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Feasibility of T-Cell Receptor γ (TCRγ) Gene Rearrangement on Formalin-Fixed, Paraffin-Embedded Tissues by PCR Assays

Y C Tai, S C Peh

ABSTRACT

Introduction: T- and B-lymphocytes are involved in recognition of foreign antigen by the specificity of their surface T-cell receptor and immunoglobulin, generated by gene rearrangement. Each T- and B-lymphocyte carries unique rearranged TCR or immunoglobulin gene, which has been applied to detect clonal from non-clonal T- and B-cell proliferation.

Methods: Paraffin-embedded biopsy tissues of 85 T-, 24 B-cell non-Hodgkin's lymphomas (NHL) of various subtypes, and seven reactive lymphoid hyperplasia were retrieved from the archives for determining the feasibility of TCRγ gene rearrangement analysis by PCR assays in our laboratory. DNA was extracted by Proteinase K digestion. The analyses were performed by five PCR assays, and analysed on polyacrylamide gel.

Results: Clonal TCRγ gene rearrangement was demonstrated in 69/85 (81.2%) of the cases. Selective rearrangement of specific Vγ segment was observed, especially in peripheral T-cell lymphoma-unspecified and nasal NK/T-cell lymphoma. Clonal TCRγ rearranged band was also demonstrated in 4/24 (16.7%) and 2/7 (28.6%) of B-NHL and reactive lymphoid tissues respectively.

Conclusion: PCR assays were able to demonstrate clonal TCRγ gene rearrangement in a high proportion of T-NHL. However, the PCR results should be interpreted carefully. A neoplasm should only be considered as T-cell type if it does not express any B-cell marker because TCRγ is not lineage specific as shown by the presence of clonal TCRγ gene rearrangement in B-NHL. Hence, the results for TCR gene rearrangement should always be interpreted in conjunction with histology and immunophenotyping.

Keywords: TCRγ gene rearrangement, paraffin-embedded tissues, NHL

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**Department of Pathology
Faculty of Medicine
University of Malaya
50603 Kuala Lumpur
Malaysia**

Y C Tai, BSc (Hons)
Biotech
Researcher

S C Peh, MBBS,
FRCPath, PhD
Project Leader

Correspondence to:
Prof S C Peh
Tel: (603) 7950 2375
Fax: (603) 7955 6845
Email: pehsc@ummc.edu.my

INTRODUCTION

B- and T-lymphocytes participate in the recognition and elimination of foreign pathogens and macromolecules. The specificity and diversity of this function are mediated by immunoglobulin and T-cell receptor (TCR) gene rearrangement of B- and T-lymphocytes respectively. The rearrangements occur in a highly ordered fashion in the early stage of B- and T-lymphocytes development. It involves the juxtaposition of different variable (V), diversity (D), and joining (J) gene segments of the immunoglobulin or TCR gene by looping out and excising the intervening sequences. The resulting unique sequence is the antigen-binding site for immunoglobulin and TCR[1]. TCR is a glycoprotein heterodimers linked by disulfide bonds. There are four TCR chains that have been identified: alpha (α), beta (β), delta (δ), and gamma (γ) chains[2-4]. The majority of T-lymphocytes in normal population carry αβ chain (95%), and the remaining (5-10%) carry γδ chain. Rearrangements of TCRβ, γ, and δ genes precede that of TCRα gene. Even though most studies indicate that rearrangement of TCRγ genes occurs first, the exact order of the TCRβ, γ, and δ gene rearrangement is yet to be established[1].

Lineage of T-cell neoplasms could not be reliably deduced by immunophenotyping because T-cell antigens are often lost, especially in post-thymic T-cell neoplasms[2]. It is reported that about 76% of peripheral T-cell lymphoma (PTCL) lost one or more pan-T cell surface antigen[2,4]. Analyses on the TCR genes have been developed to detect clonally derived tumour cells because each neoplasm carries a unique rearranged TCR gene[5-7]. The lineage of some T-cell neoplasms such as Lennert's[8] and angioimmunoblastic lymphoproliferative disease-type (AILD)[9] lymphomas have been confirmed by such analyses. Although, the usefulness of this technique has been dampened by the unavailability of high molecular weight DNA from small biopsies and fixed tissue for Southern blot analysis[3], advancement of methods for isolation of DNA from paraffin-embedded tissues and the development of polymerase chain reaction (PCR) has enabled the analysis of such samples[3,10-12].

