the time of diagnosis, including involvement of peripheral and central lymph nodes and spleen ; the bone marrow is involved in 40% of cases. Only 33% of patients are in stage I or II at the time of diagnosis.[2] FL is generally associated with reasonably long survival, measured in years, even if initially untreated.[3] It is usually not curable with conventional treatment, which does not generally prolong overall survival.[4] There is, however, increasing evidence that the availability of newer therapeutic options may be having a positive impact on improving failure-free survival, and perhaps even overall survival, in patients with advanced stage disease.[3,5,6]

The nuclear transcription factor κB (NF-κB)/Rel family of proteins forms homo- and heterodimetric complexes that play a major role in controlling the expression of genes involved in immune, inflammatory, and acute phase responses.[7] NF-κB is activated as part of the DNA damage response to ionizing radiation or some anti-cancer drugs and is thought to orchestrate a cell survival pathway that, together with the activation of cell cycle checkpoints and DNA repair, allows the cell in cases of limited damage to be restored.[8] In several types of lymphoma, constitutive NF-κB activation is observed ; it can promote continuous lymphocyte proliferation and survival, and it has recently been recognized as a critical path genetic

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factor in lymphoma.[9] However, constitutive activity of NF-κB and its roles in FL have not been clarified and a correlation between NF-κB in cases of FL and clinical and pathological implications has yet to be demonstrated.

PATIENTS, MATERIALS AND METHODS

Patients' characteristics

Twenty-eight patients with FL were enrolled in this study and their lymph nodes were biopsied at our hospital. Their characteristics are described in Table 1.

Table 1. Patients' characteristics

Clinical features	Patients' characteristics
Gender (M : F)	10 : 18
Age (Y)	25-87 (mean 57.3)
Grade (1 : 2 : 3a)	10 : 16 : 2
FLIPI (low : int : high)	5 : 7 : 16
CD10 (+ : - : NR)	19 : 8 : 1
CD23 (+ : - : NR)	11 : 7 : 10
Light chain (κ : λ : NR)	10 : 14 : 4
Karyotype	
t(14;18)	12
Normal	4
Others	5
N.R.	7

FLIPI, follicular lymphoma international prognostic index ; N.R., not reported

Patient samples

Twenty-eight formalin-fixed paraffin-embedded tissue sections from 28 patients with FL were selected from the surgical pathology files of Kitasato University Hospital, Sagamihara for the immunohistochemical determination of active p65 (RelA).

Immunohistochemical determination of activated p65 (Rel A)

Staining was performed using a monoclonal mouse anti-human p65 (MAB3026, Chemicon International, Inc., Billerica, MA) that recognizes only the unbound, active form of p65 (RelA) dissociated from IκBa. Tissues were stained using Histofine® Simple Stain MAX-PO (Multi) secondary antibody (Nichirei Biosciences, Inc., Tokyo, Japan) in a 0.01% 3, 3'-diaminobenzidine solution (brown) and counter-stained with methyl green (green).

Statistical analysis

Data from patients and pathological samples were analyzed by t-tests, Fisher's test, and Kaplan-Meier analysis, using JMP Discovery Statistical Software (SAS Institute, Inc., Cary, NC) and are presented as means and standard errors. $P < 0.05$ indicated a statistically significant difference.

RESULTS

Positive rate of NF-κB

As positive controls for activated p65, Hodgkin lymphoma and prostate cancer samples were used (Fig. 1a, 1b). Both Hodgkin lymphoma cells and prostate cancer cells showed strong immunopositivity. Six of the 28 pathological FL specimens stained positive for activated p65 (Fig. 1c, 1d). Positive cells were scattered throughout the follicles (Fig. 1e-1h). Compared with the Hodgkin cells, their immunopositivity was weaker.

Comparison between positive and negative cases

The pathological findings of positive and negative cases were compared. FL grading (1, 2, 3a, or 3b), CD10, CD23, Bcl-2, and karyotype t(14;18) were analyzed. Results presented in Table 2 indicate that no differences between the two groups could be identified.

The clinical courses of positive and negative cases were compared, analyzing sex, mean age, Follicular Lymphoma International Prognostic Index (FLIPI), relapse-free survival after treatment (Fig. 2a), and overall survival (Fig. 2b), but no significant differences were found.

