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THE RELATIONSHIP BETWEEN NON-HODGKIN'S LYMPHOMA AND THE GENE REARRANGEMENT

Guo Sutang 郭素堂 Liu Yongchang 刘永昌 Sun Junning 孙俊宁

Department of Immunology, Shanxi Cancer Institute, Taiyuan, 030013

Objective: To investigate the pattern of clonal rearrangement of immunoglobulin heavy chain gene (IGH) and T-cell receptor γ gene (TCRγ) of Non-Hodgkin's lymphoma (NHL). Methods: Bone marrow smears of 211 patients of NHL were detected by PCR, the rearranged IGH and TCRγ gene was amplified using oligonucleotide primers. Results: The clonal rearrangement of IGH gene was detectable in 51.2% (108/211); the clonal rearrangement of TCRγ gene was detectable in 21.3% (45/211); both IGH and TCRγ was detectable in 5.7% (12/211); no clonal rearrangement in 21.8% (46/211). And compared clonal gene rearrangement with pathological type and primary site of tumor. Ten patients of NHL were investigated serially. 5/10 patients still had clonal gene rearrangement at clinical complete remission. Conclusion: It demonstrated that this assay may be useful in monitoring the minimal residual disease (MRD) and in evaluating effectiveness of therapy.

Key words: NHL, Clonal gene rearrangement, Minimal residual disease, IGH TCRγ.

Although modern chemotherapy has been used successfully for treating patients with Non-Hodgkin's lymphoma (NHL), recurrence is still seen in many of cases. It has been shown that the malignant cells at recurrence often appear cytological and phenotypic characteristics similar to those seen at the primary time. Residual tumor cells have probably survived after the treatment, and it may be the reason for subsequent disease recurrence. Conventional detection techniques such as immunophenotyping, cytogenetics and morphological studies have a sensitivity of detecting residual tumor cells at the level of only 1%–5%. This detection level is inadequate in defining a "true" remission after treatment. Obviously, a highly sensitive technique in detecting the minimal residual disease (MRD) is useful in defining better the patient's remission status and evaluating the effectiveness of therapy.

The clonal rearrangement of immunoglobulin heavy chain (IGH) gene and T-cell receptor (TCR) gene can be used as tumor-specific markers for PCR detection.[1] The gene rearrangement in a polyclonal lymphoid population are heterogeneous in their nucleotide content and length, demonstrating a smear or vague bands after the PCR products are electrophored on polyacrylamide gel and stained, whereas they are homogenous in a clonal population, demonstrating a discrete or clear band. Therefor this method is ideal for the identification of minimal diseases. So far, the report about NHL in large case number have not be seen. We investigated the scientific research archival samples of NHL of our hospital in recent years.

MATERIALS AND METHODS

Cases and Normal Control

From 1990 to 1996, 211 patients with the diagnosis of Non-Hodgkin lymphoma admitted to our hospital were included in this study. 3 patients of

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reaction lymphocytosis were used as normal control. For each case, 2 to 3 slides of archived bone marrow smears were used. DNA was obtained by proteinase K digestion and salting out.

Clonal PCR Detection

The rearranged IGH and TCRγ gene was amplified using oligonucleotide primers, respectively. The DNA sequences of the primers were designed as described.[2] 1 unit Taq DNA polymerase (Promega), 1.5 mMM gcl and 50 pmol of each primer pair in a total reaction volume of 25 μl were used. 32 cycles of 60s at 93°C, 60s at 55°C, 120s at 72°C were performed in a PTC-100 DNA Thermal cycler (MJ. research Inc.). PCR products were electrophored on 6% polyacrylamide gels and stained with silver. There was discrete band at 80 to 120bps (IGH) or around 400bps (TCRγ) in cases of malignancies with clonal rearrangement.

RESULTS

Clonal Gene Rearrangement

Archived bone marrow smears of 211 patients of NHL were monitored. By PCR analysis, the results showed that 108 (51.2%) patients had clonal IGH gene rearrangement, 45 (21.3%) patients had clonal TCRγ gene rearrangement, 12 (5.7%) patients showed both IGH and TCRγ gene rearrangement (both positive), 46 (21.8% patients had no clonal gene rearrangement. Total positive rate is 78.2%. 3 patients of reaction lymphocytosis all had no any clonal rearrangement.

The Relationship of Pathological Types, Primary Sites and the Clonal Gene Rearrangement

Partial patients with well-defined pathological types and primary sites recorded in research archives are summarized in Table 1.

For pathological types, the clonal rearrangement of IGH and TCRγ gene are 75% and 12.5% in diffuse small cell, 59.25% and 16.6% in diffuse mixed, 60.4% and 37.5% in immunoblastic. The rearranged TCRγ gene in immunoblastic was compared with that in diffuse mixed ($P<0.05$). For primary sites, the clonal rearrangement of IGH and TCRγ gene as follow: 42.8% and 50% in Nasal and Waldeyer's ring; 58.76% and 14.7% in lymph node and gastrointestinal; 62.5% and 25% in others. Clonal rearrangement of TCRγ gene Nasal and Waldeyer's was compared with lymphnode and gastrointestinal ($P<0.001$).

The Relationship of Patient's Status and the Clonal Gene Rearrangement

10 patients were monitored serially. The results were summarized in Table 2.

