Re: B-Cell Non-Hodgkin’s Lymphoma: Evidence for the t(14;18) Translocation in All Hematopoietic Cell Lineages

The chromosomal translocation t(14;18)(q32;q21) can cytogenetically be detected in 80%-85% of follicular lymphomas and in up to 30% of diffuse large-cell lymphomas (I). Pathogenetically, the translocation t(14;18) juxtaposes the bcl-2 (also known as BCL2) gene in 18q21 to the immunoglobulin heavy-chain locus in 14q32. This subsequently leads to overexpression of the mitochondria membrane protein Bcl-2, which prevents cells from undergoing apoptosis.

In a recent issue of the Journal, Yarkoni et al. (2) presented data of a combined cytogenetics and fluorescence in situ hybridization (FISH) study of the translocation t(14;18). By FISH, the t(14;18) was detected in eight of 10 bone marrow aspirates from patients with B-cell lymphomas, including a patient with immunoblastic lymphoma. The t(14;18) has so far been reported only in seven patients with immunoblastic lymphoma, three of them presenting a Burkitt translocation as well (3). In non-Hodgkin’s lymphomas that were diagnosed according to the updated Kiel classification, the t(14;18) is restricted to centroblastic lymphomas and rare cases of Burkitt’s lymphoma but is never found in immunoblastic lymphoma [(4); our unpublished data].

In their recent article, Yarkoni et al. (2) reported that in seven of these eight t(14;18)-positive cases, the percentage of total bone marrow cells carrying the translocation was nearly 100%. Logically, since almost all of the unfractinated bone marrow cells carry the translocation, the authors were also able to detect the t(14;18) in nearly 100% of fluorescence-activated cell sorter (FACS)-fractionated CD10+ and CD19+ B cells, CD3+ T cells, CD13+ myeloid cells, CD41a+ megakaryocytic cells, and glycophorin A+ erythroid cells. Since even more than 97% of the CD34+ hematopoietic stem cells contained the translocation also, these spectacular data suggest that the t(14;18) occurs in a rather early multilineage progenitor stem cell.

To improve the detection of the translocation t(14;18) on the single-cell level, we recently established a FISH assay with yeast artificial chromosome (YAC)–DNA probes for the immunoglobulin heavy-chain locus and bcl-2 genes (5). Large YAC–DNA probes are advantageous compared with 3-4-kilobase (kb) plasmid probes, because they produce bright and distinct signals in nearly every cell. If 3-4-kb plasmid probes are used in standard FISH, the hybridization efficiency is too low to give reliable results in interphase cytogenetic studies. Our FISH assay turned out to be more sensitive to detect the t(14;18) translocation than polymerase chain reaction (PCR) and chromosome analysis. Moreover, to investigate the phenotype of the aberrant cells with t(14;18), we applied our technique of combined fluorescence immunophenotyping and interphase cytogenetics (FICTION technique) (6,7). Principally, the FICTION method corresponds to the FACS assortment of cells with subsequent FISH analysis. With the use of FICTION studies of 30 affected lymph nodes, we were able to attach the cells carrying the t(14;18) to CD22+ B cells. Among the B-cell population, 30%-92% of the cells carried the translocation. In no case did CD3+ T lymphocytes ever contain the t(14;18) signal constellation (5).

Our results obviously contrast those obtained by Yarkoni et al. (2) who reported the t(14;18) to be present in 100% of bone marrow cells and in cells of all hematopoietic lineages. Their data suggest that the common precursor cell of the majority of t(14;18)-positive B-cell lymphomas is not committed to B lineage but a pluripotent hematopoietic stem cell. Until now, the t(14;18) was regarded to be the initial event in the pathogenesis of t(14;18)-positive B-cell lymphomas and to take place during the immunoglobulin heavy-chain locus rearrangement occurring physiologically in B-cell-committed precursor cells (8), which is also in line with our FICTION results.

The data of Yarkoni et al. (2) would substantially influence the standard cytogenetic approaches in the diagnostic procedures in B-cell lymphomas. All peripheral blood cells derive from bone marrow stem cells. If nearly all of the hematopoietic stem cells harbor the t(14;18), the vast majority of peripheral blood cells should also uniformly carry this translocation. Thus, the detection of the t(14;18) in B-cell lymphoma should be possible simply by studying peripheral blood. By chromosome analysis, it is usually not possible to detect any cell with the t(14;18) in peripheral blood. Even in the bone marrow, cells with t(14;18) can only rarely be observed. As a rule, a considerable infiltration is required to find cytogenetically aberrant metaphases in the bone marrow.

With regard to the excellent literature on cytogenetics and molecular genetics of the t(14;18) and in view of the experimental data obtained by us and others, we had to conclude that in B-cell lymphomas, there is obviously more evidence against a generally high percentage of t(14;18)-positive cells in all hematopoietic lineages. Therefore, it seems unlikely that in the majority of B-cell lymphomas, the t(14;18) translocation occurs in a very early multilineage progenitor cell.
was fully characterized by cytogenetic analysis, showing both the t(14;18) and another translocation (Fig. 1).

Re-examination of the data of cytogenetic analysis performed on five patients revealed that the majority of the cells (65%-85%) carry the t(14;18), even when this sensitive detection method of chromosomal analysis was used (data not shown in our original paper).

We also separated CD13-CD34+ progenitor cells from the peripheral blood leukocytes (PBLs) of two patients and detected the t(14;18) in nearly 100% of these stem cells. Thus, not only can the t(14;18) be detected in PBLs but also it was detected directly in a subset of cells characterized by common and accepted methods as progenitor stem cells.

By chromosome analysis, it is usually not possible to detect cells carrying the t(14;18) in peripheral blood, since it contains almost no cells at metaphase. More sensitive methods, such as FISH analysis and PCR, are performed today in numerous studies on peripheral blood and in bone marrow because they do not require dividing cells. Such translocations are detected even in patients in complete remission (1). PCR analysis is also used to detect residual disease in patients (2).

All of our studies were performed with the use of small 3-4-kb plasmid probes. However, with regard to excellent and numerous studies on FISH analysis performed with similar small plasmid probes (3), or even as small as a 1-kb probe (3), and in view of our well-controlled studies, small DNA probes are reliable and are used commonly in standard FISH analysis.

Poetsch et al. (4) used large YAC–DNA probes for FISH analysis. Although very strongly labeled and giving bright signals, YAC–DNA probes have disadvantages, e.g., the very low ratio of human to yeast sequences causes low hybridization efficiency. Using two large YAC–DNA probes for detecting translocations decreases hybridization efficiency even more.

With the use of the FICTION technique, Poetsch et al. (4) were able to attach the cells carrying the t(14;18) only to CD22+ B cells. However, the FICTION analysis was applied on only five and not all of the 30 affected lymph nodes studied and in four of the 32 cases of non-neoplastic lymphoproliferations. Moreover, only CD3+ T cells were examined for the t(14;18), with no direct attempt to study other hematopoietic cell subpopulations. In our study, all cell lineages were presorted (double FACS sorting) and analyzed directly by FISH analysis.

Our results led us to conclude that the t(14;18) occurs most probably in very early multilineage progenitor cells, suggesting a new insight into our understanding of the origin of B cells of patients with non-Hodgkin’s lymphoma. Our data, along with other studies, will most probably influence the standard diagnostic procedures in B-cell lymphomas.

Notes

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Response

One of our patients, diagnosed with immunoblastic lymphoma, was clearly shown to carry the t(14;18). This case was fully characterized by cytogenetic analysis.

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References


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