Nucleophosmin (NPM) Gene Rearrangements in Ki-1-positive Lymphomas

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Abstract

The (2;5)(p23;q35) translocation which occurs in the fusion of the NPM (nucleophosmin) gene on chromosome 5q35 with the novel ALK (anaplastic lymphoma kinase) gene on chromosome 2p23 [S.W. Morris et al., Science (Washington DC), 263: 1281-1284, 1994] is associated with Ki-1 (CD30)-positive anaplastic large cell lymphomas (ALCL); a group of morphologically and immunophenotypically heterogeneous high grade large cell lymphomas (LCL), which share many characteristics with Hodgkin’s disease (HD), including the presence of variable numbers of Reed-Sternberg-like cells and the expression of CD30 antigen.

Using a DNA probe immediately 5’ to the NPM coding sequences, we have examined NPM gene rearrangements by Southern blotting in 5 Ki-1-positive lymphoma cell lines carrying a translocation involving the 5q35 breakpoint and in 25 Ki-1-positive lymphoma tumors, including 9 HD. Using this method, we detected rearrangements in all cell lines with apparent clustering of the breakpoints. Analysis of 25 Ki-1-positive lymphomas indicated that only 4 neoplasms, including two HD, had NPM gene rearrangements. Thus, our findings suggest that only a subset of ALCL has detectable involvement of the NPM gene. In addition, the presence of NPM gene rearrangements in HD indicates the involvement of this gene in a fraction of HD. Thus, NPM gene rearrangements may identify a certain subtype in ALCL and HD which may be closely related.

Introduction

Ki-1 (CD30)-positive ALCL 3 are high grade malignancies which constitute a distinct subgroup of large cell lymphomas (2–4) comprising approximately 25 and 10%, respectively, of large cell lymphoma and NHL (5). The CD30 antigen is present on more than 80% of neoplastic cells in Ki-1-positive ALCL (5). The protein was first identified as a surface marker of Reed-Sternberg cells of HD (6, 7). Cloning of the CD30 complementary DNA identified it as a member of the nerve growth factor receptor superfamily with conserved cysteine-rich motifs in the extracellular domain which are implicated in ligand binding (8). The CD30 ligand is a membrane-associated protein similar to ligands for members of the nerve growth factor receptor family (9). The human T-lymphotropic virus type I and T-cell receptor (TCR), T-cell receptor.

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3 The abbreviations used are: ALCL, anaplastic large cell lymphoma; LCL, large cell lymphoma; NHL, non-Hodgkin’s lymphoma; HD, Hodgkin’s disease; B-ALCL, B-cell ALCL; T-ALCL, T-cell ALCL; RT-PCR, reverse transcription-polymerase chain reaction; SDS, sodium dodecyl sulfate; SSC, standard saline citrate; Ig, immunoglobulin; TCR, T-cell receptor.

Burkitt’s lymphoma (13). The CD30 antigen is normally found on the surface of activated T- and B-lymphocytes (5–7) which constitute the neoplastic cells in Ki-1-positive ALCL (5, 14–17). Most cases exhibit T or null cell phenotype, although B-ALCL is not uncommon (5, 14–19).

Although no chromosomal abnormality has been consistently associated with HD, recurrent chromosomal translocations have been shown to play a role in the etiology of several types of NHL. The t(14;18) translocation associated with 80 to 85% of follicular lymphoma and the t(8;14) found in Burkitt’s lymphoma result in the deregulation of cellular protooncogenes bcl-2 and c-myc, respectively, through their juxtaposition with immunoglobulin gene enhancers on chromosome 14q32 (20–22). Rearrangements of chromosome 5 at band q35 are often associated with Ki-1-positive lymphomas (14–17, 23–25). The most common rearrangement is a t(2;5)(p23;q35) translocation, but variants such as the t(3;5)(q12;q35) have been described (16).

Recently, Morris et al. (1) have shown that the t(2;5) chromosomal translocation results in the fusion of the NPM (nucleophosmin/B23) gene on chromosome 5q35 with the novel ALK (anaplastic lymphoma kinase) gene on chromosome 2p23. Protooncogene activation through the formation of chimeric proteins is at the basis of malignant transformation in chronic myelogenous leukemias with the Philadelphia chromosome (26), acute pre-B leukemias with the (1;19) translocation (27), acute promyelocytic leukemias with the t(15;17) translocation (28), and in acute leukemias with translocations involving 11q23 (29).

