Large-cell anaplastic lymphoma-specific translocation (t(2;5) (p23;q35)) in Hodgkin's disease: Indication of a common pathogenesis?

Karin Orschenshak, Hartmut Morz, Jan Heli, Thomas Binder, Heinrich Bartels, Alfred Christian Felder

Summary: Chromosomal aberrations are characteristic and specific events; the detection of chromosomal abnormalities often provides information on diagnosis and prognosis of disease. Some patients with large-cell anaplastic lymphoma (Ki-1 lymphoma) have the translocation t(2;5) (p23; q35), involving a possible growth-regulating tyrosine kinase. We found this translocation in 13 patients with Hodgkin's disease of nodular sclerosis and mixed-cellularity types. This finding has implications for understanding of the relation between large-cell anaplastic lymphomas and Hodgkin's disease, diseases with morphological and immunophenotypical similarities.

Study of this translocation may help understanding of the origin of cancer and tumor growth. It also allows a more precise definition of Hodgkin's disease and may be used as an indicator for the disease—which has long been sought.

Lancet 1995, 345: 87-90

Introduction: Many cancers have been found to be characterised by specific chromosomal aberrations, which help in defining those with similar biological background. Detection of specific chromosomal aberrations can also be used for diagnosis or to assess prognosis.

Philadelphia chromosome (Ph1) is diagnostic of chronic myeloid leukaemia, and can be detected in more than 95% of cases. The molecular correlate of Ph1 is an aberrant joining of sequences from chromosome 22q11 to the c-Ab1 proto-oncogene at 9q34. This hybrid gene encodes a chimeric protein with tyrosine kinase activity.

Similar chromosomal aberrations have also been described in malignant lymphomas.

Large-cell anaplastic lymphoma (LCAL) has characteristic morphological, constant expression of the CD30 antigen, and is composed of large blasts, sometimes biculated or multilobated, pleomorphic cells with prominent, often elongated nuclei and abundant cytoplasm, similar to Hodgkin and Sternberg-Reed cells. Some T-cell-derived LCALs have a specific translocation, t(2;5) (p23q35) which may regulate cell growth, and be involved in tumorigenesis. This translocation has been shown to be a fusion product of the nuclear phosphoprotein nucleophosmin (NPM) and the anaplastic lymphoma kinase gene (ALK), resulting in a hybrid protein with tyrosine kinase activity. Other proteins with tyrosine kinase activity are the insulin receptor kinase, which have been shown to have transforming potential.

Phenotypically and immunohistochemically, the lymphoma most closely related to LCAL is Hodgkin's disease.

In both entities there are variable numbers of Hodgkin and Sternberg-Reed cells, a pattern of epitheliod-grotes, few of cells, and a variable mixture of histiocytes, epithelioid cells, neutrophils, eosinophils, lymphocytes, and plasma cells. Cases have been described that evolved from Hodgkin's disease into LCAL. Morphologically, the cellular phase of nodular sclerosis type II Hodgkin's is sometimes difficult to differentiate from LCAL; there are no criteria for distinguishing LCAL from lymphocyte-depleted Hodgkin's disease. We and others have observed patterns with Hodgkin's disease and LCAL who have the same pattern of T-cell receptors.

Furthermore, immunophenotypically both tumours have similar expression of T-cell, B-cell, activation antigens and cytokines.

Do these diseases have a common origin in early steps of tumorigenesis and is their subsequent growth varied by other cellular factors or different immunological microenvironmental factors? We investigated whether the t(2;5) translocation specific to LCAL could be detected in Hodgkin's disease.

