

Molecular Mechanisms of Lymphomagenesis through Transcriptional Disregulation by Chromosome Translocation

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Abstract

Chromosome translocation plays an important role for lymphomagenesis. Transcriptional disregulation type of chromosome translocation involves a majority of B-cell lymphoma. These include *BCL1*/cyclin D1 translocation in mantle cell lymphoma, *BCL2* in follicular lymphoma and *BCL6* in diffuse large B-cell lymphoma. It is known that the transcriptional disregulation type causes aberrant gene expression at the stages where those genes are down-regulated in normal counterpart cells. Normal B-cells at mantle zone stage down-regulate the expression of *BCL1* gene and become resting in cell cycle. However, *BCL1* expression from translocated allele with immunoglobulin gene is not down-regulated at the mantle zone stage, preventing cells from entering in resting state, and puts cells in cell cycle, leading to development of mantle cell lymphoma. *BCL2* expression from altered allele also keeps its expression at the germinal center stage where normal counterpart cells down-regulate *BCL2* expression, and makes cells to resist apoptosis, leading to development of follicular lymphoma. The same scenario can apply to diffuse large B-cell lymphoma with *BCL6* translocation; i.e. aberrant *BCL6* expression at the post germinal center stage takes place where normal counterpart cells down-regulate *BCL6* expression. Although a strong association of specific translocations with specific disease types is found, these translocations by themselves are not sufficient for malignant transformation. The factors other than chromosome translocations will be discussed.

Chromosome translocation has been known to play a major role in lymphoma development. Mantle cell lymphoma has *BCL1* translocation, t(11;14)(q13;q32), that defines an important molecular disease entity (Yatabe et al., 2000). More than 70% of follicular lymphoma have *BCL2* translocation, t(14;18)(q32;q21) (Albinger-Hegyri et al., 2002). These two translocations almost exclusively involve immunoglobulin (*Ig*) genes for translocation. Thirty to 40% of diffuse large B-cell lymphoma (DLBCL) has translocations involving chromosome 3q27 where *BCL6* is located (Ye et al., 1993). A half of the *BCL6* translocations also involve *Ig* genes (Ohno and Fukuhara, 1997). It should be noted that these three types of translocation result in different types of lymphoma although they use the same *Ig* gene as disregulator, indicating that the type of lymphoma is defined by the target genes, *BCL1*, *BCL2* and *BCL6* (Table 1).

Chromosome translocations are classified into two types. One is a fusion gene type that produces chimeric

product consisting of parts of two gene products such as *BCR-ABL* in chronic myelogenous leukemia and *PML-RAR α* in acute promyelocytic leukemia. The other is transcriptional disregulation type that produces normal

Table 1.

Malignant lymphoma and chromosome translocation.

Lymphoma	Chromosome translocation	Disregulator	Target genes
Mantle cell lymphoma (MCL)	t(11;14)(q13;q32)	IgH*	<i>BCL1</i> / cyclin D1
Follicular lymphoma (FL)	t(14;18)(q23;q32)	IgH	<i>BCL2</i>
Diffuse large B-cell lymphoma (DLBCL)	t(3;14)(q27;q32)	IgH	<i>BCL6</i>

*immunoglobulin heavy chain gene

gene product that is under control of the juxtaposed gene such as *Ig* or T cell receptor gene. It is believed that the transcriptional dysregulation type causes overexpression of the target genes. In deed, *BCL1* expression of B-cell lymphoma with *BCL1* translocation is significantly higher when compared with that of B-cell lymphoma without *BCL1* translocation (Seto *et al*, 1992; Suzuki *et al*, 1999). However, the overexpression is not found in *BCL2* and *BCL6* translocations. For example, the level of *BCL2* mRNA expression of lymphomas with *BCL2* translocation is almost the same as that in pre-B cell lines (Graninger *et al*, 1987). *BCL6* mRNA transcription level in lymphomas with *BCL6* translocation is not always as high as that in DLBCL cell lines without *BCL6* translocation (Pittaluga *et al*, 1996). Thus, the questions arise why the normal products with normal level of expression lead to lymphoma development and why these characteristic chromosome translocations strongly associate with specific disease types.

In order to understand the mechanisms of lymphomagenesis by transcriptional dysregulation, it is important to understand the gene expression regulation of these translocation junction genes during normal B-cell development. From the viewpoints of *Ig* rearrangement, somatic mutation and phenotypes of various lymphoid malignancies, the developmental stages can be divided as shown in Figure 1 although B-cell developmental pathway is still controversial. *BCL2* gene is differentially expressed during B-cell development. *BCL6* gene is also differentially expressed, being most strong at the follicular center cell stage (GC stage) where *BCL2* expression is down regulated in normal development (Figure 1) (Graninger *et al*, 1987; Pittaluga *et al*, 1996). Although *BCL1/cyclin D1* expression regulation is not well understood, it is well accepted that the gene is expressed in the same fashion as Ki67, a marker of cell proliferation (Liu *et al*, 1992). Of note is that expression of the translocation target gene for respective malignancies is down-regulated in the normal counterpart cells, namely, *BCL1/cyclin D1* at the mantle zone stage, *BCL2* at the germinal center stage and *BCL6* at the post germinal center stage (marginal zone stage). Because normal counterpart cells of these tumors do not express those genes at specific developmental stages under normal development, it is suggested that the altered gene expression caused by chromosome translocation has molecular bases leading to lymphomagenesis.

