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Activation-induced cytidine deaminase expression in follicular lymphoma : association between AID expression and ongoing mutation in FL

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【 77 】

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【 学位論文題目 】

Activation-Induced Cytidine Deaminase Expression in Follicular Lymphoma: Association between AID expression and ongoing mutation in FL
（ろ胞性リンパ腫におけるＡＩＤ発現と継続変異の相関）

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INTRODUCTION

Activation-induced cytidine deaminase (AID) is required for somatic hypermutation (SHM) and class switch recombination (CSR) of the immunoglobulin (Ig) gene. Follicular lymphomas (FLs) are thought to originate from GC B cells, based on the somatic hypermutation (SHM) in their immunoglobulin (Ig) genes. The tumor growth of FLs may be driven by antigen selection, which involves an active SHM process reflected by ongoing mutations that result in considerable intraclonal microheterogeneity. Ongoing mutation has also been reported to play a role in the resistance of some FLs to a certain therapy. The mutational analysis reports of the Ig gene in FLs have revealed some heterogeneity in SHM characteristic in terms of ongoing mutation and antigen selection. Based on the reported heterogeneity of the SHM pattern in FL, and on the possibly involved role of AID gene, AID expression was investigated here. AID mRNA was investigated in the fresh cells of 15 FL cases and 4 FL cell lines. Furthermore, SHM in immunoglobulin variable heavy chain (IgV_H) genes was analyzed in 12 FL cases and all cell lines.

MATERIALS AND METHODS

Reverse Transcription Polymerase Chain Reaction (RT-PCR) for AID

Nineteen FL materials comprising 15 fresh cells derived from lymph node tissues and 4 cell lines (FL18, 218, 318 and 518) were examined for AID mRNA expression by Reverse Transcription Polymerase Chain Reaction (RT-PCR). The primer used can also detect splice variants reported to perturb the functionality of wild-type AID. Densitometry of AID / β-actin mRNA levels used Scion Image-Release Beta 4.0.2. for Windows.

Somatic hypermutation and ongoing mutation study

A seminested PCR was performed with 500ng of genomic DNA extracted from each sample. One hundred microlitres of the PCR product was electrophoresed in a 3% low melting agarose gel in 1x TAE buffer and recovered from the gel, purified using a MinElute Gel Extraction Kit (QIAGEN, Maryland, USA) and ligated into the pCR 2.1 vector to transfect TOP10 competent cells (TA cloning kit; Invitrogen, Carlsbad, CA). Following an overnight culture on Luria Bertani (LB) agar, 15 colonies were picked based on X-Gal screening (Nacalai Tesque.Inc, Kyoto, Japan). After the insert check by PCR, 5 to 10 positive colonies were sub-cultured overnight in 5ml of LB medium. Minipreps of plasmid were extracted by alkaline lysis and purified on DNA affinity column by QIAprep Miniprep (QIAGENGmbH, Germany). Sequencing was done on an ABI Prism 310 sequencer (Applied Biosystems, Fostercity, CA) using the dye terminator cycle sequencing method (BigDye version 3, Applied Biosystems).

Final sequences were compared to the data in the V-Base (http://www.mrc-cpe.cam.ac.uk/). The consensus sequence was derived from the most dominant sequences among clones. The number of SHM within the complementarity determining region (CDR)2 and framework (FR)3 in the consensus sequence of each sample were determined. The R(replacement) to S(silent) mutation ratio in the FR3 less than 1.6 indicates an antigen selection. Sequences from the same DNA insert were compared within clones, and aligned with the consensus one.

Ongoing mutation was determined by dividing the cumulative number of partially shared mutations and unique mutations, with the expected number of mutations based on the PCR error rate ($4.5x10^{-4}$ change/base/PCR cycle, in our system). The difference of the ongoing mutation between AID-positive and AID-negative FL fresh

cell categories was analyzed by *unpaired Student-t test* (P <0.05). The correlation between AID expression and ongoing mutation was determined by the *two tailed Pearson correlation coefficient* (r >0.5). Both tests were performed using SPSS version 10 for Windows.

RESULTS

AID mRNA expression

The AID transcript by was detected in 13/19 FL materials, representing 10/15 fresh cells (case 1-3,7-10)(66.6%) and in 3/4 cell lines (FL 18, 218 and 318)(75%).

IgV$_H$ gene usage, mutational analysis and ongoing mutation analysis

The SHM analysis of IgV$_H$ gene was done for 16 materials, consisting of 12 out of 15 fresh cells and 4 cell lines. Among the 12 fresh cells, 7 were AID-positive and 5 were AID-negative.

