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NEW BIOLOGY

Molecular biology of lymphoma in the microarray era

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Summary

This review will focus on the molecular biology of lymphoproliferative disorders with emphasis on lymphomas. The spectrum of known recurrent gene rearrangements found in lymphomas will be outlined and their relevance to diagnosis and subclassification of disease will be discussed. Finally, a survey of the current trends in gene expression profiling of lymphomas by microarray technology will be presented with reference to implications for diagnosis, classification, prognosis and treatment.

Key words: Lymphoma, molecular pathology, microarray, gene rearrangements, transcription factors, antigen receptors.

Abbreviations: ALL, acute lymphoblastic leukaemia; ALCL, anaplastic large cell lymphoma; ABC, activated B-cells; D, diversity genes; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; GCB, germinal centre B cell; GEP, gene expression profiling; HL, Hodgkin lymphoma; IGH, immunoglobulin heavy chain; J, joining genes; LBL, lymphoblastic lymphomas; MALT, mucosa-associated lymphoid tissue; MCL, mantle cell lymphoma; RSS, recombination signal sequences; TCR, T-cell receptor; V, variable genes.

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INTRODUCTION

The classification of lymphoproliferative disorders, perhaps more than any other area of human malignancy, has been plagued by confusion. A plethora of competing and often incompatible classification schemes emerged during the 20th century, and until recently focused primarily on histopathological features. Over the last 10 years, the approach to classification has shifted and broadened considerably. The most recent World Health Organization (WHO) classification,[1,2] a refinement of the earlier Revised European-American Classification of Lymphoid Neoplasms,[3] provides the most comprehensive approach to lymphoproliferative disorder classification yet devised, by incorporating clinical, pathological, immunological, cytogenetic and molecular genetic parameters. The benefits of precise classification include improved communication amongst clinicians, pathologists and scientists, improved identification of optimal therapeutic strategies, and the potential to facilitate the development of lesion-specific targeted therapy to further improve outcomes and to reduce toxicity.

The importance of any pathological classification of malignancies is to divide a large and diverse group of diseases into smaller, more homogeneous and clinically relevant subgroups. Within any one of these subgroups, patients should ideally have a similar prognosis and disease biology. Novel therapeutic approaches to a malignant disease are often targeted to the biological causative factors. Incorporation of cytogenetic and molecular genetic characteristics into new classifications of haematological malignancies[4] may be the first step in developing classifications based solely on molecular characteristics of the disease. Ultimately, the genes expressed, whether normal or mutated, determine the phenotype of a tumour. The current WHO classification of haematopoietic and lymphoid tumours recognises that the biological features of some lymphomas and lymphoblastic leukaemias, with similar morphological and genetic properties, allow them to be classified as single diseases.[1,2,4] Current classifications of haematological malignancies include mutations and specific gene rearrangements which are characteristic of particular clinically and morphologically distinct diseases.

In addition, there are now several examples of DNA microarray techniques being used to examine the gene expression profiles of lymphomas. Ultimately, gene expression profiles may be used to better classify tumours from patients into more biologically and clinically relevant groupings.[5]

Using the WHO classification as a framework, the first part of this review will focus on the molecular lesions that contribute most to the recognition of distinct clinical entities within the lymphoproliferative disorders. In the second part, we will attempt to summarise the role that microarray-based technology is beginning to play in increasing our understanding of lymphoproliferative disorder pathobiology, and the potential it has for defining critical therapeutic targets.

1. MOLECULAR PATHOLOGY OF LYMPHOPROLIFERATIVE DISORDERS

Clonality and lymphoproliferative disorders

In order to generate significant diversity in the immune system's antigen recognition repertoire, normal B lymphocytes and T lymphocytes undergo a unique process of sequential antigen receptor gene rearrangement involving the immunoglobulin (IG) and T-cell receptor (TCR) genes, respectively. The process of gene rearrangement occurs early in B-cell and T-cell development, and is initially antigen-independent. There are four TCR loci (TRA, TRB, TRG and TRD) and three IG loci (IGH, IGK and IGL) located on various chromosomes (see Table 1).

The generation of antigen binding diversity in IG genes and TCR genes involves the same mechanism of V(D)J

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TABLE 1 Diversity of human antigen receptor loci

Antigen receptor locus		Chromosomal location	Functional genes[135–138]
IGH	14q32.33	Immunoglobulin heavy chain	38–46 IGHV 23 IGHD 6 IGHJ 9 IGHC
IGL	22q11.2	Immunoglobulin lambda light chain	29–33 IGLV 4–5 IGLJ 4–5 IGLC*
IGK	2p11.2	Immunoglobulin lambda light chain	31–35 IGKV 5 IGKJ 1 IGKC
TRA	14q11.2	T-cell receptor alpha	44–46 TRAV 50 TRAJ 1 TRAC
TRB	7q34	T-cell receptor beta	40–48 TRBV 2 TRBD 12–13 TRBJ 2 TRBC
TRG	7p14	T-cell receptor gamma	4–6 TRGV 5 TRGJ 2 TRGC
TRD	14q11.2	T-cell receptor delta	7–8 TRDV 3 TRDD 4 TRDJ 1 TRDC

