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REVIEW

Genomic signatures in B-cell lymphoma: How can these improve precision in diagnosis and inform prognosis?

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ABSTRACT

Current genomic technologies have immensely improved disease classification and prognostication of major subtypes of B-cell lymphomas. This novel genetic information has not only aided in diagnosis, but has also revealed a landscape of critical molecular events that determine the biological and clinical behavior of a lymphoma. In this review, we summarized the genetic characteristics of major subtypes of B-cell lymphomas, including diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), Burkitt lymphoma (BL), and mantle cell lymphoma (MCL). We illustrated how genomic profiling had identified molecular subgroups in DLBCL with varied clinical outcomes, and how a subset of genes defined prognosis in MCL and aided in BL diagnoses. We also highlighted some Phase II/III clinical trials using new therapeutic agents to determine clinical efficacy in novel molecular subgroups with distinct gene expression patterns. We believe that refinement of genomic signatures will require more intensive efforts from the biomedical research community to improve targeted therapy designs and bring a substantial change in the treatment decisions. In the next era of genomic medicine, we anticipate that a clinically and biologically relevant molecular profile of each tumor will be obtained at diagnosis to guide therapy.

1. Introduction

The classification of non-Hodgkin lymphoma (NHL) has changed considerably over the last several decades with the advances in immunology and introduction of newly developed molecular techniques. The initial, morphology-based classification scheme by Rappaport [1] in 1966 was followed by Lukes and Collins [2] and later by the Kiel classification systems [3]. An international effort to summarize different classification systems resulted in the Working Formulation [4], and later led to the Revised European American Lymphoma (REAL) system. The currently used World Health Organization (WHO) [5] classification system has a broader consensus among the clinical and biomedical community. Though immensely fruitful, these refinements have not translated into better treatment response or improvement in survival outcome for patients, suggesting further biological heterogeneity not being captured by the classification system.

Tumor formation is initiated by a genetic lesion due to an error occurring during normal cellular function or from unrepaired physical or chemical damage to the genome [6]. The genetic abnormalities accumulate during clonal evolution and lead to unique gene expression profiles (GEPs), which characterize tumor biology and clinical behavior. Screening cancers for known recurrent genetic abnormalities, such as *BCR-ABL* fusion in chronic myelogenous leukemia (CML), $BRAF^{V600E}$ mutation in melanoma, or t(8;14) in Burkitt Lymphoma (BL) [7–9], is now considered as a standard practice in cancer diagnosis and management. In the past three decades, the breakpoints from translocations known to be associated with specific lymphoma subtypes have been cloned and their mechanisms of action are being elucidated [10]. They often serve as important diagnostic and prognostic markers and are even used for monitoring treatment response or early relapse in treated patients.

With the development of high throughput genomic technologies, like GEP, microRNA (miRNA) profiles, genome-wide copy number abnormalities (CNAs) [11] and global methylation and mutation spectrum, it is now possible to dissect out genome-wide genetic or epigenetic changes and decipher the biology of lymphoid malignancies [12,13]. Techniques like next-generation sequencing (NGS) for the global analysis of the genome and epi-genome have matured in the past few years. It is now possible to examine mutations and other structural changes in the cancer genome (including point mutations, deletions, insertions, inversions and translocations) [14,15] at a reasonable cost.

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Although, whole genome re-sequencing is still expensive, whole exome and transcriptome re-sequencing are more accessible and starting to yield interesting findings [16–19]. We anticipate that some form of diagnostic and prognostic genome-wide analysis assay will be adopted in clinical practice to provide additional molecular information for improving patient management.

