Functional signatures identified in B-cell non-Hodgkin lymphoma profiles

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Abstract
Gene-expression profiling in B-cell lymphomas has provided crucial data on specific lymphoma types, which can contribute to the identification of essential lymphoma survival genes and pathways. In this study, the gene-expression profiling data of all major B-cell lymphoma types were analyzed by unsupervised clustering. The transcriptome classification so obtained, was explored using gene set enrichment analysis generating a heatmap for B-cell lymphoma that identifies common lymphoma survival mechanisms and potential therapeutic targets, recognizing sets of coregulated genes and functional pathways expressed in different lymphoma types. Some of the most relevant signatures (stroma, cell cycle, B-cell receptor (BCR)) are shared by multiple lymphoma types or subclasses. A specific attention was paid to the analysis of BCR and coregulated pathways, defining molecular heterogeneity within multiple B-cell lymphoma types.

Keywords: Non-Hodgkin lymphoma, classification, heterogeneity, B-cell receptor

Introduction
B-cell lymphomas are currently diagnosed on the basis of integrated morphological, immunophenotypic, and cytogenetic findings, following the recommendations of the World Health Organization (WHO) [1]. The adoption of these criteria has been a significant and useful step forward, as it enables multiple entities to be defined more precisely, provides specific molecular markers, and facilitates treatment assignment. In spite of this progress, there remain gray areas where the criteria for distinguishing different entities overlap. More importantly, the knowledge gained about lymphoma pathogenesis so far has not yet resulted in the hoped-for development of therapies directed at molecular targets that inhibit essential lymphoma survival mechanisms.

Gene-expression profiling has been applied to many non-Hodgkin lymphoma (NHL) entities, and has revealed molecular heterogeneity within existing diagnostic categories. Also, clinical studies have shown a striking variability within specific tumor types. For example, Monti et al. defined three categories of diffuse large B-cell lymphoma (DLBCL) using B-cell receptor (BCR), host response, and oxidative phosphorylation signatures [2], while Alizadeh et al. [3] and
Lenz et al. [4] defined two main types: (i) germinal center (GC) B-like DLBCL based on GC origin, t(14,18) translocation, c-rel amplification, mir-17–92 cluster amplification and deletion of phosphatase and tensin (PTEN) homolog; and (ii) activated B-like cluster amplification and deletion of phosphatase and (14,18) translocation, c-rel amplification, mir-17–92 center (GC) B-like DLBCL base do nG Cor i g i n,

Lenz et al [4] defined two main types: (i) germinal center (GC) B-like DLBCL based on GC origin, t(14,18) translocation, c-rel amplification, mir-17–92 cluster amplification and deletion of phosphatase and tensin (PTEN) homolog; and (ii) activated B-like (ABC) DLBCL type, based on nuclear factor kappa B (NFκB) activation, Spi-B transcription factor (SPIB) and forkhead box P1 (FOXP1) oncogene, presence of trisomy 3 and INK4a/ARF deletion. These authors demonstrated that tumor signatures can be ascribed to specific cell functions, related to the clinical variability of patients with lymphoma, and used to identify potential targets. Subsequently, it has been demonstrated that functional signatures can be ascribed to tumoral cells or specific compartments of the microenvironment, and associated with lymphoma outcome [5–8]. Progress toward identifying functional signatures critically depends on the availability of databases for specific lymphoma types, whereby the relationship between precise functions and related genes may be established. Such efforts make it possible to break down the molecular pathogenesis of some lymphoma types into precise functional signatures that provide information about the relationships between a range of oncogenic alterations. Lymphoma is shown to be the result of the integration of multiple cross-talking circuits derived from tumoral cells and the microenvironment.

In this study, we have combined the expression profiles of all the major types of B-cell NHL and used an unsupervised clustering method for identifying the functional signatures that recognize the different B-cell lymphoma types [9]. To this end, the transcriptome classification obtained was explored using gene set enrichment analysis (GSEA) (MIT v.2) and the annotated databases, BioCarta Pathways and Lymphoma/Leukemia Molecular Profiling Project Database (LMMPP DB) [10]. This enabled us to identify some of the major pathways common to multiple B-cell lymphoma types, and to characterize some of the biological determinants of B-cell lymphoma heterogeneity. Some of the biologically relevant signatures identified here recognize cell cycle, cytokine signaling, GC, BCR pathway, AKT cluster, stroma and NFκB pathway, and apoptosis. We have finally explored the expression of BCR and associated pathways.

