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ARTICLE

Genotypic Analysis in Primary Systemic Anaplastic Large cell Lymphoma

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This report presents an experience of polymerase chain reaction (PCR) analysis of T-cell receptor γ- and β gene (TCR γ, TCR β), and immunoglobulin heavy chain (IgH) gene rearrangements in 9 cases of primary systemic anaplastic large cell lymphoma. We showed 2 clonal IgH, 2 TCR γ, 1 TCR β rearrangements. The genotype was B/T-cell in 1, T-cell in 1, B-cell in 1 and null cell-type in 6 cases. We used reverse transcriptase PCR (RT-PCR) to detect t(2;5)(p23;q35) and t(1;2)(q25;p23) translocations. T(2;5) translocation was demonstrated in 2 cases, there was no t(1;2) translocation. In most cases the molecular genetic results were found to be in agreement with immunophenotypic data. (Pathology Oncology Research Vol 9, No 2, 104–106)

Keywords: anaplastic large cell lymphoma, TCR γ- and β gene rearrangement, IgH gene rearrangement, t(2;5)(p23;q35) and t(1;2)(q25;p23) translocation

Introduction

Anaplastic large cell lymphoma (ALCL) originaly was described in 1985 by Stein.[9] Large tumor cells strongly express cytokine receptor CD30 and show characteristic growth pattern (paracortical involvement of lymph node and intrasinusoidal dissemination). Recently three main subgroups have been identified according to molecular genetic, immunohistological and clinical behaviour: primary systemic anaplastic lymphoma kinase (ALK) positive, primary systemic ALK negative and primary cutaneous ALCL.[1,7,8] ALK positive ALCL occurs in young male patients with advanced disease, but has favourable prognosis. ALK negative ALCL affects older patients in both gender and the prognosis is more unfavourable. In larger studies about 50 % of ALCL cases are ALK positive. WHO system classifies anaplastic large cell lymphoma into T/0-cell type lymphomas,[2] whereas B-cell type ALCLs remain variants of diffuse large cell lymphoma. Several studies reported polymerase chain reaction to determine the genotype of the lymphoid neoplasms. We evaluated a PCR analysis on immunoglobulin heavy chain (IgH) gene, T-cell receptor (TCR) β and TCR γ gene rearrangement and RT-PCR to demonstrate t(2;5) and t(1;2) translocations – typical in ALK positive ALCL.[3-5]

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Materials and Methods

We have examined 9 cases of primary anaplastic large cell lymphoma. Eight of the samples were frozen lymph node and one bone marrow aspiration (lymphoid cells after Ficoll separation). The age of the patients ranged from 3 to 66 years. The diagnosis of ALCL was based on immunomorphologic criteria.[1,8] The standard immunphenotype analysis panel was used to prove the existence of ALCL (CD45, CD3, CD45R0, CD15, CD20, CD30, ALK1).[1,7,8] Polymerase chain reaction (PCR) were performed to show TCR β and γ gene rearrangements, and IgH gene rearrangement. We have also performed reverse transcriptase (RT)-PCR to demonstrate the characteristic translocations in ALCL: t(2;5)(p23;q35) and t(1;2)(q25;p23). We isolated DNA and RNA with phenol-chloroform and RNeasy Mini Kit (Quiagen) technics.

The following primers were used[3-4]

IgH

FR1c: 5'-AGG TGC AGC TG(G/C) (A/T)G(G/C) AGT C(G/A/T)G G-3'
FR2a: 5'-TGG (A/G)TC CG(C/A) CAG (G/C)C(T/C) (T/C)CN GG-3'
FR3a: 5'-ACA CGG C(C/T)(G/C) TGT ATT ACT GT-3'
LJH: 5'-TGA GGA GAC GGT GAC C-3'
VLJH: 5'-GTG ACC AGG GTN CCT TGG CCC CAG-3'

*TCR*β

$D_{\beta1}$: 5'-CAA AGC TGT AAC ATT GTG GGG AC-3'
$J_{\beta2}$: 5'-AGC AC(C/T/G) GTG AGC C(T/G)G GTG CC-3'
V_{β}: 5'-TGT A(C/T)C TCT GTC GTG CCA GCA G-3'