TCR gene rearrangement is more difficult to demonstrate when compared to immunoglobulin heavy

chain (IgH) gene rearrangement. Amplification of IgH gene rearrangement is targeted at two framework regions, which are highly conserved[13-15]. In contrast, the genomic organisation of TCR genes is highly complex[1,4]. PCR analysis of TCR gene rearrangement has been focused on TCRβ[6,16,17], and TCRγ[10-12] due to the complex genomic organisation of TCRα gene, while TCRδ gene is deleted in most of the mature T-lymphocytes[1,11,12]. TCRγ gene rearrangement analysis is favoured largely due to its simpler genomic structure. Though TCRγ chain is only expressed in about 10% of T-lymphocytes, the TCRγ gene is rearranged in all normal and neoplastic T-lymphocytes[1,3,18]. In a study of T-cell non-Hodgkin's lymphoma (NHL) by Diss et al[11], clonal TCR gene rearrangements were detected in 44% (24/55) and 78% (43/55) of T-NHL using PCR analysis of TCRβ and TCRγ genes respectively. Although Krafft et al[12] reported that analysis with TCRβ (121/169, 72%) and TCRγ (116/169, 69%) genes yielded similar results, in the same study, TCRγ gene analysis was able to detect clonal TCR gene rearrangement in 32 cases that were negative by TCRβ gene analysis. Hence, TCRγ gene analysis is able to enhance the sensitivity of detection.

The TCRγ chain gene contains 15 Vγ, 2 Cγ and 5 Jγ gene segments[4]. The Vγ gene segments are named according to their order in the genomic organisation. Five of the Vγ gene segments are pseudogenes, and the remaining 10 Vγ gene segments that are rearranged are grouped into four families according to their sequence homologies[19]. Family I of Vγ gene consists of seven Vγ gene segments that are highly homologous, and therefore can be amplified using a consensus primer. However, Families II, III, and IV, each consisting of a single variable gene segment, do not share similar homology[20]. The five Jγ gene segments are divided into two groups comprising of Jγ1.1, Jγ1.2, Jγ1.3 located upstream of Cγ1, and Jγ2.1, Jγ2.3 located upstream of Cγ2. PCR analysis of the TCRγ gene rearrangement is targeted at one of the four families of Vγ gene segments and one of the five Jγ gene segments.

This study aims to evaluate the feasibility of applying PCR assays in detecting clonal TCRγ gene rearrangement in formalin-fixed, paraffin-embedded tissue from a series of T-NHL in our routine diagnosis laboratory. A number of B-cell NHL and cases of reactive lymphoid hyperplasia are added to determine the specificity of the technique.

METHODS

Patients' Materials

A series of 85 T-NHL was retrieved from the archives. These cases were evaluated and classified by a pathologist (PSC) according to the criteria in the WHO classification of lymphoid neoplasm[21]. Formalin-fixed, paraffin-embedded tissue sections were used for the studies described below. Twenty-four (24) cases of B-NHL and seven cases of reactive lymphoid hyperplasia were included to determine the specificity of the assay.

Immunohistochemical Staining

Immunohistochemical staining was used to confirm and classify the lymphoma cases. Immunohistochemical staining was performed on serial sections of 4μm with a panel of antibodies from DAKO (Denmark), unless otherwise specified. Antibodies raised against CD3 (polyclonal) and CD20 (L26) were routinely used to determine T- and B-cell lineage. Additional antibodies against CD2 (NCL-CD2-271, Novocastra, United Kingdom), CD4 (NCL-CD4-1F6, Novocastra, United Kingdom), CD5 (NCL-CD5, Novocastra, United Kingdom), CD8 (C8/144B), CD15 (Leu-M1, Becton-Dickinson, USA), CD21 (1F8), CD23 (NCL-CD23-1B12, Novocastra, United Kingdom), CD30 (BerH2), CD43 (MT1, a gift from S. Poppema), CD56 (NCL-CD56-1B6, Novocastra, United Kingdom), CD79α (a gift from D. Mason), bcl-2 (clone 124), terminal deoxynucleotidyl transferase (NCL-TdT-339, Novocastra, United Kingdom), anaplastic lymphoma kinase protein (ALK1) were applied in aid of sub-classification.

Antigens were retrieved by the microwave heat-inducing method for all antibodies. A three-step immunoenzymatic staining, with peroxidase-labelled Avidin-Biotin Complex (ABC) system (DAKO, Denmark) was used to localise the antigens, except for CD5 and CD56, where peroxidase-labelled polymer system (EnVision$^+$, DAKO, Denmark) was employed. The colour was developed using liquid DAB$^+$ substrate-chromogen system (DAKO, Denmark).

DNA Extraction

DNA extraction was based on published protocols for formalin-fixed, paraffin-embedded tissues with minor modifications[22,23]. Cell lysis was carried out on one 5μm-thick section by incubating the samples in 100-200μl of digestion buffer containing 200μg/ml Proteinase K (Roche Molecular Biochemicals, Germany) in 1X PCR buffer (GIBCO BRL, USA) at 55ºC for overnight. Proteinase K was inactivated by heating at 95ºC for 10 minutes. The samples were then centrifuged to pellet cell debris and the supernatant was used directly for PCR assays.