Materials and methods

Patients and controls

Gene-expression profiling of 187 cases of B-cell NHL from adults, including Burkitt lymphoma (BL) (n = 9), DLBCL (n = 36) [11], mantle cell lymphoma (MCL) (n = 38) [12], chronic lymphocytic leukemia (CLL) (n = 38) [13], follicular lymphoma (FL) (n = 33) [14], nodal marginal zone lymphoma (NMZL) (n = 6), and splenic marginal zone lymphoma (SMZL) (n = 27) [15], was collected for the study. The expression data for most of these lymphomas have been previously published by our group [11–15]. The samples from BL and NMZL were new hybridizations. In all cases, lymph node samples were collected from untreated patients. Reactive lymph nodes from eight patients and spleen from three patients were also profiled. Normal B-cell-sorted subpopulations (mantle zone, marginal zone, and centroblasts) were also analyzed, following described protocols [12,15]. In all cases, fresh frozen samples, provided by the member hospitals of the CNIO Tumour Bank Network, were collected according to standard protocols. The cases were diagnosed using the WHO Classification criteria, combining the appropriate morphological and molecular diagnostic tests [1]. They were reviewed by a team of pathologists and hematologists, and the diagnosis was confirmed. The study was conducted under the supervision of the Instituto de Salud Carlos III Institutional Ethic Committee. The microarray data (GSE9327) are available from the Gene Expression Omnibus (GEO) public repository, subject to MIAME regulations: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=ftenzyytkicq6&acc=GSE9327.

RNA extraction and amplification

Total RNA was isolated in two steps using Trizol (Life Technologies, Grand Island, NY) followed by RNeasy (Qiagen, Valencia, Spain) purification as previously described [11]. RNA was amplified from 4 μg of total RNA using the Superscript System for cDNA synthesis (Gibco/Invitrogen, Paisley, United Kingdom) and the T7 Megascript in vitro transcription kit (Ambion, Austin, TX).

Labeling and hybridization

Amplified RNA (2.5 μg) was directly labeled with Cy3-conjugated dUTP. aRNA (2.5 μg) from the Universal Human Reference RNA (Stratagene, La Jolla, CA) was labeled with Cy5-conjugated dUTP as reference. Hybridizations were performed using the CNIO OncoChipTM. Slides were scanned using a Scanarray 5000 XL (Agilent, Palo Alto, CA), and images were analyzed with the GenePix 5.1 program (Axon Instruments, Union City, CA).

Normalization and data preprocessing

To adjust for differences in the red and green labeling, Cy3/Cy5 ratios were normalized with the diagnosis and normalization of Microarray data.
(DNMAD) tool available in the GEPAS package (http://www.gepas.org) on the basis of standard print-tip loess [16,17]. To convert patterns to a symmetrical scale, ratios were log-transformed (base 2), and mean values were assigned to duplicated spots on the OncoChip. Only those genes that represented in 90% of cases were considered in the subsequent analysis. To obtain the expression profile of each tumor we compared the ratios of the tumors to the mean of the ratios of reactive lymph nodes from eight patients. For SMZL, values were normalized against normal spleen (three cases).

Unsupervised classification of cases and genes

The methodology followed is summarized in Figure 1 for ease of understanding. Unsupervised hierarchical clustering analysis of genes was carried out using the Unweighted pair-group method arithmetic averages (UPGMA) method as implemented in the Stanford Clustering and Tree View tools [18].

Cases were analyzed using the self-organizing tree algorithm (SOTA) [19] available in Gene Expression Pattern Analysis Suite (GEPAS) (http://www.gepas.org), based on squared Euclidean distances. SOTA was used in combination with the Environment of Tree Exploration (ETE) Tree Viewer to produce a function-constrained classification of the cases.

Definition of functional signatures and functional profiling

Unsupervised clustering was used to identify the molecular signatures that underlie common pathways in the various types of B-cell NHL [18]. Using GSEA (http://www.broad.mit.edu/gsea/) [20] on a ranked list of genes that are differentially expressed in distinct molecular categories of lymphoma types we were able to identify pathways differentially expressed in these categories.

To do this, we used selected gene sets from BioCarta pathways and the LLMPP DB (http://lymphochip.nih.gov/signaturedb), which included a compilation of curated gene-expression signatures [10]. We sorted the pathways that are known to be most relevant to the study of lymphomas and normal lymphoid cells, essentially as both gene sets have been previously applied to the analysis of different lymphoma types [2], and the LLMPP DB additionally provides cell-of-origin signatures. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) pathways were not considered as they have confounded the results.