To understand molecular mechanisms of lymphomagenesis by chromosome translocation, it is also important to know when translocation takes place. It has been known by the breakpoint analysis that the translocation of *BCL1* and *BCL2* with *Ig* occurs at the pre-B-cell stage because the JH and DH segments of *Ig* are involved in the translocation (Figure 2) (Bakhshi *et al*, 1987; Tsujimoto *et al*, 1985). This indicates that the stage of cells that undergo chromosome translocation and the stage of resulting lymphomas are different. When these two genes are translocated, the gene expressions from the altered alleles caused by chromosome translocation do not appear to be aberrant for the cells at pre B-cell stage because those genes are expressed from normal alleles at this stage. Since the both gene products, expression from normal and translocated alleles are identical, expression from translocated allele does not give any growth advantage to the cells with translocation at the pre B-cell stage. After the other *Ig* allele is successfully rearranged, these cells undergo normal cell dif-

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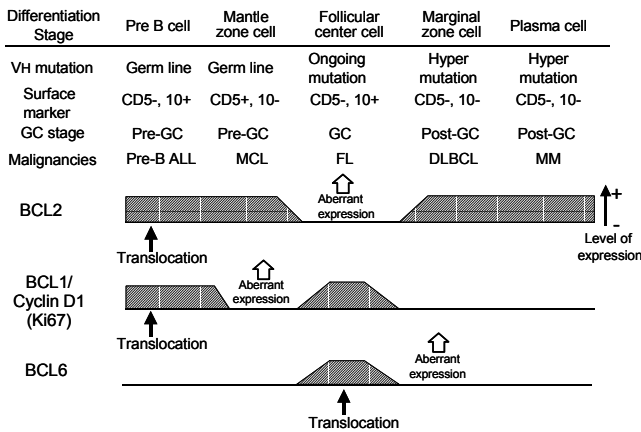


Figure 1. Regulated expression of translocation target genes in normal B-cell development and dysregulated expressions by chromosome translocation. B-cell development is divided by somatic mutation of immunoglobulin heavy chain variable (VH) gene, cell surface markers and resulting B-cell malignancies. Level of gene expression was depicted below with shaded square (▨). Closed arrows indicate the stage when translocations take place during the B-cell development. Aberrant expressions resulting from chromosome translocations are shown in open arrows. Pre-GC, pre germinal center stage; GC, germinal center stage; post-GC, post germinal center stage. Pre B ALL, pre B cell acute lymphoblastic leukemia; MCL, mantle cell lymphoma, FL, follicular lymphoma, DLBCL, diffuse large B-cell lymphoma, MM, multiple myeloma..

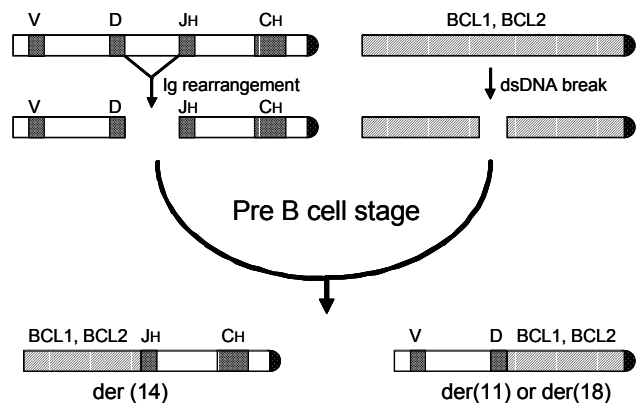


Figure 2. Chromosome translocation of *BCL1* and *BCL2*. Breakpoint analyses revealed that DH and JH of immunoglobulin gene are involved in translocation, indicating that the translocation took place when DH and JH joining, the first step of immunoglobulin rearrangement, occurred, which is in the pre-B-cell stage of B-cell development.

