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HISTOGENESIS OF CD5-POSITIVE AND CD5-NEGATIVE B-CELL NEOPLASMS ON THE ASPECT OF SOMATIC MUTATION OF IMMUNOGLOBULIN HEAVY CHAIN GENE VARIABLE REGION

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Abstract: The immunoglobulin heavy chain (IgH) gene of B-cells dramatically alters twice in their differentiation to memory or plasma cells; VDJ recombination at B-cell precursor and somatic hypermutation, class switch recombination and receptor revision at germinal center (GC) B-cells. Among them, somatic hypermutation of the IgH gene variable region (VH gene) is a powerful tool for detection of B-cell differentiation. B-cells and B-cell neoplasms have been divided into following; 1) pre-GC B-cells and neoplasms with a germline VH gene and 2) GC and post-GC B-cells and neoplasms with a somatically mutated VH gene. In this article, we review normal B-cell differentiation and histogenesis of various types of B-cell neoplasms on the aspect of somatic mutation of the rearranged VH gene. In particular, differences between $CD5^+$ and $CD5^-$ B-cell neoplasms, using our own data of over 100 cases with B-cell neoplasms, are discussed. Although $CD5^+$ B-cells are included in pre-GC B-cells for the reason of germline VH gene in most of $CD5^+$ B-cells, an about 5% of $CD5^+$ B-cells show somatically mutated VH gene. The rearranged VH gene of $CD5^+$ B-cell neoplasms shows heterogeneity, whereas $CD5^-$ B-cell neoplasms possess somatically mutated VH gene with a mean of 8～12%. Both $CD5^+$ B-cell chronic lymphocytic leukemia and $CD5^+$ diffuse large B-cell lymphoma display that about half of cases show a germline or low frequency of somatic mutation and the others possess somatically mutated VH gene. $CD5^+$ mantle cell lymphoma constitutes most cases with germline and a small number of cases with mutated VH gene. Therefore, $CD5^-$ B-cells & $CD5^-$ B-cell neoplasms are distinct from $CD5^+$ B-cells and $CD5^+$ B-cell neoplasms in somatic mutation of VH gene. It suggests that each of $CD5^-$ and $CD5^+$ B-cells independently has its own differentiation.

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INTRODUCTION

Recent advance in the molecular biologic technique has provided new insights into biological characterization of B-cells and B-cell neoplasms[1-8]. The availability of a complete map of immunoglobulin heavy chain (IgH) variable region gene (VH gene) repertoire and of the PCR technique for amplification of the VH gene allow us to analyze the degree of somatic mutation of the VH gene of B-cells. On this basis, the degree of somatic mutation of the VH gene depends on the level of B-cell differentiation during the immune response. It can be also possible to define the clonal origin of B-cell neoplasm by the analysis of rearranged VH gene.

Our purpose in this review is to clarify histogenesis of CD5-positive ($^+$) and CD5-negative ($^-$) B-cell neoplasms on the aspect of somatic mutation of the VH gene. We describe here 1) alteration of the IgH gene according to normal B-cell differentiation with peculiarity of somatic hypermutation of the VH gene, 2) the stereotype of normal B-cell differentiation and its problem, and finally 3) normal B-cell differentiation and histogenesis of $CD5^+$ and $CD5^-$ B-cell neoplasms.

OVERVIEW OF ALTERATION IN IMMUNOGLOBULIN HEAVY CHAIN GENE OF NORMAL B-CELLS

During normal B-cell differentiation, B-cells have two major phases with dramatic alteration for antigen molecule in the IgH gene[1-7,9-11]. First, VDJ recombination of the Ig gene occurs in B-cell precursor in the bone marrow. Germlines of the IgH gene are comprised of 50 functional segments of Variable region, 27 segments of Diversity region and six segments of Joint region[8]. The Ig light (IgL) gene lacks D segments. D and J segments are first joined together and a V segment is subsequently rearranged to a DJ joining for generating antibody variants as shown in Fig. 1. The IgL genes are assembled from V and J elements (VJ recombination). In-frame rearrangements of VDJ in IgH and VJ in IgL are necessary for expression of Ig (B-cell receptor, BCR) and B-cells with the expression of antibody (BCR) can survive and differentiate. When the trial of VDJ recombination failed to make in-frame sequence, the second trial of the VDJ recombination is given on the other IgH allele.