*TCR*γ

VγI cons: 5'-CTG GTA CCT ACA CCA GGA GGG GAA-3'
Vγ 9.2: 5'-GAA AGG AAT CTG GCA TTC CG-3'
Vγ 10: 5'-GCA GCA TGG GTA AGA CAA GC-3'
Vγ 11: 5'-GAT TGC TCA GGT GGG AAG AC-3'
Jγ 2S2: 5'-CCT GTG ACA ACA AGT GTT GT-3'
JP: 5'-TTG TTC CGG GAC CAA ATA CC-3'
JP1/2: 5'-CCA GGT GAA GTT ACT ATG AG-3'

We have created two mixes from the primers:

Pmix1: VγI cons, Jγ2S2, JP and JP1/2,
Pmix2: Vγ 9.2, Vγ 10, Vγ 11, Jγ2S2, JP and JP1/2.

NPM/ALK

5'NPM: 5'-TCC CTT GGG GGC TTT GAA ATA ACA CC-3'
ALK1: 5'-GCC AGC AAA GCA GTA GTT GGG GTT G-3'

For the second PCR: the same 5'NPM primer plus:

ALK2: 5'-GTC GAG GTG CGG AGC TTG CTC AGC-3'

TPM3/ALK

cTPM1: 5'-CGA GAA GTT GAG GGA GAA AGG 3'
ALK1: 5'-GCC AGC AAA GCA GTA GTT GGG GTT G-3'
cTPM3: 5'-CTG GCA GAG TCC CGT TGC CGA G-3'
ALK2: 5'-GTC GAG GTG CGG AGC TTG CTC AGC-3'

The PCR conditions were the followings:

IgH: 35 cycles with 10 min hot start (93°C), denaturation 93°C, annealing at 50°C for FR2a/J_H, at 54°C for FR1c/J_H and FR3a/J_H, elongation at 72°C. Every steps 1 minute, final elongation 10 min at 72°C.

TCR β: 30 cycles, denaturation at 93°C 1 min, $D_{\beta1}$/ $J_{\beta2}$: annealing at 59°C, Vβ/$J_{\beta2}$: annealing at 53°C 1-1 minute, elongation at 73°C 1 minute, and a final 10 min elongation. The expected length of the PCR products are between 55-100 bp.

TCR γ*:* In both cases denaturation at 93°C, annealing at 55°C, elongation at 72°C. We have used semi-nested PCR, the first PCR product provided the template for the second PCR (1% of the first amplification product) another 35 cycles, the conditions were as in the first PCR.

t(2;5)(p23;q35) NPM/ALK: We used semi-nested PCR, the first PCR product provided the template for the second PCR (1-2% of the first amplification product) 5 min hot start at 94°C, then the program: 30 sec at 94°C, 3 min at 68°C for 35 cycles, then 8 min final elongation at 72°C. The conditions of the second PCR were similar to those of the first one, the denaturation was shorter (20 sec at 94°C), the annealing and the elongation at 68°C lasted for 5 sec.

t(1;2)(q25;p23) TPM3/ALK: After 5 minutes DNA denaturation and AmpliTaq Gold DNA polimerase activation (at 94°C) followed 30 cycles of 45 sec denaturation at 94°C, 45 sec annealing at 62°C and 45 sec elongation at 72°C, then final 8 min elongation at 72°C. During the second PCR we used 1 product of the first PCR as template, the conditions were the same. The length of the PCR products were 300 bp.

Results

We have analysed 9 cases of primary systemic anaplastic large cell lymphoma with several PCR reactions. Eight of them had null-cell phenotype, and one was T-cell type. The molecular genetic results are shown in ***Table 1.*** In six of these cases the final genotype was null-cell, 1 was T-, 1 B- and 1 case was B/T hybrid genotype. The sample which had B-cell genotype was probably Hodgkin's disease, rich in tumor cells. We could demonstrate 2 cases with t(2;5) translocation (***Figure 1***), but we did not find any case with t(1;2) translocation. The average age of 7 male and 2 female patients was 36.7 years (3-68). The age of 7 ALK negative cases was 44.3 years, while the age of the two ALK positive cases was 3 and 27 years. Three of seven ALK negative patients died of disease progression, whereas the two ALK positive patients are alive free of any symptoms of the disease.