2. Transition phases in genomic era and impact on DLBCL pathogenesis

Both T and B cells rearrange their antigen receptor genes during maturation using RAG-1, 2 recombinases [18] and chromosomal breaks encountered during these processes increase the chance of illegitimate recombination events [19,20]. For B-cells that participate in the germinal center (GC)-reaction, additional double strand DNA breaks are introduced during class switch recombination(CSR) [21]. GCB cells also undergo somatic hypermutation (SHM) that mainly affect the Ig gene loci [22]. For B-cell lymphomas that are arrested at the GCB cell stage of differentiation, this process continues to be active and may introduces mutations in genes not normally identified as targets of GC-SHM [23,24], thus promoting tumor progression. During the past three decades these events were observed in clinical laboratories through laborious approaches; however major technological breakthroughs such as modern cytogenetic techniques, immunophenotyping, and molecular genetic analysis have also evolved during these years, and are briefly summarized in Table 1. To a certain degree these techniques improved our understanding of lymphoid biology, aided in diagnosis, and informed prognosis. In present era, a more detailed characterization of molecular features is increasingly critical for the definition of specific entities. In this review, we highlighted the impact of the genome-wide studies in B-cell lymphomas, especially common ones like diffuse large B-cell lymphoma (DLBCL) [25–28], mantle cell lymphoma (MCL) [27], follicular lymphoma (FL) [28], and Burkitt lymphoma (BL) [29], and rare ones like marginal zone B-cell lymphoma (MZL) [30]. Some basic morphological (Fig. 1 A–H), immunophenotypic and molecular features of major B-cell lymphomas are summarized in Table 2.

3. Genomic signatures delineated lymphomas into molecular subtypes with unique biological characteristics

3.1. Diffuse large B-cell lymphoma

This heterogeneous disorder shows a diffuse architecture of mature B-cell phenotype and large cells with two major morphological subtypes (e.g. centroblastic vs. immunoblastic) [31]. GEP has identified two major molecular subtypes related to the cell-of-origin (COO). One of them expressing a set of genes that are typically expressed by GCB cells is named the germinal center B-cell (GCB) like DLBCL (Fig. 2 A) [25,26]. The other subgroup, activated B-cell like (ABC) DLBCL, expresses a set of genes that are upregulated in activated peripheral blood B-cells, and exhibits poor clinical outcome compared to GCB-DLBCL (Fig. 2 B–D) [25,26,32,33]. A small subset could not be confidently classified into either GCB- or ABC-DLBCL subgroups and is termed as unclassifiable. These studies also identified gene expression predictors of survival outcome including a group of genes associated with cell proliferation (the proliferation signature), the tumor microenvironment (the lymph node stromal signature, and the major histocompatibility complex class I & II molecules (the MHC signature). High expression of the proliferation signature and low expression of the MHC signature are associated with poor survival whereas high expression of the lymph node stromal signature is associated with a better outcome. A follow-up study performed by Lenz and co-workers on a rituximab-treated DLBCL cohort [32] refined the lymph node stromal signature into two signatures (stromal I and II signature). Stromal signature I reflects extracellular matrix deposition and cellular infiltration and high expression is associated with better outcomes, whereas stromal signature II reflects angiogenesis and high expression is associated with poorer survival [32]. Using a different analytical approach Monti et al. [33] identified 3 groups of DLBCL

Table 1
Commonly used technologies in molecular diagnosis in pre-genomic era and post-genomic era.

	Technique	Bio molecular test	Remarks
Pre-genomic era	Southern blot	Genomic DNA	– Translocation detection – Clonal rearrangements of T or B-cell receptor gene, – amplification or deletions of genomic locus – requires good quality and quantity of bio specimen – Time and labor intensive
	Polymerase chain reaction	DNA RNA (RT-PCR)	– Requires only a small amount of tissue – Archival paraffin embedded material can be used – Detection of minimal residual disease (MRD) – Can be gene specific
	Quantitative real time PCR	DNA RNA (RT-PCR)	– Can provide quantitative information in tracking MDR – Increased precision, accuracy and standardization – Amenable to high throughput
	DNA sequencing analysis	DNA	– Mutation of specific genes (e.g. FLT3, JAK2, NPM1, TP53, ATM) for diagnostic or prognostic significance in tumor biopsies SSCP, DGGE or TGGE
	Fluorescence in-situ hybridization (FISH)	DNA RNA	– Specific location of aberration to particular cells or tissues – Standard technique for identification of *BCL2*, *BCL6*, *MYC* and other Translocations
Genomic era	DNA microarray	RNA	– High-throughput – Quantitative analysis of global gene expression – Bioinformatics expertise required
	SNP arrays	DNA	– High-throughput – Detection of global DNA copy number alterations – Bioinformatics expertise required
	Next generation sequencing	DNA RNA	– High-throughput – Detection of mutations present in the genome or exome – Quantitative analysis of global gene expression – Bioinformatics expertise required
	High throughput qPCR for miRNA	miRNA	– High-throughput – quantitative analysis of expression of large numbers of miRNA