Table 2. Difference between NF-κB positive and negative

Clinical features	+	-	Statistical significance
Patient number	6	22	
Gender (M : F)	1 : 5	9 : 13	N.S.
Age (mean)	48.5	57.3	N.S.
FLIPI (low : int : high)	2 : 0 : 4	4 : 7 : 11	N.S.
CD10 (+ : -)	5 : 1	14 : 7	N.S.
CD23 (+ : -)	2 : 1	9 : 6	N.S.
Bcl-2 (+ : -)	6 : 0	21 : 1	N.S.
Light chain (κ : λ)	2 : 2	8 : 12	N.S.
G-banding			
t(14;18)	3	9	
Normal	1	3	N.S.
Others	2	3	
Transform	3	8	N.S.

FLIPI, follicular lymphoma international prognostic index ; N.S., not significant ; Transform, transformed to aggressive lymphoma

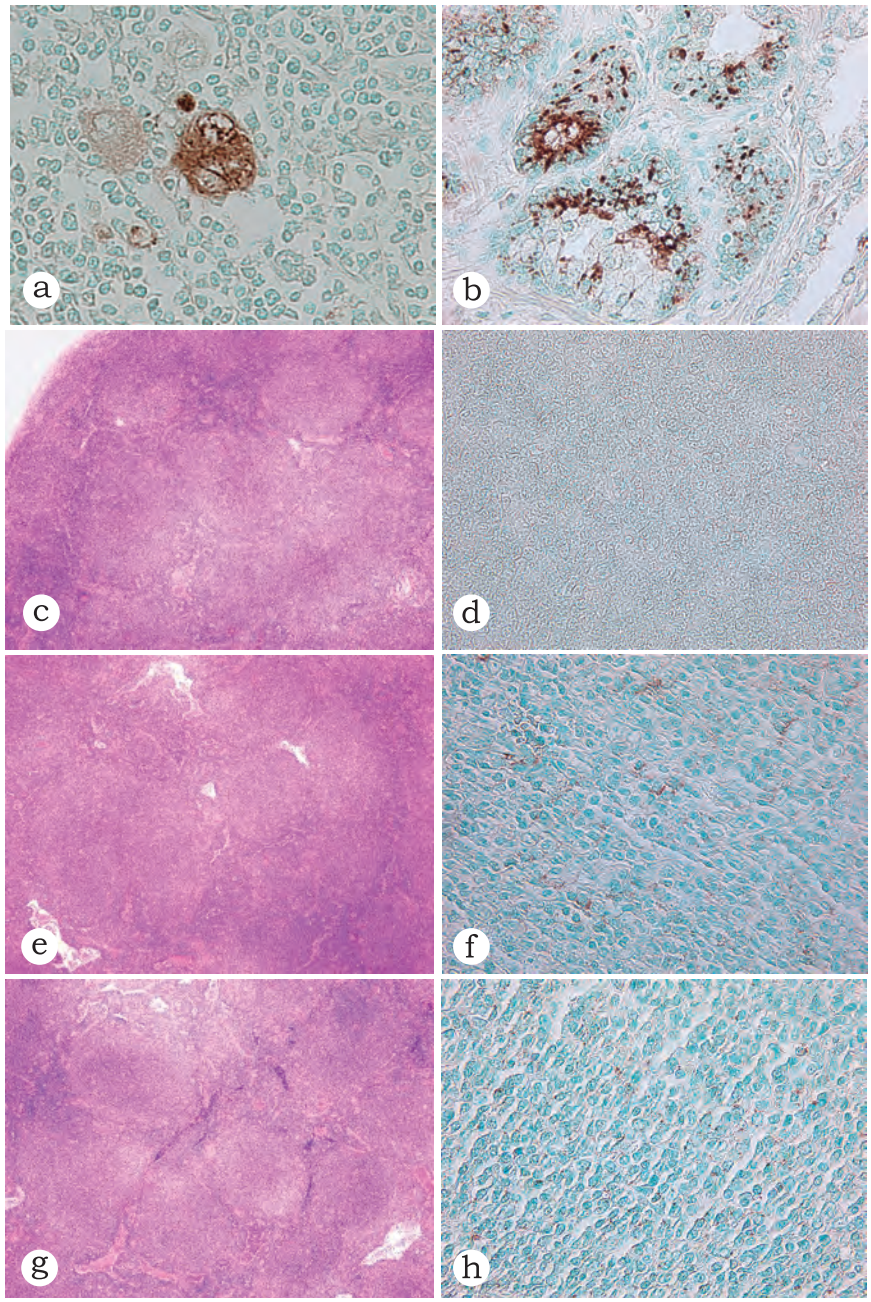

Fig. 1. Histological and immunohistochemical findings of resected lymph nodes. (***1a***) Hodgkin and Reed Sternberg cells showing strong immunopositivity for active p65 (relA). (***1b***) Prostate cancer cells showing strong immunopositivity for active p65 (relA). (***1c***) Follicular lymphoma (H&E stain). (***1d***) Follicular lymphoma cells showing negative for active p65 (relA). (***1e***) Follicular lymphoma (H&E stain). (***1f***) Follicular lymphoma cells showing weak immunopositivity for active p65 (relA). (***1g***) Follicular lymphoma (H&E stain). (***1h***) Follicular lymphoma cells showing weak immunopositivity for