Primers and PCR Conditions

The assays were carried out according to the method described by Diss et al[11]. Five assays were designed to cover all functional Vγ and Jγ gene segments as shown in Table I. The conditions for each mixture were optimised empirically. Each 25-μL reaction mixture contained 0.4 mmol/L dNTPs (MBI Fermentas, Lithuania), 0.5 mmol/L of each primers (synthesised by Invitrogen-GIBCO BRL, USA), 1.0U *HotStarTaq*

Table I: PCR assays for demonstration of clonal TCRγ gene rearrangements in T-NHL.

Assay	Vγ Gene Family	Vγ Gene Segment	Jγ Gene Segment	Annealing Temperature	Product Size
I	VγI,VγIII/IV	Vγ(1-8), Vγ(10/11)	Jγ1.1/2.1	63°C	70bp-95bp
II	VγI,VγIII/IV	Vγ(1-8), Vγ(10/11)	Jγ1.2/2.2	55°C	80bp-110bp
III	VγI,VγIII/IV	Vγ(1-8), Vγ(10/11)	Jγ1.3	55°C	70bp-95bp
IV	VγII	Vγ9	Jγ1.1/2.1	55°C	150bp-180bp
V	VγII	Vγ9	Jγ1.2/2.2	55°C	160bp-190bp

DNA polymerase (QIAGEN, Germany), and 3.0 mmol/L $MgCl_2$. The reaction was cycled at 15 minutes at 95ºC to activate the DNA polymerase, followed by 40 cycles of 95ºC for one minute, 63ºC or 55ºC for one minute, and extension at 72ºC for one minute.

PCR amplification products were run on 10% polyacrylamide gel (acrylamide:bis-acrylamide ratio, 29:1, BIO-RAD, USA) with 110V for two hours, using a vertical gel system (Owl Scientific, USA). The gel was then stained with 5µg/ml ethidium bromide (GIBCO-BRL, USA) before viewing on UV illuminator.

Direct Sequencing of PCR Product

Two representative samples from each assay that yielded clonal band were subjected to direct sequencing to confirm the specificity of the assays. The PCR products were separated on 3% low-melting point agarose gel (HISPANLAB, Italy), and the specific band of interest was excised from the gel and purified using Agarose Gel DNA Extraction Kit (Roche Molecular Biochemicals, Germany) according to the manufacturer's instruction. The sequencing was carried out by a service provider, Research Biolabs Sdn. Bhd. The sequences were compared to the deposited sequences in the PubMed Centre by using BLAST programme.

RESULTS

Immunophenotype Expression

All the cases classified as T-NHL expressed at least one of the T-cell markers, i.e. CD3, with or without additional expression of CD2, CD5 or CD43 (MT1). Ten cases (10/85, 11.8%) expressed TdT and were classified as precursor T-lymphoblastic lymphoma (Table II). Seventeen (17/85, 20.0%) of the peripheral T-NHL expressed CD56, a marker for natural killer (NK)-cell and its derivatives. These cases were sub-grouped into nasal (13) and nasal-type (4) NK/T-cell phenotypic lymphoma according to the primary site of presentation. The nasal-type NK/T-cell lymphomas were from the gastrointestinal tract (3) and testis (1). The remaining 58 (58/85, 68.2%) cases of peripheral T-NHL were subtyped into 26 peripheral T-cell lymphoma (PTCL)-unspecified, 19 CD30-positive anaplastic large cell lymphoma (ALCL) (11 T-cell and eight null-cell), six T-angioimmunoblastic lymphoproliferative disease (T-AILD), four primary cutaneous T-cell lymphoma, and three immunoblastic T-cell lymphoma. Of the 19 ALCL, 72.7% (8/11) and 100% (8/8) of the T- and null-cell ALCL expressed ALK1 protein respectively.

TCRγ Gene Rearrangement Pattern

When one or two distinct bands were detected after PAGE from the amplification product of any one of the five assays, these cases were regarded as demonstrating clonal TCRγ gene rearrangement. PCR analysis by combination of five assays demonstrated clonal TCRγ gene rearrangement in 81.2% (69/85) of the T-NHL in this series (Table II). The frequency differs in different subtypes, i.e. in 100% of T-lymphoblastic lymphoma (10/10), null-cell ALCL (8/8), primary cutaneous T-lymphoma (4/4), and T-immunoblastic lymphoma (3/3); 90.9% (10/11) of T-cell ALCL; 84.6% (22/26) of PTCL-unspecified; and

Table II. The pattern of TCRγ gene rearrangement of T-NHL subtypes by PCR analyses.