B-cells with the expression of surface IgM and IgD (naïve B-cells) transfer to peripheral (secondary) lymphoid tissue of lymph nodes and spleen. Naïve B-cells around sheathed capillary in the spleen or paracortex in the lymph node are stimulated by binding cognate antigen with their antigen receptors and by interaction of T helper cells (antigen stimulation), following that B-cells form or migrate into

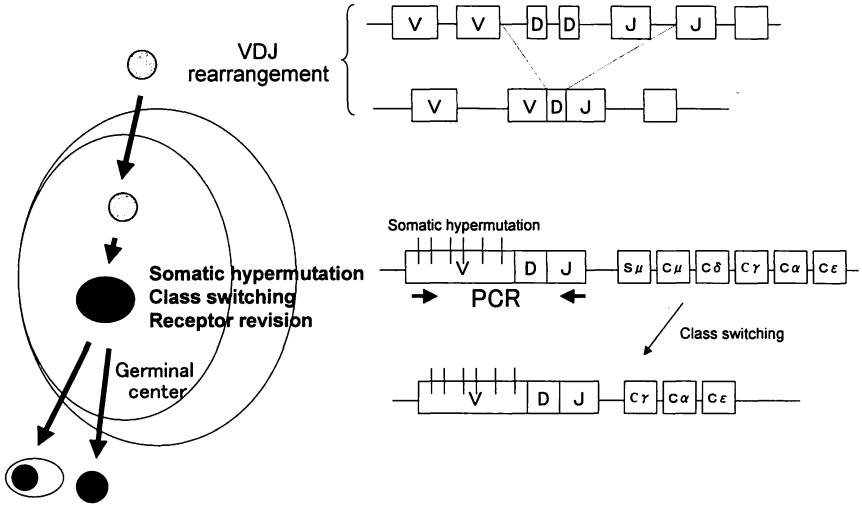

Fig. 1. Alteration of the immunoglobulin heavy chain gene during B-cell differentiation, VDJ recombination, somatic hypermutation, class switching and receptor revision.
During normal B-cell differentiation, B-cells have two major phases with dramatic alteration for antigen molecule in the IgH gene. First, VDJ recombination of the Ig gene occurs in B-cell precursor in the bone marrow. Antigen-stimulated B-cells form or migrate into germinal center (GC) of secondary follicle. The B-cells proliferate several rounds and maturate for increasing affinity of a BCR to the respective antigen by somatic hypermutation of the rearranged VH gene. Class switch recombination is undergone in the IgH gene of B-cells in the GC independent of somatic hypermutation. Changing the isotype of a BCR altered effecter functions of the antibody. A BCR is replaced by Cγ (IgG), Cα (IgA) or Cε (IgE) instead of Cμ (IgM) and Cδ (IgD). Receptor revision (editing) is also a process by which an originally expressed antibody polypeptide chain, usually the IgL is replaced by another one.

germinal center (GC) of secondary follicle. In GC, antigen-stimulated B-cells proliferate several rounds and maturate for increasing affinity of a BCR to the respective antigen (affinity maturation). Affinity maturation is achieved by nucleotide substitutions of the rearranged VH gene. They are randomly introduced into the VH gene at a very high rate (10^{-3} to 10^{-4}/bp/generation) and called as somatic hypermutation. B-cells with high affinity of a BCR for a distinct antigen can survive by contact to follicular dendritic cells (FDCs), escape outside GC and finally differentiate into memory B-cells or plasma cells. When B-cells fail to increase affinity or have a BCR with reactive for self-antigen, these cells proceed to apoptotic death. Macrophages immediately clean the dead cells off the GC. Class switch recombination is undergone in the IgH gene of B-cells in the GC independent of somatic hypermutation. Changing the isotype of a BCR altered effecter functions of the antibody. Before entering into GC, B-cells express Cμ (IgM) and Cδ (IgD). A BCR is replaced by Cγ (IgG), Cα (IgA) or Cε (IgE). Receptor revision (editing) is also a process by which an originally expressed antibody polypeptide

chain, usually the IgL is replaced by another one.