active p65 (relA). Fig. *1c* and *1d* are taken from a same patient. Fig. *1e* and *1f* are taken from the other same patients. The case of Fig. *1g* and *1h* are same as that of Fig. *1c*. (*1a*), (*1b*), (*1d*), (*1f*) & (*1h*), Counterstained with methyl green ; original magnification, (*1a*), (*1b*) & (*1d*)×200 ; (*1c*), (*1e*) & (*1g*)×40 ; (*1f*) & (*1h*)× 400.

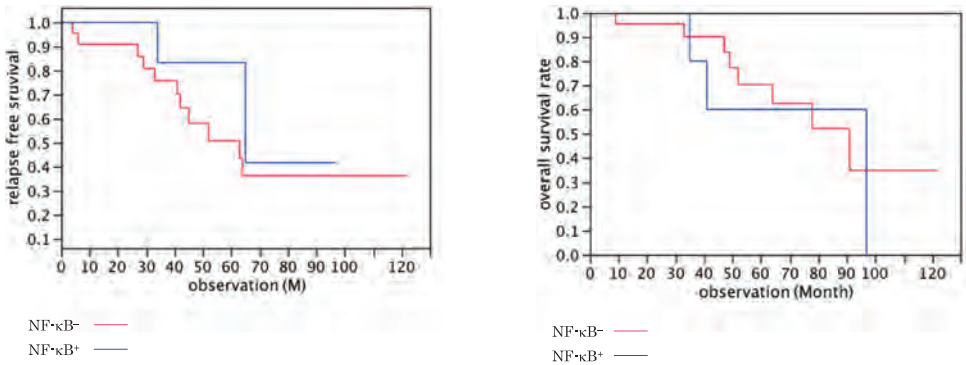

Fig. 2. Survival analysis. (***2a***) Relapse-free survival of positive and negative groups. There was no significant difference. (***2b***) Overall survival of positive and negative groups. There was no significant difference observed between the groups.

DISCUSSION

Because most studies have analyzed the activation of NF-κB in FL using molecular genetic methods and constitutive activation of NF-κB has been analyzed only infrequently, we assessed constitutive NF-κB activity using a histopathological approach. Six of the 28 FL cases (21.4%) in our study exhibited constitutive NF-κB activity. The antibody used recognizes an epitope overlapping the nuclear location signal of the p65 subunit of NF-κB and selectively binds to its activated form.[10] Therefore, the results confirm that constitutive NF-κB activity exists in FL.