The ALK gene is homologous to tyrosine kinase receptors and is likely to be membrane associated (1). NPM, a previously characterized nucleolar phosphoprotein (30), is thought to be involved in ribosomal protein assembly and nuclear/cytoplasmic transport (31, 32).

In order to examine the involvement of the NPM gene in Ki-1-positive lymphomas, we have used a DNA probe immediately 5’ to the NPM (1) gene to analyze DNA samples from Ki-1-positive ALCLs by Southern blotting. Because of morphological and immunophenotypic similarities between ALCL and some HD subtypes and because of the occurrence of ALCL with or subsequent to HD (secondary ALCL) (4, 5) we have extended our studies to include several cases of HD. Our results indicate that NPM gene rearrangements occur in only a fraction of primary ALCL and that they also occur in a small fraction of HD.

Materials and Methods

Patient Samples. Sixteen ALCL cases and 9 cases of HD (2 of lymphocyte predominant, 2 of mixed cellularity, and 5 of nodular sclerosis subtype) were categorized according to Stein and Dallenbach (5) and according to the Rye classification. These cases were taken from the files of the Institute of Pathology, Klinikum Steglitz, Free University of Berlin, Germany, and from the University of Bologna, Italy. All cases were immunophenotyped using the monoclonal antibodies Ber-H2 specific for CD30, L26 specific for CD20, polyclonal CD3 (all obtained from Dako, Glostrup), and β-F1 specific for TCR β chain (purchased from T Cell Sciences) in conjunction with the immunalkaline phosphatase method (33).
Patient DNA Isolation. DNA was extracted from snap-frozen samples by using the DNA extractor (Applied Biosystems; Model 341A) using the reagents and protocols from the same supplier. The DNA was resuspended in 50–100 µl of water, and the concentration was assessed spectrophotometrically.

Cell Lines. K98-2-7F (K98) is a lymphoblastoid cell line developed from normal female peripheral blood lymphocytes by T. Henderson (Fels Research Institute) and was used as a negative control for NPM gene rearrangements. The Karps 299 (K299) (17), SU-DHL-1 (SU) (34), DEL (35), and JB6 (kindly provided by M. Kadin) cell lines all derive from Ki-1-positive NHL and carry a breakpoint on chromosome 5 at band q35.

RT-PCR. Total RNA was extracted by the guanidinium thiocyanate method (36). Cellular RNA (2 µg), 100 ng of 3' ALK or 3' NPM primer (1), and 0.2 mm deoxynucleotide triphosphates (Boehringer Mannheim) were incubated in 1× RT Buffer (BRL) in a total volume of 18 µl for 2 min at 94°C, followed by 30 min at 65°C in a 9600 Thermal Cycler (Perkin Elmer). Superscript II (400 unit); (2 µl) reverse transcriptase (BRL) were added to the reaction mix, which was then incubated at 37°C for 1 h. Amplification was carried out with an additional 200 ng of 3' ALK or 3' NPM primer and 300 ng of 5' NPM primer (1), 1 unit of Taq polymerase (Boehringer Mannheim), and 0.075 mm deoxynucleotide triphosphates in 1× PCR buffer (with MgCl₂, Boehringer Mannheim) in a 100-µl volume. Thirty cycles of amplification were performed as follows: 94°C, 1 min; 65°C, 1 min; and 72°C, 2 min. PCR products were separated in 2.5% agarose gels, denatured, neutralized, and transferred to Hybond-C nylon membranes (Amersham). Oligonucleotide end labeling was performed with polynucleotide kinase (Boehringer Mannheim) as recommended by the manufacturer using 50 µCi [γ-32P]dCTP (Amersham). Filters were processed as described below.

DNA Probes. TCR gene rearrangements were evaluated using the Cαβ probe (ONCOR), and IgH gene rearrangements were evaluated with a @H probe (ONCOR), and IgH gene rearrangements were evaluated with a @H probe (ONCOR) recommended by the manufacturer using 50 µCi [γ-32P]dCTP (Amersham). Filters were processed as described below.