Department of Pathology, Medical University of Lübeck (K Orschenshak, H Morz, J Heli, F C. Feiler); 3rd Department of Internal Medicine, University of Ulm (H Birrer, W Pettit, and Department of Pathology, and Oncology, Städtische Krankenhaus Süd, Lübeck (H Morz, W Pettit); Germany

Correspondence to: Prof Alfred Christian Felder, Department of Pathology, Möncheberg University of Lübeck, Ratzeburger Allee 260, 23305 Lübeck, Germany.
Materials and methods
Lymphomas and non-infective lymphoid tissues were taken from patients under investigation and autopsied or removed as, were peripheral blood leukocytes and cultured cell lines, including a CDS (ampy 0.001 cell) line (P2) from a chronic myelogram of a patient with LCAL, carrying the specific (cDNA) translocation. As control, we included two B-cell lymphomas (cometastatic lymphomas), two T-cell lymphomas (lymphometastatic lymphomas), two lymph nodes with T-cell hyperplasia, as well as the three lines Karpas299 and HUT102, the B-cell lines Ramos and Raji, all myeloid leukemic cell lines KM10 and U937 (all cell lines except L2 were from DSM, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). Normal peripheral blood leukocytes were either used unstimulated or stimulated for 48 h with phorbol-myristate acetate (PMA) 10 ng/ml, and calcium ionophore A23187 100 ng/ml (Sigma, Deisenhofen, Germany).

Extraction of cellular RNA and reverse-transcription polymerase chain reaction (RT-PCR) were done as previously described.1 RNA integrity was checked by agarose gel electrophoresis and ethidium bromide staining. A beta-actin fragment of 1800 bp was amplified to monitor first-strand synthesis and to control cDNA amplification.4 Bands for beta-actin were scored: +/+ (appearing after 20 cycles), +/− (appearing after 25 cycles), − (not appearing after 50 cycles or not at all). 1/0 of the reverse-transcribed cDNA was used for PCR with 100 pmol 5′ and 5′ primer (P1/P2 or P3/P4, figure), and 1 u Taq polymerase (Boehringer, Mannheim, Germany) added to a final volume of 50 μl. Visible bands were scored: +/− for strong positive bands, +/− for detectable bands, − for smears or no detectable band.

Each experiment was repeated at least two times, in different laboratories by two independent investigators from our staff with new sets of reagents to exclude contamination.

The amplified specific cDNA fragments from cell line L2, the LCAL case 18 and Hodgkin’s disease cases 4, 6, 10, and 13 were hybridized with the probe (see figure) with the TAC antibody (Enzoquest, Lesh, Netherlands). Sequence analysis for specific cDNA was done on deuterated double-stranded plasmid by the / major chain immobilization method with Sequence Kit (US Biochemical, Cleveland, Ohio, USA). Sequence analysis for cDNA was done on deuterated double-stranded plasmid by the / major chain immobilization method with Sequence Kit (US Biochemical, Cleveland, Ohio, USA). The probes used for Southern blot analysis of PCR products were the 37-14 kDa bands of specific cDNA cloned from the cell line L2, which was confirmed by DNA sequencing. DNA bands of 100% sequence identity with the published sequence4 and a breakpoint spanning oligonucleotide P7. 10 μl of PCR products was subcloned to electroborides and cloned into a nitro membrane (Hybond N+; Amersham Corp, Arlington Heights, Illinois, USA). The probes were labelled with digoxigenin-dUTP by random priming reaction (for cDNA) or terminal deoxynucleotidyl transferase reaction (for oligonucleotides) with digoxigenin-labelling and detection kit (Boehringer, Mannheim, Germany).

The limit of detection for specific cDNA was determined by diluting L2 cells down from 10,000 to 1 cell into 99,000 to 100,000 peripheral blood leukocytes. RNA extraction, RT-PCR analysis, and Southern blot hybridization for confirmation of specific cDNA were done as described above.

Additionally, one case of Hodgkin’s disease (the most intensely positive case) was analyzed by semi quantitative RT-PCR analysis. For this purpose 5 μg of total cellular RNA of this case and from L2 cells were extracted and reverse transcribed. First-stranded cDNAs were then adjusted to equal β-actin amplifications. to which an approximately similar band intensity has been obtained after 20, 25, and 30 cycles of amplifications to plot densitometer identical cDNA loadings. Equivalecuted amounts of the cDNAs were then subjected to specific amplification and compared after different numbers of cycles (20, 25, 30, 35). The results were again confirmed by Southern blot hybridization.