These mechanism, VDJ recombination, somatic hypermutation, class switch and receptor revision are closely related to oncogenesis of B-cell lymphoma. These remodelings of the Ig gene are explained as leading double strand breaks to the Ig gene, so that chromosomal translocation involving Ig gene may happen in both two stages of B-cell progenitors and GC B-cells. For examples, translocation of the *bcl*-2 gene and the IgH gene ; t (14 ; 18) (q32 ; q21) is speculated to occur during VDJ recombination in B-cells progenitors in the bone marrow, because a breakpoint of the IgH gene is located in VDJ segments[12]. Translocation of the *c-myc* gene and the IgH gene ; t (8 ; 14) (q24 ; q32) may generate during the class switch recombination in GC B-cells because a breakpoint of the IgH gene is located in C segments[12].

PECULIARITY AND ANALYSIS-METHODS OF SOMATIC MUTATION OF THE IGH GENE

Somatic hypermutation of the VH gene is found in a region about 1-2 kb downstream of the transcriptional promoter, so that somatic hypermutation depends on Ig enhancers and on transcription of the Ig gene. This unique mechanism is not a misreading in DNA synthesis, but associated with double-stranded break as similar as VDJ recombination and class switch. The *bcl*-6 gene subsequently reported to show somatic mutation on GC B-cells and some B-cell neoplasms[13]. Diffuse large B-cell lymphoma (DLBCL) possessed somatic mutation not only in VH and *bcl*-6 genes, but also the other several genes such as *c-myc* and *pim*-1[14]. Somatic mutation frequencies in these genes other than the VH gene are, however, very low in comparison to the VH gene.

The VH gene is comprised of Frame Work (FW) 1, Complementary Determinating Region (CDR) 1, FW2, CDR2, FW3 and a part of CDR3. CDR3 constitutes V-N-D-N-J. Random nucleotides are inserted to two sites between V and D and between D and J by Terminal deoxy-Transferase (TdT) activity, so B-cells have a variable length of 300～400 bps VH gene dependent on their VDJ recombination[15]. The VH gene can be amplified using DNA from cell suspension, frozen material, as well as paraffin-embedded material. The VH gene is normally amplified by two times PCR methods, such as semi-nested PCR using FR1C as sense primer and LJH and VLJH as anti-sense primers[16]. Because a length of PCR-amplified VH gene of each B-cell depends on its VDJ rearrangement, a size of PCR product of each B-cell is different. An amplified single and discrete band indicates a clonal proliferation of VDJ rearranged B-cells, whereas smear bands suggest non-neoplastic B-cells. If primers failed to anneal a template DNA because of mutations in the primers area of the template DNA, a VH gene may not be amplified. A polyacrylamide gel electrophoresis with 4-6% concentration instead of agarose gel electrophoresis is needed for measuring precise size of the PCR products or comparing two samples.

Nucleotide sequences of the amplified IgH gene are analyzed by a direct sequence method of the PCR products. Alternatively, the PCR products are cloned

to an appropriate vector devised for the cloning of the PCR products and sequenced. The nucleotide sequences analyzed are compared to registered IgH germlines in Gene banks such as the Genbank in the National Cancer Institute (http://www.ncbi.nlm.nih.gov/blast/ or http://www.ncbi.nlm.nih.gov/igblast/). The closest germline with highest homology to the nucleotide sequence of examined case is regarded as the germline usage for the case. Somatic mutations of two types exist. Replacement (R) mutation is single-base substitutions resulting in amino acid replacement and silent (S) mutation is single-base substitutions without amino acid replacement. Somatic mutation frequency represents as percentage of the number of R- and S- mutations divided the number of examined nucleotides. Insertions and deletions may be observed. The EB virus transformed peripheral blood lymphocytes cell lines show a VH gene utilization roughly proportional to the estimated family sized and functional VH genes dispersed into 7 families and germline complexity of these family is reported to be 25-30% of VH1 family, 5-10% of VH2 family, 50-55% of VH3 family, 5-18% of VH4 family, 1-2% of VH5 family and 1% of VH6 family[17].