	Total No. of Cases	Assay I (%)	Assay II (%)	Assay III (%)	Assay IV (%)	Assay IV (%)	Total (%)
T-NHL							
Precursor T-lymphoblastic lymphoma	10	8 (80.0)	4 (40.0)	4 (40.0)	5 (50.0)	0 (0.0)	10 (100.0)
PTCL-unspecified	26	17 (65.4)	4 (15.4)	9 (34.6)	6 (23.1)	0 (0.0)	22 (84.6)
ALCL	19	14 (73.7)	0 (0.0)	14 (73.7)	5 (26.3)	0 (0.0)	18 (94.7)
T-cell	11	8	0	7	3	0	10
Null-cell	8	6	0	7	2	0	8
T-AILD	6	3 (50.0)	2 (33.3)	3 (50.0)	0 (0.0)	0 (0.0)	5 (83.3)
Primary cutaneous T-cell lymphoma	4	2 (50.0)	0 (0.0)	1 (25.0)	3 (75.0)	0 (0.0)	4 (100.0)
T-immunoblastic lymphoma	3	1 (33.3)	2 (33.3)	2 (66.7)	2 (66.7)	0 (0.0)	3 (100.0)
T/NK cell lymphoma	17	4 (23.5)	0 (0.0)	0 (0.0)	4 (23.5)	0 (0.0)	7 (41.2)
Nasal	13	0	0	0	3	0	3
Nasal-type	4	4	0	0	1	0	4
Total	85	49 (57.6)	12 (14.1)	33 (38.8)	25 (29.4)	0 (0.0)	69 (81.2)
B-NHL	24	0 (0.0)	0 (0.0)	0 (0.0)	4 (16.7)	0 (0.0)	4 (16.7)
Reactive lymphoid hyperplasia	7	0 (0.0)	2 (28.6)	0 (0.0)	2 (28.6)	0 (0.0)	2 (28.6)

83.3% (5/6) of T-AILD. Interestingly, clonal TCR-γ gene rearrangement was demonstrated in 23.1% (3/13) and 100.0% (4/4) of the nasal and nasal-type NK/T-cell phenotypic lymphoma respectively.

Assay I demonstrated clonal TCRγ gene rearrangement in the majority (57.6%, 49/85) of the T-NHL, followed by assay III (38.8%, 33/85), assay IV (29.4%, 25/85), and assay II (14.1%, 12/85). Assay V was unable to detect clonal TCRγ gene rearrangement in any of the cases in this series. Of the 49 cases that showed clonal TCRγ gene rearrangement by assay I, 36.7% (18/49) of these cases were clonal for assay I only, while the remainder 63.3% (31/49) of these cases were clonal together with either one of the other assays or a combination of other assays (two together with assay II only 15 together with assay III only, seven together with assay IV only, four together with assays II and III, two together with assays III and IV, and one together with assays II, III and IV). Comparison of the assays showed that some of the tumours demonstrated selective rearrangement of specific Vγ gene segments. Most of the precursor T-lymphoblastic lymphoma (8/10, 80.0%), ALCL (14/19, 73.7%), and PTCL-unspecified (17/26, 65.4%), and all of the nasal-type NK/T-cell phenotypic lymphoma (4/4; 100.0%) demonstrated clonal pattern in assay I, whereas all nasal NK/T-cell phenotypic lymphoma (3/3, 100.0%) were demonstrated by assay IV.

Clonal TCRγ gene rearrangement was demonstrated in four (16.7%) of the 24 B-NHL, with no specific association with any subtype. These cases included two Burkitt's lymphoma, one mucosa-associated lymphoid tissue-type lymphoma and one diffuse large B-cell lymphoma. Clonal TCRγ gene rearrangement in all these four cases of B-NHL were demonstrated with assay IV. Clonal TCRγ gene rearrangement was demonstrated in 2/7 (28.6%) reactive lymphoid hyperplasia by both assays II and IV. None of the B-NHL and lymphoid reactive hyperplasia showed clonal TCRγ gene rearrangement with assay I, which is in sharp contrast to the T-NHL.

Sequencing of two representative samples of clonal PCR product per assay showed that the sequences were homologous to the sequences of TCRγ gene deposited in the PubMed Centre.

Fig. 1: Amplification of TCRγ Gene in T-NHL by five assays targeting all the functional Vγ and Jγ gene segments.

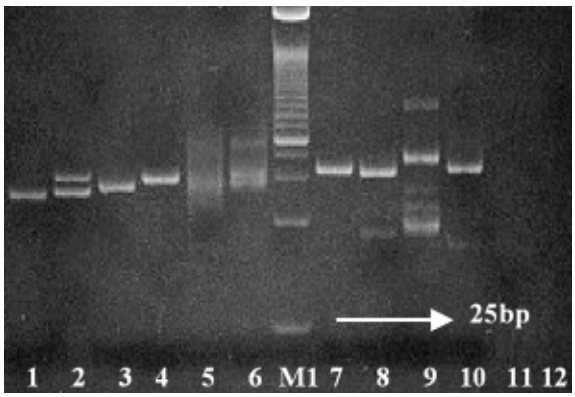

Fig. 1a: Amplification of assay I (Lanes 1-6) and assay II (Lanes 7-12). M1 denotes 25bp DNA ladder. Lanes 1-4 represent clonal rearrangement from T-NHL, while Lanes 5 and 6 represent polyclonal amplification from a T-NHL and reactive lymphoid hyperplasia respectively. Lanes 7-10 are clonal amplification for assay II, and Lanes 11 and 12 do not have detectable product.