To clarify the significance of constitutive NF-κB activity in FL, the association of NF-κB positivity with various pathological and clinical prognostic factors was examined. Bcl-2 expression in the germinal center was observed in 21 of 22 cases. However, there were no statistical differences between the two groups. FL grade,[11] FLIPI,[12] and CD10[13] are widely thought to be prognostic factors in FL. FL tumors are graded on a scale from I to III and this grade appears to have some prognostic value. The FLIPI is based on an international study of long-term survival in more than 4,000 patients with FL and it is useful in assessing its prognosis. CD10 is a marker for germinal center B cells and it is generally expressed in FL cells. Karube *et al*. reported that patients with CD10-MUM1$^+$ FL showed a relatively poor prognosis.[13] However, in this study, no pathological and clinical information was found to be associated with constitutive NF-κB activity in FL.

t(14;18)-negative FL showed an increased level of NF-κB activity within the subset identified by gene expression signature.[14] In the present study, three of six cases that showed constitutive NF-κB activity were t(14;18)-negative, and the other three were t(14;18)-positive ; thus, constitutive NF-κB activity was observed for both t(14;18)-positive and -negative groups. Why was constitutive NF-κB activity observed in t(14;18)-positive FL cases in our study? Constitutive NF-κB activity may be induced by several things in lymphoma.[8,15-20] First of all, constitutive NF-κB activity is induced by DNA damage, often from anti-cancer drugs or radiation therapy,[8,15] but none of our patients with FL had been treated with either anti-cancer drugs or radiation therapy. In addition, deleterious mutations in the IκBα gene have been reported in cases of Epstein-Barr virus-negative Hodgkin's lymphoma. These mutations may lead to nuclear translocation of NF-κB and transcriptional upregulation of its target genes.[20,21] Mutation of the IκBα gene may be a candidate for the activation of NF-κB in FL. Furthermore, microenvironments around the tumors have been a focus of some studies. In particular, macrophages that infiltrate tumors, called tumor-associated macrophages, secrete various cytokines[16] and may induce constitutive NF-κB activity. Necrosis and hypoxia of tumors have been shown to induce the production of hydrogen peroxide and subsequent activation of NF-κB in colon cancer.[17] Cells around the lymphoma and its microenvironment are also candidates for causing NF-κB activation in FL. As a cause of activation of NF-κB in B cell lymphoma, truncated p100 proteins with a partially deleted C-terminal ankyrin domain has been focused, but this is associated with the non-canonical pathyway, instead of the canonical pathway that we observed.[18]

Eleven cases transformed to aggressive lymphoma among 28, six of which were re-biopsied after the transformation for immunostaining purposes. Three of these 6 transformed FL cases were positive for activated NF-κB. Interestingly, one case that was negative at the onset of disease stained positive for NF-κB only after transformation, while another two stained positive both before and after transformation. The case that was positive only after diffuse large B-cell lymphoma transformation was biopsied just after chemotherapy because this was a multi-drug-resistant case. Constitutive NF-κB activity itself may have contributed to the resistance to

chemotherapy or chemotherapy may have damaged the DNA of the lymphoma and induced constitutive NF-κB activity. The patient in this case died 5 months after the second biopsy. Because constitutive NF-κB activity was observed in one case only after diffuse large B-cell lymphoma transformation, constitutive NF-κB activity may be associated with the transformation of FL into an aggressive lymphoma. Because only a few transformed cases were examined in this study, analysis of additional cases is necessary to confirm the significance of these findings.

Only nodal FL cases were analyzed in this study. There are definitive differences between nodal and extranodal follicular lymphomas. Patients with extranodal FL may enter complete remission and they show a more favorable long-term prognosis compared to those with nodal FL.[22] Therefore, analysis of NF-κB activation in extranodal FL may be another means to identify a significant relationship between NF-κB activation and clinicopathological features.

Signaling pathways leading to constitutive NF-κB activation in FL are currently unknown. The recently developed microarray technology allows large-scale parallel analyses of gene expression and permits simultaneous comparison of relative expression levels of thousands of genes in different cell types.[23] FL have also been analyzed recently by this method.[24,25] In FL, the activation of many molecules such as p38 MAPK, PLA2, platelet-derived growth factor receptor β (PDGFR3), and Rab6 has previously been reported.[26] Most of these molecules do not appear to be associated with NF-κB activation.

Constitutive NF-κB activity is observed in FL, but as of yet its significance is not fully understood. NF-κB and pathways involving NF-κB are possible targets for successfully treating lymphoma. If constitutive NF-κB activity can be stopped, then it is likely that lymphomas that depend on this activity can be successfully treated. Thus, the drug dehydroxymethylepoxyquinomicin, a novel NF-κB inhibitor, may be a successful treatment for lymphoma.[27] Further research on the importance of NF-κB in FL appears to be warranted.

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