B-CELL DIFFERENTIATION AND HISTOGENESIS OF B-CELL NEOPLASMS

1) The stereotype and its problem

Fig. 2 shows differentiation of normal B-cells with their immunophenotype and counterpart cells of various types of B-cell neoplasms. It is mainly explained by changing of immunophenotypic markers of CD5, CD10 and CD38. The aspect of somatic mutation of the VH gene has given the concept that normal B-cells are divided into antigen-stimulated B-cells and un-stimulated B-cells. Un-stimulated B-cells situate at pre GC. The rearranged VH gene of pre-GC B-cells, consisting of precursor B-cells and naïve B-cells, shows germline. Antigen-stimulated B-cells mean GC B-cells and post GC B-cells including memory B-cells and plasma cells, and both GC B-cells and post GC B-cells possess a somatically mutated VH gene. Somatic mutation analysis of the rearranged VH genes of neoplastic B-cells has been extensively published so far and that theory seems to be generally accepted for B-cell neoplasms. B-cell neoplasms derived from pre-GC B-cells have an unmutated VH gene, whereas those derived from GC B-cells and post GC B-cells have a hypermutated VH gene.

Several questions, however, remain. The most important question, which is our purpose of this article, is the existence of a unique system of CD5+ B-cells. Although CD5 is regarded as a differentiation marker in pre-GC B-cells and CD5+ B-cells were set in pre-GC naive B-cells (Fig. 2), it may be incorrect. We have investigated a large scale of somatic mutation analysis in the rearranged VH gene of B-cell neoplasms and literature review for normal B-cells with a special attention to CD5+ and CD5- B-cells and their neoplasms. Somatic mutation frequencies of various types of B-cell neoplasms are shown in Table 1 and the histogram of somatic

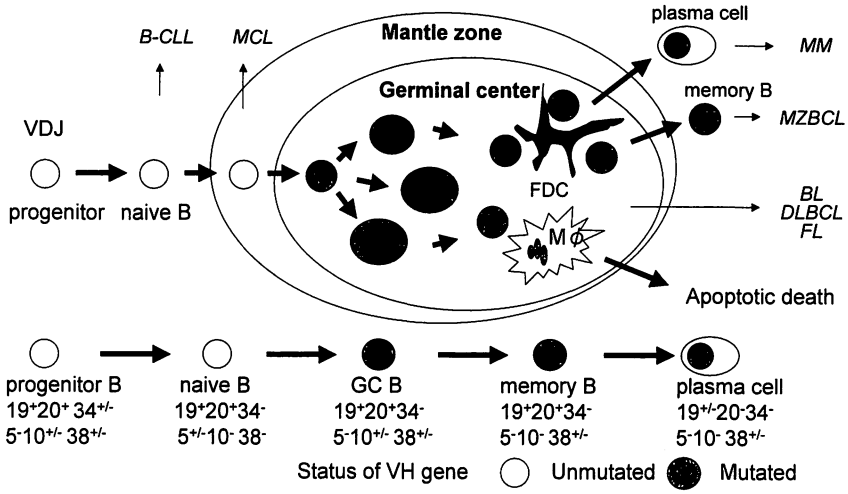

Fig. 2. Differentiation of B-cells and histogenesis of B-cell neoplasms. (sterotype)
During normal B-cell differentiation, antigen-stimulated naive B-cells form or migrate into germinal center (GC) of secondary follicle. In GC, antigen-stimulated B-cells proliferate and maturate for increasing affinity of a BCR to the respective antigen (affinity maturation) achieved by somatic hypermutation of the rearranged VH gene. B-cells with high affinity of a BCR for a distinct antigen can survive by contact to follicular dendritic cells (FDCs), escape outside GC and finally differentiate into memory B-cells or plasma cells. When B-cells fail to increase affinity or have a BCR with reactive for self-antigen, these cells proceed to apoptotic death.
B-CLL/SLL, B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma ; MCL, mantle cell lymphoma ; PCM, plasma cell myeloma ; MZBCL, marginal zone B-cell lymphoma ; BL, Burkitt lymphoma ; DLBCL, diffuse large B-cell lymphoma ; FL, follicular lymphoma.