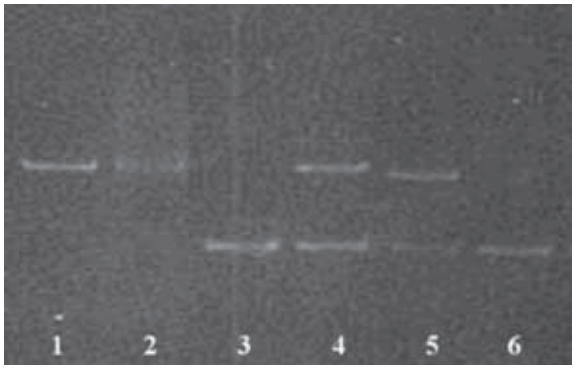

Fig. 1b: Amplification of assay III. Lanes 1, 4, 5 represent clonal amplification, Lane 2 is a polyclonal smear and Lanes 3 and 6 do not have detectable product.

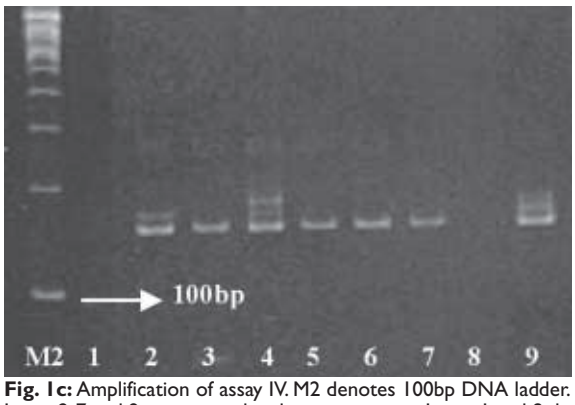

Fig. 1c: Amplification of assay IV. M2 denotes 100bp DNA ladder. Lanes 2-7 and 9 represent clonal rearrangement, Lanes 1 and 8 do not have detectable product. Lanes 4 and 9 are considered as clonal as a distinct band can be identified in the background polyclonal smear.

DISCUSSION

Distinction between reactive and malignant lymphoproliferation is of utmost importance in the management of patients. However, it is difficult to distinguish between these two conditions in some instance, especially in cutaneous lymphoproliferation[24,25]. Frequent lost of T-cell surface antigen during development of neoplasm has hampered the use of immunophenotyping in demonstrating clonal T-cell proliferation[2,4]. Previous studies on immunoglobulin heavy chain (IgH) and TCR genes rearrangement have indicated the usefulness of these techniques in demonstrating clonal proliferation of B- and T-cell respectively[7,10-15].

The results from this study indicate that clonal TCRγ gene rearrangement in most of the T-NHL can be demonstrated using assay I and assay III, which target at the VγI family. This is especially true in

precursor T-cell lymphoblastic lymphoma, ALCL and PTCL-unspecified, where most of the cases were detected by these assays. This may be explained by the fact that a great majority (approximately 70%) of TCRγ rearrangements in lymphoid malignancies preferentially use members of the VγI family of the TCRγ gene[26,27]. However, supplement with assay IV is necessary to achieve a satisfactory detection rate as assay IV demonstrated clonal TCRγ gene rearrangement in 30.6% of the cases. Assay IV significantly improved the sensitivity of detection in certain subtypes such as ALCL and NK/T-cell phenotypic lymphomas. Fodinger et al reported similar findings where 73% (52/71) of the cases were amplified by VγI family, 15% (11/71) by Vγ9, 6% (4/71) each by Vγ10 and Vγ11[28].

NK-cells are known to lack TCR gene rearrangement. Hence, the cases of nasal NK/T-cell phenotypic lymphomas that showed clonal TCRγ gene rearrangement might represent cases of γδ T-cell lymphomas. γδ T-cell lymphoma is a distinct entity first identified in the spleen and liver[29], and expresses CD56. This group of hepatosplenic γδ T-cell lymphomas show distinct histology and clinical behaviour. Studies reported that some extranodal T-cell lymphomas, especially those presented in the nose, gastrointestinal tract and skin fit into this category[30-33], and are indeed γδ T-cell lymphoma rather than true NK-cell lymphoma[32-37]. In addition, these cases were preferentially amplified by assay IV, in contrast to PTCL-unspecified, in which the majority were clonal by assay I. Assay IV targets at the Vγ9 and Jγ1.1/2.1 gene segments of the TCRγ gene. This is in accordance with report that most human γδ T-cells express Vγ9/Vδ2 TCR chain[38].