ferentiation until specific developmental stages depending on the translocation target genes. Naïve B-cell with successful *Ig* heavy chain (*IgH*) and *Ig* light gene rearrangements come to mantle zone where the cell cycle is in resting stage. *BCL1*/cyclin D1 gene at this stage is down-regulated by yet unknown mechanisms, but the B-cell with *BCL1* translocation keeps *BCL1*/cyclin D1 expression under the influence of neighboring *Ig* gene. This provides aberrant cell cycle signal to the cell with *BCL1* translocation, and this would become driving force for the cell to grow, leading to the development of mantle cell lymphoma. The same scenario can apply to the follicular lymphoma. The B-cell that underwent *BCL2* translocation at pre-B-cell stage would express *BCL2* from the translocated allele where B-cells in the normal development also express *BCL2* gene. The B-cell with *BCL2* translocation undergoes normal differentiation pathway and will arrive at mantle zone. *BCL2* gene expression is not aberrant for cells at mantle zone stage in normal development and *BCL2* gene product does not give growth signal. When the cell with *BCL2* translocation at mantle zone stage is stimulated with a specific antigen, the cell becomes activated and proliferates in germinal center where a majority of B-cells undergo apoptosis that is known to be selection force for high affinity antibody producer. The *BCL2* expression of germinal center B-cells is down-regulated by yet unknown mechanism under normal development. Although the mechanism of *BCL2* down-regulation is unknown, those who are not producing high affinity antibodies die by mechanism of apoptosis, leaving the high affinity cells to survive. When the B-cell with *BCL2* translocation is stimulated with a specific antigen, the cell gets into germinal center. The *BCL2* gene remains expressed from the translocated allele, which provides resistance to apoptotic signal in the germinal center, giving the cell to growth advantage at this stage of development. The scenario common to both translocations is aberrant expression of the target genes where these genes are down regulated in the normal developmental pathway.

Is this mechanism only applicable to *BCL1* and *BCL2* *BCL6* translocation has been known to be associated with 30 to 40% of DLBCL. It is well known that DLBCL may contain several subtypes but association of *BCL6* translocation with specific DLBCL subtype has not been well understood. However, a majority of DLBCL has cell surface phenotype of CD5-CD10- and is believed to be of post GC stage origin by the somatic mutation analysis (Lossos *et al*, 2000). Translocation of *BCL6* has been known to take place at the germinal center stage where the *BCL6* is expressed (Yoshida *et al*, 1999; Ohno and Fukuhara, 1997). When the normal germinal center goes out of the germinal center after selection, *BCL6* gene becomes down-regulated. The B-cell with *BCL6* translocation would keep *BCL6* expression and this expression becomes aberrant for the cells of post GC stage. Although why aberrant expression of *BCL6* causes malignant transformation remains to be explored, the same scenario is applicable that the aberrant gene expression is forced by chromo-

some translocation at the stage where the gene expression of normal counterpart cells is down-regulated. The common mechanisms for chromosome translocation of transcriptional dysregulation type are not the overexpression of the target genes but the transcriptional dysregulation at the appropriate stages of B-cell development.

Next question is if the chromosome translocation by itself is sufficient for malignant transformation. It is well accepted that the *BCL2* translocation is one of the most likely causative gene alteration for follicular lymphoma. *BCL2* functions anti-apoptotic against various death signals. As mentioned above, aberrant expression of *BCL2* give growth advantage at the GC stage where cells die by the mechanism of apoptosis. However, it should be noted that *BCL2* does not give any growth signal for cells to proliferate. Then, what is the driving force for the cells with *BCL2* translocation to proliferate? The clue was given by the observation that only lymphoma cells in the germinal center are proliferating (Dogan *et al*, 1998). Kagami *et al* (2001) also reported the establishment of follicular lymphoma cell line FLK-1, the growth of which is dependent on follicular dendritic cell line HK. As shown in Figure 3, the cells can only grow in the presence of FDC. Both of these reports suggest that growth signals for follicular lymphoma cells are given by the FDC. Although the molecular bases for this growth signal remain to be explored, the lymphoma cells and FDC interaction is one of the important factors for malignant transformation of follicular center cells.

In conclusion, chromosome translocation of transcriptional dysregulation type plays an important role for lymphomagenesis. Consequence of this type of translocation is not simply causing the overexpression of target

FLK-1 cell proliferation depends on HK (FDC) cells

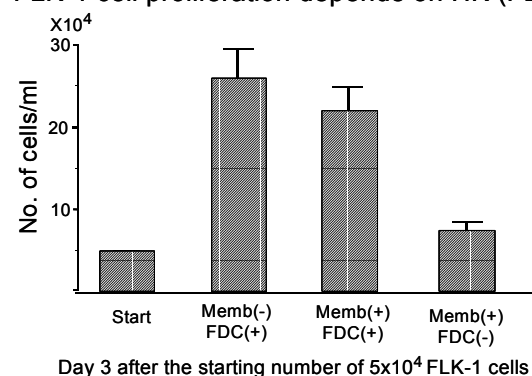


Figure 3. Follicular dendritic cell line, HK, dependent growth of follicular lymphoma derived cell line, FLK. 5×10^4 cells are co-cultured in the presence or absence of HK. Cell number was counted on day 3. Continuous growth was observed with HK and FLK-1 without separating $0.4 \mu\text{m}$ Transwell membrane, while no significant growth was shown without HK cells. Although FLK-1 demonstrated cell growth with separating membrane (without direct contact), cells eventually stop growing (See more detail in Kagami *et al*, 2001).

genes but causing transcriptional dysregulation of gene expression where normal counterpart cells do not express these genes. It is also important to understand the interaction of lymphoma and stroma cells that will lead to specific type of lymphoma with specific chromosome translocation.

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