Table 1. Somatic mutation frequencies of the VH gene of various types of B-cell neoplasms

	examined cases	distribution (%)	mean (%)
Lymphoblastic lymphoma (LBL)	6	0- 0.5	0.1
Chronic lymphocytic leukemia (B-CLL)	11	0-15.0	6.8
Mantle cell lymphoma (MCL)	11	0- 6.8	2.1
Follicular lymphoma (FL)	5	7.9-12.9	10.2
Diffuse large B-cell lymphoma (DLBCL)	39	0.9-25.9	10.6
Burkitt lymphoma (BL)	7	0.5- 9.8	4.5
MALT lymphoma	11	6.1-12.6	8.6
Plasma cell myeloma (PCM)	5	4.8-16.3	9.0

mutation frequencies of each B-cell neoplasm is shown in Fig. 3.

2) CD5$^+$ B-cells and CD5$^+$ B-cell neoplasms

CD5$^+$ B-cells, which are called B-1 cells, are known to constitute of the major subpopulation of B-cells by their distinct differences to CD5$^-$ B-cells in anatomic

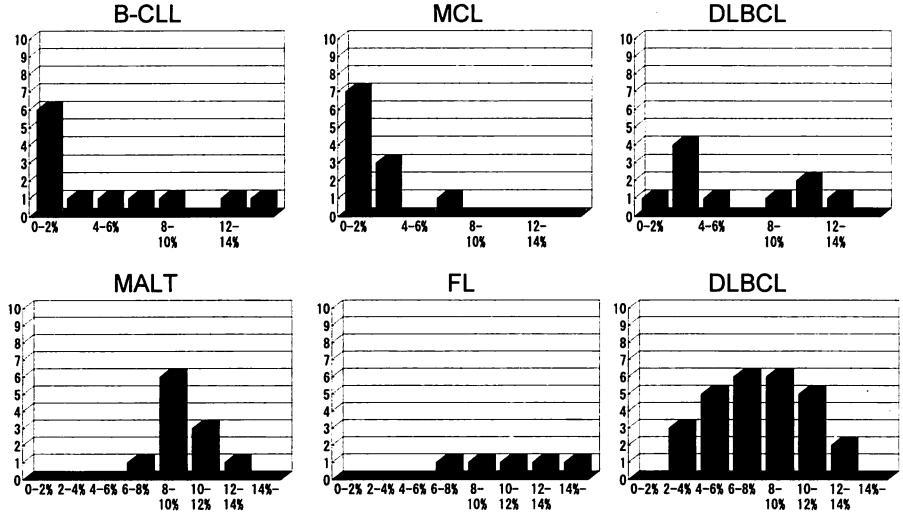

Fig. 3. Histogram of somatic mutation frequency of CD5+ and CD5- B-cell neoplasms.
CD5+ B-cell neoplasms (upper) versus CD5- B-cell neoplasms (lower).
Each of CD5+ B-cell neoplasms indicates heterogeneous pattern, whereas all of CD5- B-cell neoplasms are somatically hypermutated. The histogram of CD5- DLBCL excluded DLBCLs with more than 16%-mutation frequency.

localization, immunophenotype, gene usage and function[18-22]. The CD5 antigen is expressed on varying number of B-cells throughout life; 40-60% in fetus, 20% in adult peripheral blood and spleen, and 30% in adult lymph nodes and tonsils[19,20]. In secondary lymphoid tissue such as the lymph node, CD5+ B-cells locate in mantle zone of secondary follicle, but not in GC. CD5+ B-cells constitute a distinct developmental lineage and may produce a disproportionate level of low affinity and polyspecific autoantibodies[18,21]. Differences in selection and somatic mutations between CD5+ B-cells and CD5- B-cells have been also reported[23]. Although most CD5+ B-cells carry non-mutated VH and VL genes, indicating that CD5+ B-cells are naive B-cells, about 5% of PB CD5+ B-cells possess somatically mutated VH gene and co-express CD27, memory B-cell marker[22]. Moreover, somatic mutations in autoantibody-associated VH genes of circulating IgM+ IgD+ B cells have been described[24]. Thus, CD5+ B-cells are comprised of naïve B-cells and memory B-cells.