Clonal TCR gene rearrangement is useful in determining clonal nature and T-cell lineage of some tumours. The T-cell lineage of Lennert's and AILD lymphomas was first confirmed through the TCR gene rearrangement studies[8,9]. Likewise, null-cell ALCL, which lack both T- and B-cell antigenic expressions, speculated to be derived from T-cell because it usually expresses cytotoxic molecules such as perforin, granzyme B and TIA-1 was confirmed as T-cell genotype[39]. In this study, clonal TCRγ gene rearrangement was demonstrated in all cases of null-cell ALCL, in accordance with the report by Foss et al[40].

However, TCRγ gene rearrangement is not lineage-specific, and its interpretation should be supported by immunophenotyping. Dual rearrangement involving IgH gene and T-cell receptor (TCR) gene had also been reported previously on both T-cell and B-cell lymphomas[7,12,25,41,42]. Clonal TCRγ gene rearrangement was found in 16.7% (4/24) of B-NHL in this study. In a study of 80 NHL, Garcia et al also reported lineage infidelity in 21% of B-NHL, and 16% of T-NHL[43]. It is postulated that dual rearrangement is due to aberrant activation of the recombinase machinery[2,5,25]. This hypothesis is supported by the existence of similar structural configurations and a common recombinase enzyme for both IgH and TCR genes rearrangements[3,25,41]. Therefore, the tumour cell should only be considered as T-cell type if it does not express any B-cell surface antigen.

A disadvantage of TCRγ gene rearrangement analysis is the narrow range of the PCR product size. The PCR products for assay I, II, and III range from 70bp to 110bp, whereas assay IV and V range from 150bp to 190bp. Polyclonal or oligoclonal infiltrating T-cells that give a restricted smear may appear as a single band on agarose gel. Several separation methods have been employed to achieve higher resolution. These methods include polyacrylamide gel electrophoresis[10-12], denaturing gradient gel electrophoresis (DGGE)[44,45], heteroduplex analysis[3,46], PCR-single-stranded conformational polymorphism (SSCP) analysis[47] as well as sequencing[26] and automated high-resolution analysis[48,49]. Sequencing and automated high-resolution analysis is expensive and requires dedicated equipment, whereas DGGE, PCR-SSCP and heteroduplex analysis are technically more demanding than PAGE. Therefore, most studies apply PAGE and it is a technique which is easier and feasible for our need.

CONCLUSION

This study showed that TCRγ gene rearrangement by PCR could be applied in paraffin-embedded tissues. It is a useful technique in distinguishing reactive and malignant lymphoproliferation. However, TCRγ gene rearrangement is more complex than IgH gene rearrangement and the results should always be interpreted in compliance with histology and immunophenotype findings. Designation of T-cell lineage by the use of clonal TCRγ gene rearrangement should only be made if the tumour cell does not express any B-cell marker. This study also identifies a group of lymphoma that express CD56 antigen, but carry rearranged TCRγ gene. Further study is being carried out to describe this group of lymphoma.

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REFRENCES

1. Kronenberg M, Siu G, Hood LE, Shastri N. The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. Ann Rev Immunol 1986; 4:529-91.
2. Knowles DM. Immunophenotypic and antigen receptor gene rearrangement analysis in T cell neoplasia. Am J Pathol 1989; 134:761-85.
3. Channabhai M, Adomat SA, Gascoyne RD, Horsman DE. Clinical utility of heteroduplex analysis of TCR-gamma gene rearrangements in the diagnosis of T-cell lymphoproliferative disorders. Am J Clin Pathol 1997; 108:295-301.