Southern Blotting Analysis. DNA was isolated from cell lines by conventional methods (37). Cell line (10 µg) and patient and germline human placenta DNA (ONCOR) (5 µg) was digested with BamHI, HindIII, and PstI enzymes (Boehringer Mannheim) in suitable buffers as recommended by the manufacturer. Using the Probe Tech 2 electrophoresis apparatus (ONCOR), digested DNA was size fractionated by gel electrophoresis in 0.7% agarose, run for 16 h at 22V, depurinated, denatured, and vacuum transferred onto Sure Blot nylon membranes (ONCOR) following manufacturer’s instructions. After transfer, the membranes were baked for 1 h at 80°C and prehybridized in 5× Saline-sodium phosphate-EDTA, 5× Denhardt’s solution, 1% SDS, and 1 mg/ml salmon sperm DNA solution at 65°C for 1 h. Probes were labeled with 10 µCi of [α-32P]dCTP (3000Ci/mmol; Amersham) using the Prime It II kit (Stratagene) to a specific activity of 104–105 cpm/µg DNA. Filters were hybridized for 16 h at 65°C using approximately 5 × 106 cpm/ml hybridization solution. Filters were then washed at 65°C sequentially in 2× SSC-0.1% SDS for 30 min; 0.2× SSC-0.1% SDS for 20 min; and 0.1× SSC-0.1% SDS for 10 min.

Finally, filters were exposed to Kodak X-Omat AR film with two Du Pont Cronex Lightning-plus intensifying screens at −70°C for 16 or 96 h.

Results and Discussion

Both HD and Ki-1-positive ALCL are morphologically and immunophenotypically heterogeneous diseases which have several characteristics in common (4, 5). Indeed, it has been suggested that HD and ALCL represent a continuous disorder, especially in light of the occurrence of ALCL with or subsequent to HD (secondary ALCL) (4, 5, 38). Morphological similarities between HD and Ki-1-positive ALCL include the presence of variable numbers of Reed-Sternberg-like cells in ALCL, as well as sclerotic areas within the tumor which give an appearance similar to that of lymphocyte depleted or nodular sclerosis HD subtypes (5). Immunophenotypically, both tumors are CD30 positive and present variable CD45 (leukocyte common antigen) and epithelial membrane antigen positivity (5). Studies of lymphocyte receptor (Ig and TCR) gene rearrangements in Ki-1-positive ALCL have revealed that most are T-cell derived with only a minority of cases exhibiting B-cell antigens and Ig gene rearrangements (18, 19). A significant proportion have no detectable rearrangements in either the TCR or Ig genes. In addition, in many instances, although the lymphocytic origin of the neoplastic cells in ALCL is generally accepted (5), partial or unproductive TCR and Ig gene rearrangements and uncertain immunophenotypes do not permit a definition of cell lineage (14–19, 34). The incidence of rearrangements of chromosome 5 at band q35 in Ki-1 ALCL is not clearly established. Most t(2;5) translocations or its variants have so far been found in T or null cell phenotype ALCL (14–17, 34–35). In a study which included 12 Ki-1-positive lymphomas, 5 (42%) were found to carry the t(2;5) translocation. Four of these cases were of T-cell origin, and 1 was O-type (39). However, in another study of 31 patients, only 2 were found which had the t(2;5) translocation (24). Because of the difficulty encountered in differential diagnosis of some cases of NHL and HD subtypes and in particular of ALCL and HD (4, 5) and the implications for prognosis and treatment of the disease, it is important to determine the frequency with which NPM gene rearrangements occur in Ki-1-positive ALCL.

Because the presence of several NPM pseudogenes (40) renders the NPM complementary DNA unsuitable for use in Southern blotting analysis, we have opted to use the single copy probe 16.3.F1 located immediately 5' to NPM coding sequences. The 16.3.F1 probe is a SacI/BglII fragment derived from the 16-3/1.2S probe located approximately 7 kilobases centromeric to the 5q35 breakpoint (1). In order to test the ability of our probe to detect NPM gene rearrangements, we performed Southern blotting analysis on Ki-1-positive ALCL lymphoma cell lines carrying rearrangements at 5q35. Two of the cell lines derived from T-cell lymphomas and two are of uncertain lineage (17, 33–35). The t(5;6)(q35;p21) translocation reported in the DEL cell line (35) is demonstrated here to be a three-way t(2;5;6)(p23;q35;p21) translocation at the molecular level. The presence of rearranged bands of almost identical molecular weight in all cell lines including DEL indicates similar rearrangements (the presence of the same translocation) and clustering of breakpoints at the genomic level (Fig. 1). At the RNA level, the presence of the t(2;5) translocation was demonstrated in cell lines (Fig. 2) by an RT-PCR method which allows amplification of the NPM/ALK fusion product specifically by using primers on either side of the fusion mRNA as described previously (24). Because of the presence of several variant translocations, examination of the NPM locus rather than the chromosome 2 locus would provide a better assessment of the frequency of chromosomal rearrangements in Ki-1-positive ALCL. The clustering of breakpoints observed in the cell lines indicate that the 16.3.F1 probe should detect most NPM gene rearrangements, provided that tumor samples carry a sufficient number of cells with the 5q35 rearrangements which could then be detected by Southern blotting.