Results
RT-PCR for analysis for AMNLK
RT-PCR analysis showed the characteristic translocation (in cell line L2) of NKLK in lymphocytes. In four of five patients with LCAL (T and 0 type), whereas specimens from other malignant lymphomas, lymph nodes with T zone hyperplasia, hyperplastic, tumoral tissue, and normal peripheral blood leukocytes were negative. Hodgkin’s disease of LCAL cases differed in the band intensities for specific cDNA. RT-PCR results for patients P3/P4 were confirmed for specificity to specific with Southern blot hybridization. Hybridization patterns were identical, whether the full-length cDNA clone was used as a probe or as the junction-specific oligonucleotide. Eleven of thirteen cases of Hodgkin’s disease and five cases of LCAL showed the expected hybridization signal.

Sequenceing of RT-PCR products (in cell line L2)
Four RT-PCR-positive cases of Hodgkin’s disease and one RT-PCR-positive case of LCAL (cases 4, 6, 10, 13, and 18) had adequate cDNA available for large-scale amplification, cloning, and sequencing. Sequence analysis was done with TAC primers starting from the pCRII sequence, to confirm correct primer use. Sequence analysis of cases 4, 6, 10, 13, and 18 showed complete homology with the published specific hybrid gene.

For sensitivity of DNA detection L2 cells were used to calculate the detection limit with RT-PCR. For this purpose L2 cells were serially diluted in peripheral blood leukocytes while the total number of cells was kept constant. DNA was then photometrically quantified, and used for semi quantitative β-actin RT-PCR. After 20, 22, and 25 cycles of amplification, β-actin products were seen on ethidium bromide-stained agarose gels. CDNA were calibrated for equal β-actin loading and amplification rates. Subsequently RT-PCR was done for specific 35 cycles. There was a gradual decline in band intensities, but β-actin could be detected even in case 1 cell. These results were confirmed by Southern hybridization with oligonucleotide labelled breakpoint-specific oligonucleotide P7.

Semi quantitative RT-PCR analysis for AMNLK
DNA from Hodgkin’s disease case 4 (with the most prominent band for specific after 30 cycles) was compared with DNA from L2 cells by semi quantitative RT-PCR approach. First-strands were again equilibrated
has described a case of Hodgkin's disease with a 5q35 abnormality.

The scarcity of tumour cells is a major difficulty in representative chromosomal analyses in Hodgkin's disease, and the (t(2;5)) translocation may be missed by conventional chromosome analysis, as may other characteristic aberrations, for example (t(4;18)) in follicular lymphomas. The structure of (t(2;5)) has been resolved at the genetic level. A previously unidentified tyrosine kinase gene on chromosome 2p23 is fused to the nucleophosmin gene at 5q35, resulting in a highly transcribed, truncated, but active kinase gene. This kinase, termed ALK, is normally silent in lymphoid cells and belongs to the insulin receptor kinase family of phosphotyrosine kinase genes, which regulate cellular growth and may trigger neoplastic transformation when mutated, translocated, and expressed aberrantly.

Our study shows that eleven of thirteen cases of Hodgkin's disease and four of five cases of LCAL showed the LCAL-specific translocation (t(2;5)). Primers P12 and P34 showed the same pattern for most cases. Two cases of Hodgkin's disease showed a product only for P34. This result may be due to degradation of RNA, leading to a reduction in amplifiable cDNAs. Alternatively, a similar, but different translocation event may be responsible for this difference, allowing only the small fragment to be amplified. Such discrepancies are frequently shown for other translocations, such as (t(14;18)):MALT oncogenes, in which a major and a minor cluster breakpoint region exists. The two cases of Hodgkin's disease and the case of LCAL that were negative for both sets of primers are likely to be true negative cases.

Our experiments show that starting with high quality RNA allows for the synthesis of long cDNAs and for early detection of β-actin gene transcription. Although band intensities declined gradually from 10,000 to 1 L82 cell, a signal could be detected even for one cell, suggesting for high sensitivity of speckled detection.