4. Griesser B. Gene rearrangements and chromosomal translocations in T cell lymphoma — diagnostic applications and their limits.Virchows Arch 1995; 426:323-8.
5. Asou N, Matsuoka M, Hattori T, Kawano F, Maeda S, Shimada K, et al. T cell γ gene rearrangements in hematologic neoplasms. Blood 1987; 69:968-70.
6. McCarthy KP, Sloane JP, Kabarowski JHS, Matutes E, Wiedemann LM. The rapid detection of clonal T-cell proliferations in patients with lymphoid disorders. Am J Pathol 1991; 138:821-8.
7. Trainor KJ, Brisco MJ, Wan JH, Neoh S, Grist S, Morley AA. Gene rearrangement in B- and T-lymphoproliferative disease detected by the polymerase chain reaction. Blood 1991; 78:192-6.
8. O Connor NTJ, Feller AC, Wainscoat JS, Gatter KC, Pallesen G, Stein H, et al. T-cell origin of Lennert s lymphoma. Br J Haematol 1986; 64:521-8.
9. Lipford EH, Smith HR, Pittaluga S, Jaffe ES, Steinberg AD, Cossman J. Clonality of angioimmunoblastic lymphadenopathy and implications for its evolution to malignant lymphoma. J Clin Invest 1987; 79:637-42.
10. Lorenzen J, Iux G, Zhao-H hn M, Kl ckner A, Fischer R, Hansmann ML. Detection of T-cell clonality in paraffin-embedded tissues. Diagn Mol Pathol 1994; 3:93-9.
11. Diss TC, Watts M, Pan LX, Burke M, Linch D, Isaaccson PG. The polymerase chain reaction in the demonstration of monoclonality in T cell lymphomas. J Clin Pathol 1995; 48:1045-50.
12. Krafft AE, Taubenberger JK, Sheng ZM, Bijwaard KE, Abbondanzo SL, Aguilera NSI, et al. Enhanced sensitivity with a novel TCRγ PCR assay for clonality studies in 569 formalin-fixed, paraffin-embedded (FFPE) cases. *Mol Diagn* 1999; 4:119-33.
13. Ramasamy I, Brisco M, Morley A. Improved PCR method for detecting monoclonal immunoglobulin heavy chain rearrangement in B cell neoplasms. J Clin Pathol 1992; 45:770-5.
14. Diss TC, Peng H, Wotherspoon AC, Isaacson PG, Pan L. Detection of monoclonality in low-grade B-cell lymphomas using the polymerase chain reaction is dependent on primer selection and lymphoma type. J Pathol. 1993; 169:291-5.
15. Essop MF, Blakolmer K, Close PM, Manuel YE, Cornelius S. Analysis of low and high grade B-cell lymphoma subtypes using semi-nested PCR and two primer sets. Eur J Haematol 1997; 59:136-41.
16. Griesser H, Feller A, Lennert K, Minden M, Mak TW. Rearrangement of the β chain of the T cell antigen receptor and immunoglobulin genes in lymphoproliferative disorders. J Clin Invest 1986; 78:1178-84.
17. Zemlin M, Hummel M, Anagnostopoulos I, Stein H. Improved polymerase chain reaction detection of clonally rearranged T-cell receptor β chain genes. Diagn Mol Pathol 1998; 7:138-45.
18. Benhatter J, Delacretaz F, Martin P, Chaubert P, Costa J. Improved polymerase chain reaction detection of clonal T-cell lymphoid neoplasms. Diagn Mol Pathol 1995; 4:108-12.
19. Lefranc MP and Rabbitts TH. The human T-cell receptor γ (TRG) genes. TIBS 1989; 14:214-8.
20. LeFranc MP, Baer FR, Stinson MA, Rabbitts TH. Diversity and rearrangement of the human T cell rearranging γ genes: Nine germ-line variable genes belonging to two subgroups. Cell 1986; 45:237-46.
21. Jaffe ES, Harris NL, Stein H, Vardiman JW editors. World Health Organisation classification of tumours: Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press 2001.
22. Zhang DX and Hewitt. Isolation of DNA from preserved specimens. In: Karp A, Isaac PG, Ingram DS editors. Molecular tools for screening biodiversity. London: Chapman & Hall 1998; 41-5.
23. Pan LX, Diss TC, Peng HZ, Isaacson PG. Clonality analysis of defined B-cell populations in archival tissue sections using microdissection and the polymerase chain reaction. Histopathology 1994; 24:323-7.
24. Ashton-Key M, Diss TC, Du MQ, Kirkham N, Wotherspoon A, Isaacson PG. The value of the polymerase chain reaction in the diagnosis of cutaneous T-cell infiltrates. Am J Surg Pathol 1997; 21:743-7.
25. Th riault C, Galoin S, Valmary S, Selves J, Lamant L, Roda D, et al. PCR analysis of immunoglobulin heavy chain (IgH) and TcR-γ chain gene rearrangements in the diagnosis of lymphoproliferative disorders: results of a study of 525 cases. Mod Pathol 2000; 13:1269-79.
26. Kneba M, Bolz I, Linke B, Bertram J, Rothaupt D, Hiddemann W. Characterization of clone-specific rearranged T-cell receptor γ-chain genes in lymphomas and leukemias by the polymerase chain reaction and DNA sequencing. Blood 1994; 84:574-81.
27. Van Dongen JJM, Wolvers-Tettero ILM. Analysis of immunoglobulin and T cell receptor genes. Part I: Basic and technical aspects. Clin Chima Acta 1991; 198:1.
28. Fodinger M, Buchmayer H, Schwarzinger I, Simonitsch I, Winkler K, Jager U, et al. Multiplex PCR for rapid detection of T-cell receptor-gamma chain gene rearrangements in patients with lymphoproliferative diseases. Br J Haematol 1996; 94:136-9.