Using the 16.3.F1 probe, we have analyzed a total of 16 Ki-1 ALCL patient samples (Fig. 3; Table 1), twelve of T-cell phenotype, 1 HD-like O-type ALCL, 1 null phenotype, and 2 B-ALCL (Table 2). We detected TCR gene rearrangement in 3 of the 12 T-ALCL and NPM gene rearrangements in only 1 T-ALCL and 1 B-ALCL (Fig. 3; Table 1). These findings are in contrast to previous observations which suggest that a majority, if not all, of T-ALCL carry the t(2;5) translocation (5, 38) and indicate that only a small fraction of Ki-1-positive ALCL have NPM gene rearrangements. However, most reports are based on patient samples selected on the basis of the

4 F. Bullrich and S. W. Morris, unpublished observation.
NPM GENE REARRANGEMENTS IN KI-1-POSITIVE LYMPHOMAS

Sample 665 (Fig. 3, panels B, Lane 9, and panel C, Lane 4) are similar to those observed in the cell lines (Fig. 1), indicating the presence of the t(2;5) translocation in this patient’s lymphoma. The rearrangement in patient 61 with a B-cell phenotype (Fig. 3) represents a new finding since thus far all neoplasms with the t(2;5) chromosomal translocation were found to be T- or O-ALCL. However, the NPM rearrangement in the B-ALCL appears to be different at the genomic level from characteristic t(2;5) NPM gene involvement with a BamHI rearranged band of higher molecular weight.

An additional novel observation is the detection of an NPM gene rearrangement in two of nine cases of HD (Fig. 3; Table 3). The NPM rearrangement in the two cases of HD is similar to that observed in the B-ALCL. The similarity in these breakpoints indicates a second cluster of rearrangements different from the one observed in the cell lines and in the T-ALCL tumor sample. The detection of NPM rearrangements in HD is even more surprising because it was found in two types of HD (lymphocyte predominance HD and nodular sclerosis.

Fig. 1. NPM gene rearrangements in cell lines with a 5q35 breakpoint detected with the 16.3.F1 probe. DNA (10 µg) was digested with HindIII (A), BamHI (B), and PstI (C) and electrophoresed through 0.7% agarose gels in a ProbeTech 2 electrophoresis apparatus (ONCOR).

Fig. 2. A, ethidium bromide-stained gel of normal NPM and NPM/ALK fusion RT-PCR products. B, NPM/ALK RT-PCR products in cell lines carrying the 5q35 breakpoint detected by Southern blotting and hybridization of the filter with an end-labeled oligomer containing the NPM/ALK junction sequence (1). RNA (2 µg) was used for each reaction. K98 cell line RNA was used as a negative control.

Fig. 3. NPM gene rearrangements in Ki-1-positive lymphomas detected with the 16.3.F1 probe. DNA (5 µg) was digested with HindIII (A), BamHI (B), and PstI (C). Negative controls are K98 cell line (A, Lane 1) and human placenta (A, Lane 8; B, Lane 1; and C, Lane 10). (A) Lanes 2–7, HD; Lanes 9 and 10, B-ALCL. (B) Lanes 2, HD; Lanes 3 and 4; B-ALCL, and Lanes 5–9; T-ALCL. (C) Lanes 1–4; T-ALCL; and Lanes 5–9, HD.
HD) generally believed to be very different from each other (41). Our findings point to the possibility that NPM gene rearrangement may identify subgroups in ALCL and HD, which have in common a similar molecular and other features. This underscores the importance of molecular approaches for the characterization of these rearrangements in all cell lines known to carry the t(2;5) chromosomal translocation. The finding of a rearranged NPM gene in two of nine cases of HD might indicate that a 5q35 breakpoint is more frequently present in HD than in ALCL. Further studies are needed to show whether the HD cases with a rearranged NPM gene represent ALCL which mimic HD or whether they are variant cases of HD which are closely related to ALCL in terms of molecular and other features.

### References


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