
Preface

The molecular investigation of hematological malignancies has always fore-run that of other cancers. There are a number of potential reasons for this, some of which are more logistical than theoretical, and include the ready access to pathologic material with the presence of malignant cells generally in great excess in comparison with the normal. These factors are combined with a set of diseases for which potentially curative systemic therapies are available, making the precise subclassification for prognostic and therapeutic purposes even more important. In general, studies of the leukemias have first started with immunophenotyping, classical cytogenetics, restriction digest analysis, polymerase chain reaction studies (PCR), fluorescent *in situ* hybridization (FISH), and comparative genomic hybridization. Most recently, gene expression arrays have been tested first in leukemia, closely followed by the application of these techniques to lymphoma.

The different techniques described in *Lymphoma: Methods and Protocols* have evolved in parallel over the last three decades. The first was classical cytogenetics and the identification of consistent structural abnormalities in the chromosomes of malignant cells, with the findings linked to morphologic and immunophenotypic entities. From chromosomal rearrangements, reverse genetics led to the characterization of key genes adjacent to breakpoints, whose function was then related back to the abnormal cellular behavior. The finding that an activating oncogene, c-Myc, was involved in the characteristic chromosome 8 breakpoint in Burkitt's lymphoma was a key moment for this field. Analysis of different breakpoints has yielded other, similarly important insights, such as identification of a failure to undergo apoptosis as a central mechanism in malignancy following the finding of the Bcl-2 gene at the chromosome 18q21 breakpoint in follicular lymphoma. The field of cytogenetics has been enhanced greatly by the development of FISH, in which insights into more sophisticated patterns of chromosomal abnormality can be understood, and there has subsequently been a steady rise in the number of regions for study.

An increase in our understanding of lymphocyte biology, particularly B-cell ontogeny and the germinal center reaction has led to the application of immunoglobulin gene rearrangements as markers of clonality and to patterns of somatic mutation in the variable regions being used as indicators of the stage in development at which transformation occurred. Study of these patterns of

mutation in low-grade lymphoma has yielded the fascinating information that follicular types are preferentially mutated at glycosylation sites, suggestive of a ligand-related selection pressure in the germinal center during pathogenesis. In chronic lymphocytic leukemia (CLL) the more “immature” unmutated/nongerminal center types show a considerably more aggressive course than the post-germinal center mutated cases. There are interesting parallels in diffuse large B-cell lymphoma, which is always mutated in the immunoglobulin V genes, but in which a germinal center immunophenotype and pattern of mRNA expression conveys a better prognosis. The application of gene arrays to the question of biological heterogeneity in morphologically similar diseases has been especially rewarding. Different subtypes of large B-cell lymphoma have been clearly defined, and the grey zone between Hodgkin lymphoma and sclerosing mediastinal lymphoma is being steadily illuminated.

Currently gene arrays are not a routine part of the diagnostic investigation for lymphoma, although it seems highly likely that this will be the case for subsequent editions of this book. However, it may be that the gene signatures are sufficiently paralleled by the phenotype to allow immunohistochemistry to be used instead, once a firm correlation has been established. This looks likely to be the case for large B-cell lymphoma with the use of Bcl-6 and CD10 as germinal center markers, and certainly broadens the applicability of the classification when fresh biopsy material is difficult to obtain.

The process of classifying and reclassifying lymphoma has been a source of some frustration for clinicians and pathologists alike for many years, but the advent of the Revised European American Lymphoma (REAL) classification and subsequently its adoption into the new WHO classification have brought unprecedented harmony into this area. The recognition of REAL disease entities has been critical to the assimilation of immunophenotypic and molecular data into the classification, which has been a notable success. The process of subdividing the different entities along biological lines is well underway, with classes of CLL and diffuse large B-cell lymphoma already characterized as described. Other entities such as MALT lymphoma are being split down according to the precise translocation present, and in all these cases the subdivisions seem likely to have relevance to the type of therapy chosen. Thus, gastric MALT lymphoma with a t(11;18)(q21;q21) is much more likely to respond to the eradication of *Helicobacter pylori* than is one with t(1;14)(p22;q32).

The use of such molecular information to guide therapy is slowly gaining ground, although in other areas the benefits remain less clear. The detection of minimal residual disease using PCR techniques has been extensively studied, particularly in follicular lymphoma, with the general conclusion that absence of the pathognomonic t(14;18)(q32;q21) translocation at the time of clinical

remission is preferable, but no firm data as to whether the pursuit of such molecular remission actually conveys a survival benefit. Until the advent of rituximab antibody therapy there were few patients in whom molecular remission was achieved with anything less than myeloablative treatment, but now that there is an intervention that can achieve a high rate of conversion from PCR-positive to PCR-negative it may be possible to test the validity of this hypothesis. The use of quantitative PCR to follow progress sequentially has added a further level of refinement to this approach and made it much easier to consider therapy directed by molecular results.

As well as providing prognostic information, molecular analyses have been used to produce novel therapeutics, especially in the area of immunotherapy. Characterization of the lymphocyte cell surface molecules has been used to identify antigens against which vaccines or antibodies may be deployed, and the newer technique of serological identification by expression cloning (SEREX) has provided a wealth of information on this. The idiotype represents the truly unique antigen on a B-cell surface and here once again the application of molecular techniques has been able to provide a highly specific immunogen, in the form of a DNA vaccine with patient-specific sequences derived from the lymphoma.

In conclusion, the aim of this book is to give some practical information on the current molecular approaches being used in the understanding, classification, and therapy of lymphoma. Though it cannot be exhaustive in providing information for every chromosomal translocation and gene rearrangement, we hope that it provides a useful background to the field and a means to get started with the molecular diagnostics of a group of diseases that are both biologically interesting and medically important.

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