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

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Abstract

The (2;5)(p23;q35) translocation which results in the fusion of the NPM (nucleophosmin) gene on chromosome 5q35 with the novel ALK (anaplastic lymphoma kinase) gene on chromosome 2p23 is associated with Ki-1-positive 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 2 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 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 superfamily 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) chromosome 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 promyelocytic leukemias with the t(15;17) 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 B-F1 specific for TCR β chain (purchased from T Cell Sciences) in conjunction with the immunoalkaline phosphatase method (33).

Received 3/23/94; accepted 4/21/94.

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1 This work was supported by National Cancer Institute Grants CA 39860 (to C. M. C.), K08 CA 01702 (to S. W. M.), the Research FellowshipAward 5 F31 CA60352-02 (to F. B.), and the American Lebanese Syrian Associated Charities of St. Jude Children’s Research Hospital.

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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.
Patient DNA Isolation. DNA was extracted from snap-frozen samples by using the DNA extractor (Applied Biosystems; Model 341 A) 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 Karpas 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 15 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 Cγ3 probe (ONCOR), and IgI gene rearrangements were evaluated with a IgH probe described previously (21). The 16.3.F1 probe was purified in low melting temperature agarose after digestion of the 16-3.12S probe (1) with BglII and SacI restriction enzymes (Boehringer Mannheim) in a buffer suitable for this double digest as recommended by the manufacturer.

Southern Blotting Analysis. DNA was isolated from cell lines by conventional methods (37). Cell line (10 μg) and patient gel and cell surface (Ig and TCR) gene rearrangements in Ki-1-positive ALCL were 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 (1). 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 NPM 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...
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Sample 665 (Fig. 3, panel 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.

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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 ProbeTech2 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) Lane 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 pathogenesis and perhaps other features. These results underscore the notion that morphologically and phenotypically similar diseases and for the necessity 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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