Table 1. The relationship between pathological types, primary sites and the gene rearrangement

	Cases	IGH (%)	TCRγ (%)	IGH+TCR (%)	Negative (%)
Pathological types					
Diffuse small cell	16	12 (75)	2 (12.5)	1 (6.25)	1 (6.25)
Diffuse mixed	42	25 (59.25)	7(16.6)	2 (4.76)	8 (19.04)
Immunoblastic	48	29 (60.4)	18 (37.5)	1 (2.08)	0
Primary site					
Nasal and Waldeyer's	14	6 (42.8)	7 (50)	1 (7.1)	0
Lymphnode and gastrointestinal	97	57 (58.76)	14 (14.7)	9 (9.2)	17 (17.25)
Others	16	10 (62.5)	4 (25)	0	2 (12.5)

At primary diagnosis, 8 patients had clonal IGH gene rearrangement, 1 patient had TCRγ gene rearrangement, 1 patient had both positive. After morphological complete remission, by TCRγ analysis, patients 3, 5, 6, 7, 9 became negative, patients 1, 2, 4, 8 remained original rearrangement, patient 10 converted from both positive to TCRγ gene rearrangement. Table 2 shows only patient 5 maintain PCR negative for two and half years, the remaining 9 patients had recurred. Duration of CR varied from half

years to six years. At recurrence, 2 cases remained original rearrangement, 3 cases turned from IGH to both positive, 3 cases changed from IGH to TCRγ, 1 case converted from both positive to TCRγ.

Table 2. Clinical characteristics of patients and the gene rearrangement

Patients (No.)	Diagnosis		CR		Relapse		Duration of CR
	IGH	TCRγ	IGH	TCRγ	IGH	TCRγ	(year)
1. Liu××	+	-	+	-	+	+	1
2. Zhang××	+	-	+	-	-	+	1
3. Tian××	+	-	-	-	+	-	1.5
4. Wang××	+	-	+	-	+	+	1
5. Yang××	+	-	-	-			>2.5
6. Han××	+	-	-	-	-	+	1
7. Zhao××	+	-	-	-	-	+	1
8. Yao××	-	+	-	+	-	+	0.5
9. Wang××	+	-	-	-	+	+	1
10. Chui××	+	+	-	+	-	+	6

DISCUSSION

The pattern of clonal IGH and TCRγ gene rearrangement in 211 cases of lymphoma was investigated. It has been recognized that the clonal T-cell receptor gene rearrangement is present in both T and B-cell malignancies.[6] In this study, 5.7% patients were found to have clonal rearrangement of both IGH and TCRγ gene, 51.2% patients were found to have clonal IGH gene rearrangement, 21.3% patients were found to have clonal TCRγ gene rearrangement. That is, lymphoma of B-lineage versus T-lineage is 3:1, the figure is similar to the report in China, but different from the figure of 4:1 reported in Hongkong.[3] It is reported that accurate rate of lymphoma diagnosis is 26% morphologically. In this study, 77 patients (36%) were recorded in archives positive morphologically, but the clonal gene rearrangement was detectable in 78.2% patients. It is apparent that PCR analysis of gene rearrangement is valuable in early diagnosis and identifying lymph cell origin of NHL.

There are not difference in clonal IGH gene rearrangement in pathological types or in primary sites (Table 1). But we found the more malignant the types are the higher the percentage of clonal TCRγ gene rearrangement. Diffuse small cell 12.5%, diffuse mixed 16.6%, immunoblastic 37.5%. Similarly, the rearranged TCRγ gene in gastrointestinal and lymph node is 14.7%, in Nasal and Waldeyer's ring is 50%. There is significant difference. The finding is similar to the report (Liang R). It demonstrated that the clonal rearrangement of TCRγ gene have relation with the malignances of diseases.

10 patients were analyzed serially in PCR (Table 2). During complete remission, 5/10 patients remained PCR positive. This finding show that when patients achieved CR clinically, there are still MRD in their bone marrow.[4] As is well known, almost all patients with lymphoma who achieved complete remission, ultimately have recurrence. Our finding of minimal residual malignant cells in 5/10 patients during CR explains this observation.

9/10 patients had recurred. At recurrence, gene rearrangement turned from IGH to both positive in patients 1, 4, 9, gene rearrangement turned from original IGH to TCRγ in patients 2, 6, 7. Gene rearrangement remained original in patients 3, 8. Gene rearrangement changed from both positive to TCRγ in patient 10. Patients 1, 2, 4, 6, 7, 9 had a new clone appeared and had a remission period of less one year. Patients 3, 10 had no new clone appeared and had a relative longer remission period except for patient 8. It seems to appear a new clone which is related to the deterioration of disease.[5] This remains further to be confirmed by studies on a larger number of patients. PCR analysis of serial samples proved validity for the estimation of MRD, the evaluation of the efficacy of treatment, and assessment of the risk of an impending

recurrence.

REFERENCES

1. Ritsuo Nishiuchi, Tadashi Yoshino, Norihiro Teramoto. Clonal analysis by PCR of B-cell lymphoma with late relapse. Cancer 1996; 77(4):757.
2. 张巧花, 刘永昌, 郭素堂. 几种恶性血液病微小残留病的检测. 白血病 1997; 6(2):85.
3. Liang R, Todd D, Ho FCS. Aggressive Non Hodgkin's lymphoma: T-cell versus B-cell. Hematological Oncology 1996; 14:1.
4. Ronald A, Ghossein, Juan Rosai. Polymerase chain reaction in the detection of micromatastases and circulation tumor cell. Cancer 1996; 78(1):10.
5. M Kneba, I Bolz, B Linke. Analysis of rearrangement T-cell receptor β-chain gene by polymerase chain reaction DNA sequencing and automated high resolution PCR fragment analysis. Blood 1995; 86(10):3930.
6. Maria D Lozano, An-ne Tierens, Timothy C Greiner. Clonality analysis of B-lymphoid proliferations using the polymerase chain reaction. Cancer 1996; 77(7):1349.