The V$_H$ genes were derived from the V$_H$3 in 10 materials, V$_H$4 in 5 materials and V$_H$1 in 1 material. There is no apparent bias from that in normal peripheral blood lymphocytes (PBLs). All materials displayed SHM in their IgV$_H$ genes, with SHM rates from 4.16% to 15.6% (average of 10.07%). SHM rate was generally lower in AID-positive FL fresh cells than in AID-negative ones, except for 2 cases (case 7 and case 10). Antigen selection was detected in 8/16 materials (50%).

The ongoing mutation in 7 AID-positive FL fresh cells was generally high, except in case 7, with the average of 17.93-fold higher than the expected additional mutation by PCR error. Besides expressing the wild-type AID, all AID-positive fresh cells also expressed splice variants. The products of these splice variants in the FL cases did not seem to inhibit the function of the wild-type AID mRNA products, as seen from the presence of ongoing mutation. Among the 5 AID-negative fresh cells, ongoing mutation was generally low, with average of only 2.96-fold higher than the expected additional mutations by PCR error. Two AID-negative cases did not have any additional mutation (cases 6 and 11). The difference of ongoing mutation between the AID-positive and AID-negative FL fresh cells was significant (P=0.047). AID expression and ongoing mutation were also well correlated in the FL fresh cells (r=0.899, P=0.01). None of the 4 cell lines showed ongoing mutation.

DISCUSSION

Switch-off point of AID expression in B-lineage differentiation

Initial report showed that AID is specifically expressed in the germinal center (GC) of secondary lymphoid organ. However, the exact switch-off point of AID in B cell-lineage differentiation remains to be identified. In our previous study, the AID expression was found in 13/15 Burkitt lymphoma (BL) cell lines and 5/5 cases of fresh BL cells (Hardianti *et al.*, 2004). SHM was found in 4/4 analyzed AID-positive BL lines, whereas no SHM was found in the 2 rare AID-negative BL lines. Thus, the absence of AID in the 2 rare BL lines was of primary-negative type (negative before the primary expression). This primary-negative AID expression in the 2 rare BL cell lines is in contrast to the absence of AID expression in our FL materials, in which AID was switched off after the completion of SHM. In this situation, the absence of AID can be referred to as secondary-negative.

It appeared that the SHM rate was generally lower in AID-positive FL fresh cells than in AID-negative ones, except for 2 cases (case 7 and case 10). According to the plot of AID/β-actin of AID-positive FL fresh cells, there seemed to be a tendency for SHM rate to increase along with AID expression level. Considerable intraclonal microheterogeneity, indicating ongoing mutation, was a common feature in AID-

positive FL fresh cells, except in 1 case (case 7). In contrast, in the 5 AID-negative FL fresh cells, the SHM rate was generally high, accompanied by a low degree or lack of ongoing mutation. Case 7, which was AID-positive but had a high SHM rate and low intraclonal microheterogeneity, is of importance, as it possibly represents a borderline stage between common AID-positive and AID-negative FL cases, before AID expression is switched off. Some mechanisms to switch off the expression of AID in FL fresh cells might be affected by the level of SHM and ongoing mutation activity.

Association between AID expression and ongoing mutation in FL cells

Our primary finding was that a significant difference in intraclonal microheterogeneity indicating ongoing mutation, was found between AID-positive FL fresh cells and AID-negative ones (P=0.047). In the fresh cells of FL cases, the AID expression was associated with ongoing mutation (r=0.899, P=0.01). This could explain the reported heterogeneity in fresh FL cells. None of the FL cell lines (3 AID-positive and 1 AID-negative) showed ongoing mutation. Some additional factors may be required to maintain the ongoing process *in vitro*.

CONCLUSION

Since analyzing ongoing mutation clarifies the properties of B-cell lymphoma in disease progression and the resistance of FL to certain therapy, such analyses should be beneficial for predicting the disease prognosis. Our results suggest an association between AID and ongoing mutation in FL, and such results deserve further investigation.

論文審査の結果の要旨

受付番号	甲 第 1631 号	氏　名	Mardiah Suci Hardianti
論文題目	**Activation-Induced Cytidine Deaminase Expression in Follicular Lymphoma: Association between AID expression and ongoing mutation in FL** （ろ胞性リンパ腫におけるAID発現と継続変異の相関）		
審査委員	主　査　前田　盛 副　査　千原和夫 副　査　横野浩一		
審査終了日	平成 17 年 1 月 24 日		

（要旨は１，０００字～２，０００字程度）

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CONCLUSION

This study investigated on malignant lymphoma specifically in its Activation-induced cytidine deaminase and ongoing mutation in follicular lymphoma, and important knowledge about cell origin and prognosis in follicular lymphoma, whose investigations have rarely been conducted, has been obtained; thus recognized as important corpus. The researcher, therefore, is recognized to qualify to receive the degree of Ph.D.(Medicine).