We also introduced clusters of coregulated genes by supervised clustering of genes that have aligned biological functions. Supervised clustering shows gene sets to be differentially expressed in distinct lymphoma types, and chosen for their capacity to demonstrate a functional relationship and/or therapeutic target potential. Such gene clusters included proteasome clusters, PI3 kinase (PI3K) clusters, PIM family, ubiquitins, histones, GC, BCR, apoptosis, tubulins, follicular T-cells, macrophages, stroma, and cytokines.

Thus, the final lymphoma-enriched BioCarta (LEB) database used in this study contains selected gene sets from the BioCarta pathways and the

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Gene Expression Profiling (GEP) for 187 cases of B-cell NHL using CNIO cDNA Oncochip

Unsupervised hierarchical clustering of genes

Self-Organising Tree Algorithm (SOTA) using UPGMA Euclidean distance to identify lymphoma categorization based on expression profiling

Gene Set Enrichment Analysis (MIT) v2 linked to Biocarta pathways and M Sig Database signatures

Identification of clusters of coregulated genes

Identification of functional NHL types based on gene signatures

Deregulated pathways amongst functional NHL types (Figure 2)

Using Signal to Noise Ratio

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Figure 1. Depiction of the general analytical strategy.
LLMPP DB, and gene signatures identified in this study by unsupervised clustering of B-cell NHL (Supplementary Table 1).

GSEA was used to identify functional signatures.

(a) To identify functional pathways that are commonly deregulated in NHL types and subtypes, the genes were ranked with respect to their signal-to-noise ratio (SNR) [21] using a phenotype-based permutation-test procedure. A minimum of one single gene in a gene set was necessary to qualify it for analysis, so that all genes and pathways, up to a maximum of 500 genes per gene set, were included. The GSEA score values, which measure the maximum deviation from 0 encountered in a random walk, correspond to a weighted Kolmogorov-like statistic. Each lymphoma subtype/subclass was compared against all lymphoma cases using the SNR statistic. The normalized GSEA-enrichment scores for the pathways obtained in this way were clustered using Spearman correlation to generate a heat map representing the functional signatures.

(b) To identify heterogeneity defined by BCR genes, we used Pearson correlation statistic for ranking genes. The external sources of data were http://llmpp.nih.gov/ for MCL [22], FL [5] and CLL [23], and data from Shipp et al. for DLBCL [24].

Results

Gene-expression profiling data-cluster analysis

SOTA and ETE Tree View were used to analyze the expression-profiling data from 187 cases of B-cell NHL. The distribution of cases obtained by SOTA (unsupervised clustering) and subsequently found to differ biologically by GSEA confirmed the currently used B-cell lymphoma classification, and suggested heterogeneity for most B-cell NHL types. The actual distribution of cases classified by Euclidean distance clustering (Table I) is described below.

With very minor exceptions, the molecular subgroups identified here essentially matched the histological classification, although there was heterogeneity within the existing NHL types and overlap of different lymphoma types. For example, NMZL cases were identified within a cluster of FL cases, suggesting that common cell subpopulations and/or pathways could be present in both tumor types.

The molecular subgroups or biological phenotypes for specific lymphoma types characterized here were labeled ‘A’ or ‘B’ for the sake of simplicity, and did not conform to the staging system, and were not intended to establish a basis for a new subclassification of particular lymphoma types.

Interestingly, CLL was divided into two main groups, one of them more closely clustering with MCL and SMZL cases. It should be noted that BL remained a separate entity, possibly due, in part, to the small number of samples.

After establishing the existence of molecular subgroups reproducing B-cell lymphoma classification and establishing some additional heterogeneity, we analyzed differences in the expression signatures of NHL types.

Identification of functional signatures

Lymphoma classes, identified by SOTA unsupervised clustering, were used to explore functional gene sets using GSEA. The enrichment scores for the functional pathways were then clustered using Spearman correlation distance to determine how

Table I. Distribution of 187 cases of B-cell NHL using the Self-Organizing Tree Algorithm (SOTA) and the ETE Tree viewer; each lymphoma type, except Burkitt Lymphoma, was separated into two distinct clusters, labeled ‘A’ and ‘B’.