29. de Wolf-Peeters C, Achten R. γδ T-cell lymphomas: a homogenous entity. Histopathology 2000; 36:294-305.
30. Chan JKC, Sin VC, Wong KF, Ng CS, Tsang WYW, Chan CH, et al. Nonnasal lymphoma expressing the natural killer cell marker CD56: A clinicopathologic study of 49 cases of an uncommon aggressive neoplasm. Blood 1997; 89:4501-12.
31. Charton-Bain MC, Brousset P, Bouabdallah R, Gaulard P, Merlio JP, Dubus P, et al. Variation in the histological pattern of nodal involvement by gamma/delta T-cell lymphoma. Histopathology 2000; 36:233-9.
32. Gaulard P, Bourquelot P, Kanavaros P, Haioun C, Le Couedic JP, Divine M, et al. Expression of the alpha/beta and gamma/delta T-cell receptors in 57 cases of peripheral T-cell lymphomas. Identification of a subset of γ/δ T-cell lymphomas. Am J Pathol 1990; 137:617-28.
33. Arnulf B, Copie Bergman C, Delfau Larue MH, Lavergne-Slove A, Bosq J, Wechsler J, et al. Nonhepatosplenic γδ T-cell lymphoma: a subset of cytotoxic lymphomas with mucosal or skin localisation. Blood 1998; 91:1723-31.
34. Gaulard P, Henni T, Marolleau JP, Haioun C, Henni Z, Voisin MC, et al. Lethal midline granuloma (polymorphic reticulosis) and lymphomatoid granulomatous. Evidence for a monoclonal T-cell lymphoproliferative disorders. Cancer 1988; 62:705-10.
35. Kanavaros P, Farcet JP, Gaulard P, Haioun C, Divine M, Le Couedic JP, et al. Recombinative events of the T cell antigen receptor delta gene in peripheral T cell lymphomas. J Clin Invest. 1991; 87:666-72.
36. de Bruin PC, Kummer JA, van der Valk P, van Heerde P, Kluin PM, Willemze R, et al. Granzyme B-expressing peripheral T-cell lymphomas: neoplastic equivalent of activated cytotoxic T cells with preference for mucosa-associated lymphoid tissue localisation. Blood 1994; 84:3785-91.
37. Harabuchi Y, Imai S, Wakashima J, Hirao M, Kataura A, Osato T, et al. Nasal T-cell lymphoma casually associated with Epstein-Barr virus: clinicopathologic phenotype, and genotype studies. Cancer 1996; 77:2137-49.
38. Fisch P, Meuer E, Pende D, Rothenfusser S, Viale O, Kock S, et al. Control of B cell lymphoma recognition via natural killer inhibitory receptors implies a role for human Vgamma9/Vdelta2 T cells in tumor immunity. Eur J Immunol 1997; 27:3368-79.
39. Falini B. Anaplastic large cell lymphoma: pathological, molecular and clinical features. Br J Haematol 2001; 114:741-60.
40. Foss HD, Anagnostopoulos I, Araujo I, Assaf C, Demel G, Kummer JA, et al. Anaplastic large cell lymphomas of T-cell and null-cell phenotype express cytotoxic molecules. Blood 1996; 88:4005-11.
41. Chen YT, Godwin TA, Mouradian JA. Immunohistochemistry and gene rearrangement studies in the diagnosis of malignant lymphomas: a comparison of 152 cases. Hum Pathol 1991; 22:1249-57.
42. Griesser H, Feller AC, Lennert K, Tweedale M, Messner HA, Zalcberg J, et al. The structure of the T cell gamma chain gene in lymphoproliferative disorders and lymphoma cell lines. Blood 1986; 68:592-4.
43. Garcia MJ, Martinez-Delgado B, Granizo JJ, Benitez J, Rivas C. IgH, TCR-gamma, and TCR-beta gene rearrangement in 80 B- and T-cell non-Hodgkin s lymphomas: study of the association between proliferation and the so-called aberrant patterns . Diagn Mol Pathol 2001; 10:69-77.
44. Greiner TC, Raffeld M, Lutz C, Dick F, Jaffe ES. Analysis of T cell receptor gene rearrangements by denaturing gradient gel electrophoresis of GC-clamped polymerase chain reaction products. Correlation with tumor-specific sequences. Am J Pathol 1995; 146:46-55.
45. Wood GS, Tung RM, Haeffner AC, Crooks CF, Liao S, Orozeo R, et al. Detection of clonal T-cell receptor γ gene rearrangements in early mycosis fungiocides/Sezary Syndrome by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR/DGGE). J Invest Dermatol 1994; 103:34-41.
46. Bottaro M, Berto E, Biondi A, Migone N, Crosti L. Heteroduplex analysis of T-cell receptor γ gene rearrangements for diagnosis and monitoring of cutaneous T-cell lymphomas. Blood 1994; 83:3271-8.
47. Signoretti S, Murphy M, Cangi MG, Puddu P, Kadin ME, Loda M. Detection of clonal T-cell receptor γ gene rearrangements in paraffin-embedded tissue by polymerase chain reaction and nonradioactive single-strand conformational polymorphism analysis. Am J Pathol 1999; 154:67-75.
48. Simon M, Kind P, Kaudewitz P, Krokowski M, Graf A, Prinz J, et al. Automated high-resolution polymerase chain reaction fragement analysis. A method for detecting T-cell receptor γ-chain gene rearrangements in lymphoproliferative diseases. Am J Pathol 1998; 152:29-33.
49. Ayling JF and Iland HJ. High-resolution analysis of gene rearrangements in lymphoid malignancies. Pathology 1999; 31:252-6.