Hodgkin's disease is composed of a minority of tumour cells which may represent only a small percentage of the total cellular infiltrate. Only a few of the neoplastic cells may carry the (t(2;5)) translocation. To investigate this, we examined one case of Hodgkin's disease (4) by a semiquantitative approach. Amplification was delayed in the Hodgkin's disease case by about 5 cycles, which may be interpreted as a 32-fold lower amplification rate, suggesting a 32-fold smaller number of positive cells. This correlates well with the 3% to 5% Hodgkin and Sternberg-Reed cells in this case. Assuming that the Hodgkin and Sternberg-Reed cells express the same amount of NPM/ALK as L82 cells, it seems most likely that almost all of the neoplastic cells carry (t(2;5)). However, other cases showed weak or very weak signals, which indicate lower numbers of cells with (t(2;5)).

These observations show that LCAL-specific translocation (t(2;5)) is found in an identical pattern in most cases of Hodgkin's disease and may be confined to a varying number of cells, probably the Hodgkin and Sternberg-Reed cells. The (t(2;5)) translocation has been shown to involve a tyrosine kinase gene, which is most probably bcl-6, a pleomorphic due to this translocation and may induce cellular proliferation, indicating that LCAL and Hodgkin's disease undergo at least one identical step during tumorigenesis.

**Table: NPM/ALK RT-PCR analysis**

| Case | P2/P3- | 612 NM | P3/P4- | 618 NM | Interleukin
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HOME</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>HOME</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>HOME</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>HOME</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>LCAL</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>LCAL</td>
<td>15</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>LCAL</td>
<td>16</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>LCAL</td>
<td>17</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>LCAL</td>
<td>18</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>LCAL</td>
<td>19</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>LCAL</td>
<td>20</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>LCAL</td>
<td>21</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>20</td>
</tr>
<tr>
<td>LCAL</td>
<td>22</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>20</td>
</tr>
</tbody>
</table>

**Discussion**

The pathogenesis of Hodgkin's disease and LCAL is poorly understood; the diagnosis of both diseases is still based on complex morphological criteria, with Hodgkin and Sternberg-Reed cells embedded in a variable background of fibrous stroma and non-neoplastic cells. In Hodgkin's disease, cytogenetic analysis has shown neoploidy, hyperploidy, chromosomal translocations, and losses of, and recurring chromosomal alterations to 6q, 8q, 11q, 14q, but no constant disease-specific chromosomal abnormality. In LCAL, however, some cases, especially those of T-cell type, carry the specific translocation (t(2;5)(p23;q35)), which has not yet been detected in any other lymphoma. So far, only one report by comparison of β-actin amplifications. Normalised cDNAs of L82 cells and the Hodgkin's disease case were amplified in parallel and visualised after various numbers of cycles. An amplified band appeared for L82 cells (10 ng RNA approx 1000 cells) after 25 cycles, whereas for the Hodgkin's disease case (10 ng whole cellular RNA) bands could be visualised after 30 cycles. In Southern blots, bands were confirmed to be true NPM/ALK products.
This finding underlines the close biological relation between these two diseases, which is also shown by morphology, and genotypic and immunophenotypic analysis.

The method described for detection of this chromosomal translocation will allow better differentiation between borderline cases, most of which are T-cell lymphomas (eg, angioimmunoblastic lymphadenopathy type, lymphohistiocytic lymphoma, and plasmocytoid T-cell lymphomas) and some B-cell lymphomas, such as rare cases of chronic lymphocytic leukaemia of B-cell type with a high content of Hodgkin and Sternberg-Reed cells. It will be of interest to study transional cases developing from one entity to another, as the occurrence—or lack of—t(12;15) would allow us better to understand the biological relevance of this translocation.

It is almost impossible to detect rearrangements of the T-cell receptor or immunoglobulin genes in cases of Hodgkin's disease, because of the small number of tumour cells. The t(2;15) translocation may therefore be used as an indicator of clonality. It can also be used to monitor patients during disease-free intervals. The sensitivity demonstrated in this study allows the detection of one Epstein-positive cell in 100 000 negative cells.

This work was supported by Deutsche Krebshilfe. W55958/3.

References