CD5+ B-cell neoplasms are heterogeneous in morphology, immunophenotype and genotype. Most cases of B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (B-CLL/SLL) and mantle cell lymphoma (MCL), some cases of hairy cell leukemia (HCL) and 5-10% of diffuse large B-cell lymphoma (DLBCL) are known to express the CD5 antigen[25-27].

The rearranged VH genes of B-CLL/SLL are not uniform. Although B-CLL was initially considered to lack a significant hypermutation of the rearranged VH gene, CD5+ B-CLL with a somatically mutated V gene has subsequently been

described[28]. The frequency of somatic mutation of CD5+ B-CLL/SLL in our series varied from 0% to 15.0%, and B-CLL is apparently divided into two groups; cases with germline VH gene showing less than 2%-mutation frequency and cases with somatically mutated VH gene that distribute from 2% to 15%[29]. The B-CLL/SLL cells may arise from CD5+ B-cells that are exposed to a self-antigen, and produce auto-reactive antibodies[30], and the proportion of B-CLL with a somatically mutated VH gene is estimated at 40% of B-CLL[22].

MCL has the morphologic and immunologic profiles of tumor cells derived from B-cells of the secondary follicle mantle zone[31], and it is described as a B-cell neoplasm with the germline of a rearranged VH gene. It is widely accepted that CD5+ B-cells (naive B-cells) situated in the mantle zone with a germline VH gene are the normal counterpart of MCL. However, more than two-thirds of cases with MCL have a germline sequence within 2% -mutation-frequency, whereas the others showed 2.0%～6.8%[32]. We recently found the latter cases were memory B-cell MCLs. The case, interestingly, shared antigens of CD20, CD5, cyclin D1, a definite marker of MCL, and CD27, memory B-cell marker[33]. Somatic mutation frequency of the VH gene of this case was 6.8%. The existence of such memory B-cell MCL supports that B-cell neoplasms from CD5+ B-cells are comprised of both B-cells with a germline VH gene and with a mutated VH gene.

Several reports on the analysis of somatic mutations in CD5+ DLBCLs have showed that most cases are somatically mutated[32,34-36]. CD5+ DLBCL as well as CD5+ B-CLL showed various degrees of somatic mutation with a range of 0.7 ～12.9%[31]. An average of frequency of somatic mutation of CD5+ DLBCL was also similar to that of CD5+ B-CLL[31]. A Richter transformation must be developed from the clone of a preexisting B-CLL, so it is likely that the tumor clone of a Richter transformation showed little mutation of the rearranged VH gene. De novo CD5+ DLBCLs with little mutated VH gene were demonstrated (Fig. 4). CD5+ DLBCLs with somatically mutated VH genes were also documented in Western countries[36]. CD5+ DLBCL, as well as CD5+ B-CLL, may be divided into two groups of germline or low frequency group (0-5%) and somatically hypermutated group (5-17%) see Fig. 4. Therefore, the common characteristic of the rearranged VH gene of each CD5+ B-cell neoplasm is its heterogeneity.

To compare with germline complexity of VH gene family[17], the VH gene family usage of both CD5+ B-CLL and CD5+ DLBCL was biased. More cases of CD5+ B-CLL in the Western have VH5 and VH6 families and it is suggested that this biased VH gene usage is related to antigen selection by autoantigens. Ikematsu has reported that CD5+ B-CLL in the Japanese represents high proportion of VH4 usage when compared with the respective gene complexity for each VH family[37]. CD5+ B-CLLs with VH4 family constituted 40% of our cases, so high proportion of VH4 usage of the Japanese cases is considered to be characteristics. A half of CD5+ DLBCLs had VH4 family. Thus, this could be one of common characteristics between CD5+ DLBCL and CD5+ B-CLL in the Japanese population.