Discussion

Anaplastic large cell lymphoma is a heterogenous group among aggressive lymphomas. It accounts for 2-5 % of all lymphomas, and about 20 % of peripheral T-cell lymphomas. Proving the genotype by T-cell receptor gene rearrangement analysis with PCR is one of the most important methods. Three years ago we reported a PCR analysis on a series of NHL cases.[6] In that study we observed clonal rearrangement of TCR γ gene in 5/8 of T-

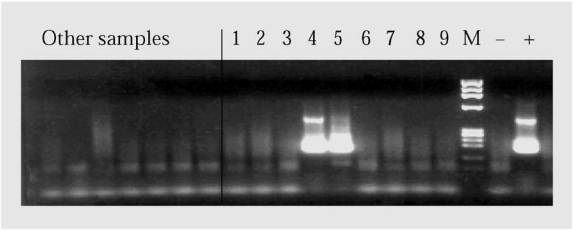

Figure 1. *RT-PCR of NPM-ALK translocation. M: molecular weight markers, –: negative control, +: positive control (from SU-DHL1 cell line), 1-9: samples from primary systemic ALCL cases, 4-5: t(2;5) positive cases, 1-3, 6-9: t(2;5) negative cases*

Table 1. **Molecular genetic results**

Number	*Age (year)*	*Gender*	*Immuno-phenotype*	*TCR γ*	*TCR β*	*IgH*	*t(2;5)*	*t(1;2)*	*Genotype*
1.	61	M	T-ALCL	C	C	C	N	N	B/T genotype
2.	68	M	0-ALCL	P	P	P	N	N	Null genotype
3.	19	M	0-ALCL	P	P	C	N	N	B genotype
4.	38	F	0-ALCL	P	P	P	N	N	Null genotype
5.	3	M	0-ALCL	P	P	P	Pos	N	Null genotype t(2;5) positive
6.	27	M	0-ALCL	P	P	P	Pos	N	Null genotype t(2;5) positive
7.	36	F	0-ALCL	P	P	P	N	N	Null genotype
8.	66	M	0-ALCL	C	P	P	N	N	T genotype
9.	22	M	0-ALCL	P	P	P	N	N	Null genotype

F: female, M: male, C: clonal, P: polyclonal, N: negative, Pos: positive.

cell ALCL and 1/12 of null-cell ALCL cases. There was no positive case of rearranged TCR γ gene among 13 B-cell lymphoproliferative samples. Unfortunately among the 180 ALCL cases of Hungarian ALCL Registry only a few frozen lymph node samples are available for molecular genetic examinations. We have examined 9 immunophenotypically T/0-cell primary systemic ALCL cases from this registry with PCR method to establish genotype. We analysed IgH- TCR β- and TCR γ gene rearrangements. In a French study[10] 144/183 (79 %) of B-cell NHL, 10/90 (11 %) of T-cell NHL cases were positive with IgH gene rearrangement. In the TCR γ gene rearrangement analysis 18/183 (10 %) of B-cell NHL and 74/90 (82 %) of T-cell lymphoproliferative cases were positive. We have used the same method completed with TCR β gene rearrangement analysis. The immunohistological and molecular genetic results showed similar data in all but three cases. In 1-1 B/T, T and B genotype case the original phenotype were T-, null- and null-cell, respectively. In the first case (primary bone marrow ALCL with B/T hybrid genotype) two malignant clones may have been coamplified during repeated PCR analysis or the tumor infiltrating oligoclonal T lymphocytes caused double genotype. The case which had B genotype was probably Hodgkin's disease. We could demonstrate two cases of t(2;5) translocation among 9 cases. Even if we exclude two cases of B genotype it is only 30 % positivity of cases (2/7), which is less than the expected 50-60 %, but the so small number of patients can explain the difference of our cases from the ones in the literature.[1,8]

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