*There are 4–5 functional IGLC genes in the sequenced haplotype. There are other haplotypes with 8, 9, 10 or 11 IGLC genes, each one probably preceded by one IGLJ gene.

recombination between consensus heptamer-nonamer recombination signal sequences (RSS) flanking the many variable (V), diversity (D) and joining (J) genes, except that the IGL, IGK, TRB and TRG genes lack D exons.[6] In B cells, V(D)J recombination occurs in the bone marrow and is initiated by the recombination activating gene 1 and 2 proteins, RAG1 and RAG2.[7] Once a functional immunoglobulin is generated, the naïve B-cells migrate from bone marrow to the germinal centres of secondary lymphoid organs (lymph nodes, spleen and mucosa-associated lymphoid tissue) where the processes of antigen-dependent somatic hypermutation and class switching occur to enhance the functionality and the avidity of the antigen receptor for its target antigen. In the majority of B-cell lymphomas, the presence of somatic mutations indicates their origin in the germinal centre or post-germinal centre cells of lymph nodes. The exceptions are mantle cell lymphomas and some cases of small lymphocytic lymphoma (chronic lymphocytic leukaemia), where unmutated V genes are found.

In T cells, an analogous process occurs for the genes which encode the α, β, δ and γ chains of the TCR complex. Each cell that undergoes antigen receptor gene rearrangement generates a distinct DNA sequence that identifies it from all other normal lymphocytes.

The progeny of a lymphoid cell that has undergone this physiological process of gene rearrangement will share an identical immunoglobulin or TCR gene rearrangement with its parent cell. In lymphoid malignancies, this phenomenon can be exploited by molecular techniques to demonstrate the presence of a monoclonal population of lymphoid cells that is highly suggestive (though not diagnostic) of a lymphoid malignancy.[8] The hypervariable portion of the IgH locus, known as CDR3, provides a tumour-specific template for amplification by PCR, and consensus primers that are directed at the V and J genes of the IgH locus will generate a DNA smear of variably sized PCR products with polyclonal DNA when visualised by electrophoresis in low-resolution gels, whereas a discrete band will be seen with monoclonal DNA. Visualisation with high-resolution techniques (for example, polyacrylamide gel electrophoresis of fluorescently labelled PCR products) provides even better discrimination between monoclonal and polyclonal cell populations (see Fig. 1) and provides accurate sizing of the tumour-specific PCR product that facilitates detection of the same clone in another tissue site.[9]

In this way, lymphoproliferative disorders (which are usually monoclonal) can be readily distinguished from reactive lymphoid cells (which are usually polyclonal).

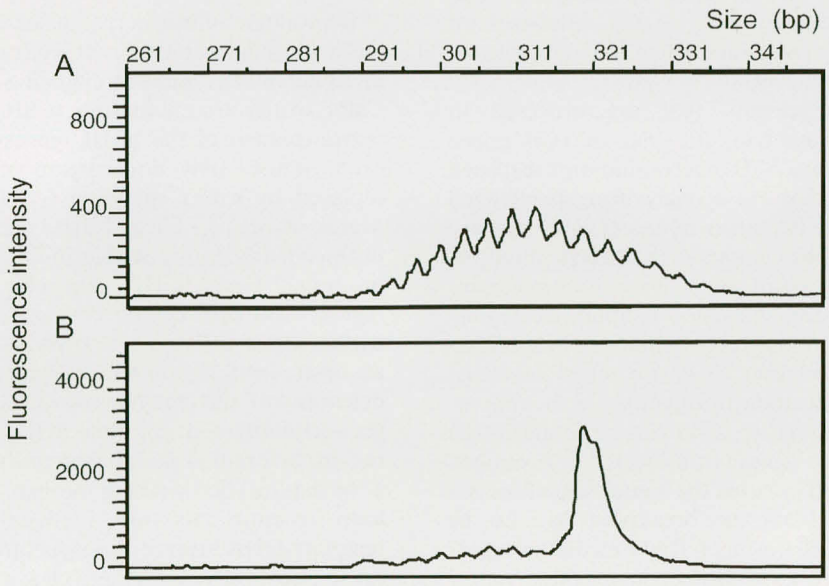

Fig. 1 High-resolution electrophoresis showing PCR products of IGH gene rearrangements; (A) a polyclonal population of B cells in peripheral blood of a healthy donor, and (B) a monoclonal population of B cells in peripheral blood of a patient with B-cell chronic lymphocytic leukemia (reproduced from Ayling and Iland[9]).