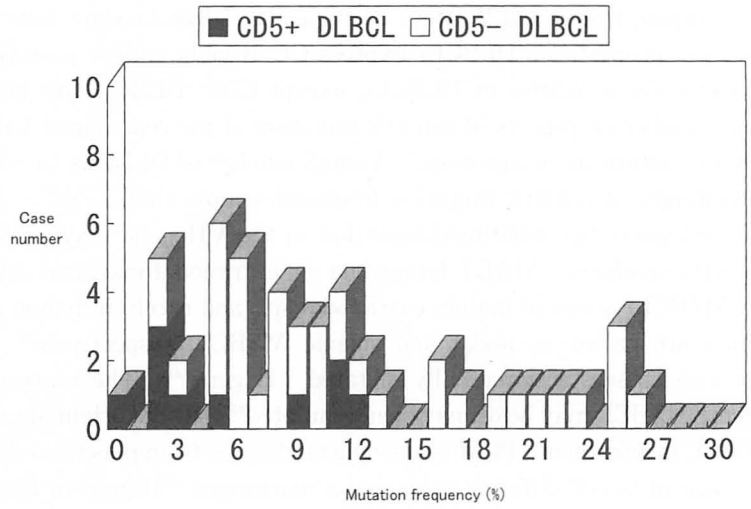

Fig. 4. Histogram of somatic mutation frequency of CD5+ and CD5- diffuse large B-cell lymphomas (DLBCL).
CD5+ DLBCL shows heterogeneity, whereas CD5- DLBCL has a peak of somatically mutated VH gene.

3) CD5- B-cells and CD5- B-cell neoplasms

Eighty-five percents of PB B-cells are of CD5-negative[22]. About half of CD5- B-cells have unmutated VH gene, whereas the others have a somatically mutated VH gene with a mean of 5-6% mutation frequency. The IgM+ IgD+ subpopulation expressing CD27 carry somatically mutated V gene[38]. In secondary lymphoid tissue, GC B-cells express CD5- and CD10+ and have somatically mutated VH gene with a similar average. The VH genes of marginal zone B-cells and plasma cells are somatically mutated[22].

CD5- B-cells neoplasms must be divided into B-LBL and other than B-LBLs. B-LBL is distinct from other CD5- B-cell neoplasms because B-LBLs are derived from precursor B-cells. B-LBL is the same disease entity as precursor B-cell acute lymphoblastic leukemia, both of which express CD79a, CD19, CD20 and CD10 in various number, but not surface Ig nor CD5[39]. These disorders involve mainly bone marrow and PB. The IgH gene in most of B-LBL is rearranged and shows germline sequence[40]. CD5- B-cells neoplasms other than B-LBL are mature B-cell neoplasms derived from GC and post GC B-cells, so most of them possess somatically mutated VH gene[27,41]. FL is believed to be GC B-cell neoplasms[41]. About 80% of FLs have a *bcl-2/Ig* translocation, t (14 ; 18) (q32 ; q21), which occurs at VDJ rearrangements[39]. They exclusively express CD79a and CD10 and constitute follicular pattern with FDC network. Because GC B-cells show intraclonal diversity by ongoing somatic hypermutation, the rearranged VH gene of FLs possessed somatic hypermutation and intraclonal microheterogeneity (ongoing mutation)[42]. DLBCL constitutes the largest category accounting for 30-40% of all lymphoma

cases among Japan, Korea and Western countries[39]. DLBCLs show heterogeneity in their biologic properties. DLBCLs express GC B-cells and/or post-GC B-cell markers. Gene abnormalities of DLBCLs, except $CD5^+$ DLBCL, are believed to occur in GC[43] and most reports of somatic mutation of the rearranged VH gene of $CD5^-$ DLBCLs confirm its oncogenesis. A small number of DLBCLs have extremely high percentages of somatic mutation frequencies more than 15%[27]. This phenomenon is unknown, but additional mutation in the VH gene may occur during lymphoma cell expansion. MALT lymphoma (=extranodal marginal zone B-cell lymphoma, MZBCL) arises in mainly extranodal site and rarely in lymph node and spleen, which are called as nodal and splenic MZBCL, respectively[39]. MALT lymphomas also possess a somatically mutated VH gene[44]. The rearranged VH gene of splenic MZBCL may be or may be not mutated[45], but the origin of unmutated splenic MZBCL is unknown. PCM is a neoplasm derived from plasma cells situated at the end stage of B-cell differentiation and a rearranged VH gene of PCM is also somatically mutated[46]. Each of a mean of somatic mutation frequencies of FL, $CD5^-$ DLBCL, MALT lymphoma and PCM are 8～11%. Although BL is a B-cell neoplasm that derived from GC B-cells, somatic mutation frequencies of BL are somewhat different from other $CD5^-$ B-cell neoplasms[47]. They are lower and distributed between 0.5～10.0%. BLs are generated by translocation of the c-*myc* gene and the IgH gene ; t(8 ; 14) (q24 ; q32) during the class switch recombination in GC B-cells[48]. BLs have CD10 and a marker of early GC phase and lower percentage of somatic mutation frequency of rearranged VH gene, so that GC B-cells at early phase of differentiation is counterpart cells of BL.

4) Appendix, Hodgkin lymphoma

Hodgkin/Reed-Sternberg (HRS) cells were suggested to be B-cells, because a small percentage of HRS cells express B-cell antigen such as CD20 in immunohistochemistry, and Southern blotting with the IgH gene probe infrequently revealed a rearrangement band in a sample of HL. Recent molecular techniques of single cell PCR have confirmed B-cell origin of HRS cells[49]. PCR amplification of the IgH gene from micro-dissected HRS cells from the HL frozen sections elegantly demonstrated monoclonal proliferation of B-cells with somatically mutated VH gene. Out-of-frame sequence of the rearranged VH gene is found in HRS cells. Those data indicated GC B-cells are the counterpart cells of HRS cells.

5) Conclusion

The common characteristic of $CD5^+$ B-cell neoplasms is heterogeneity of somatic mutation pattern in the VH gene, whereas that of $CD5^-$ B-cell neoplasm is a somatically hypermutated VH gene. $CD5^+$ B-cells and $CD5^-$ B-cells can independently differentiate and $CD5^+$ B-cell neoplasms and $CD5^-$ B-cell neoplasms are distinct in somatic mutation pattern of the IgH gene (Fig. 5).

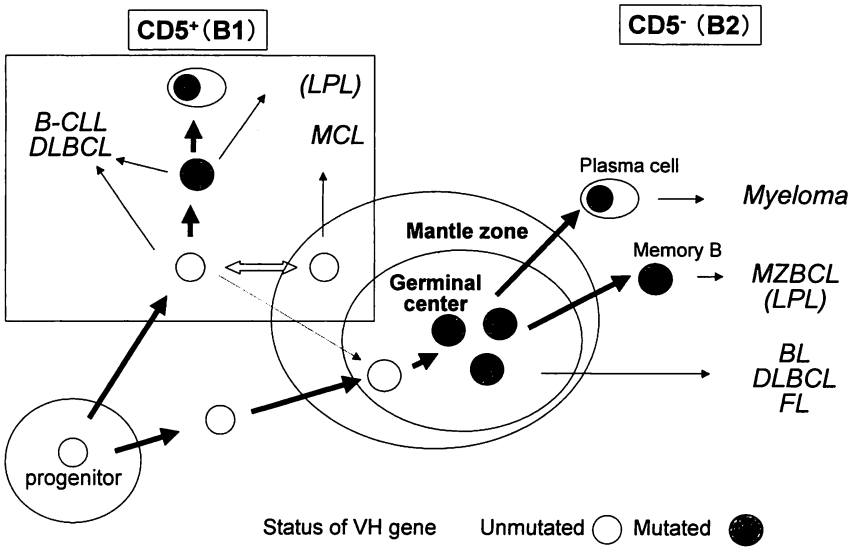

Fig. 5. Differentiation of B-cells and histogenesis of B-cell neoplasms.
This figure shows that differentiation of CD5+ B-cells and CD5- B-cells are independent.
(Modefied, Klein U et al.[12])

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