<table>
<thead>
<tr>
<th>Pathological diagnosis</th>
<th>Cases</th>
<th>Molecular profiling diagnosis</th>
</tr>
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<tbody>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>38</td>
<td>Chronic lymphocytic lymphoma-A 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chronic lymphocytic lymphoma-B 18</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>36</td>
<td>Diffuse large B-cell lymphoma-A 18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diffuse large B-cell lymphoma-B 18</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>38</td>
<td>Mantle cell lymphoma-A 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mantle cell lymphoma-B 30</td>
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<td>Splenic marginal zone lymphoma-A 11</td>
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<td></td>
<td></td>
<td>Splenic marginal zone lymphoma-B 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diffuse large B-cell lymphoma-A 1</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>9</td>
<td>Burkitt lymphoma 9</td>
</tr>
<tr>
<td>Nodal marginal zone lymphoma</td>
<td>6</td>
<td>Follicular lymphoma-B 6</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>33</td>
<td>Follicular lymphoma-A 24</td>
</tr>
<tr>
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<td>Follicular lymphoma-B 9</td>
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</table>
closely related the lymphoma classes were. The gene
sets could be assigned to two major branches: one
expressed mainly by the tumor cell (cell cycle,
cytokines, PIM/mitogen activated protein kinase
(MAPK) pathways, GC, CD40 signaling, BCR,
and AKT); and other, including NFκB and NFκB-
associated pathways along with the stroma pathways,
these being expressed by both tumoral cells and the
microenvironment (Figure 2):

(a) Cell cycle: the signature mainly included the cell
cycle, g2, RAC and CYCLIN D1 pathways,
glycolysis, Krebs cycle, tubulins, and a cluster of
proteasomes. Cell cycle signature was over-
expressed in BL and DLBCL, with intermediate
expression for MCL and SMZL, and under-
expression for CLL and FL. In this cluster,
C-MYC targets were found to be overexpressed
in DLBCL and BL. This cell cycle signature
included some unexpected pathways, such as
those of bone marrow plasma cells, T-cell
anergy, and regulatory T-cells.

(b) Cytokine signaling: the signature included nat-
ural killer (NK) cells and cytotoxic T-lympho-
cytes (CTL) that appear involved with several
important cytokines and interleukins. This
signature was overexpressed in MCL, and DLBCL,
being more heterogeneous in SMZL and CLL.

(c) PIM/MAPK: an interesting sub-cluster within
this cytokine signaling cluster included MAP
kinase, STAT3 and the PIM pathways, and
B-cell and T-cell calcium signaling. The CLL,
MCL, SMZL, and DLBCL tumor types over-
expressed these pathways. The intensity of the
expression of these pathways was strikingly
variable within MCL.

(d) CD40 signaling: this sub-cluster was comprised of
genes activated after CD40 signaling [25].
Tumor types that appeared to show increased
CD40 signaling were CLL, SMZL, MCL, and
DLBCL, with a weaker signal in FL and NMZL.

(e) GC: signature formed by B-cell (including
CD20 and TCL1), phosphorylation of cAMP
response element binding (pCREB), GC
B-cells. These pathways were overexpressed in
BL and in subsets of SMZL, CLL, and MCL.
It is interesting to note that FL-a shows lower
expression for GC B-cell than FL-b and shares
much similarity with NMZL.

(f) BCR pathway: includes the physical components
of BCR and the BCR signaling pathway, and the
 interleukin (IL)7, Phospholipase C (PLC),
and interferon (IFN); pathways. Increased expres-
sion of these genes was observed in cases
diagnosed with CLL, MCL, and SMZL, with
striking variability within each group.

(g) AKT cluster: includes the AKT and WNT
pathways, a cluster of proteasomes and ubiqui-
tins, and the PKC (protein kinase C) pathway.
Increased expression of these clusters was
observed in MCL, DLBCL, and CLL.

(h) Stroma: includes extracellular matrix, integrins,
variable cytokines, and the proteasome pathway.
These pathway clusters were highly expressed
in cases diagnosed with FL, MZL, and in a
lower intensity in DLBCL cases. It is notable
that NFκB target genes were available in
stromal signature and not in NFκB pathways
signature, probably reflecting the fact that
NFκB target genes pathway included cytokines,
interleukins, IRF (interferon regulatory factor)
genes, proteasomes, and stroma genes such as
MMP9, VCAM1, PDGF, and cell membrane
proteins (http://bioinfo.lifl.fr/NF-KB/).

(i) NFκB pathway and apoptosis: this signature
includes CD40, REL-A, the inflammatory path-
way, TALL1 (TNF and ApoL-related leukocyte
expressed ligand 1), TNFR2 (tumor necrosis
factor receptor superfamily 2), death, FAS, Fas
(TNF receptor superfamily, member 6), and
apoptotic pathways. It also includes dendritic
cells and T-cells, and their associated pathways,
and mammalian target of rapamycin (mTOR).
Weaker expression of the genes included in this
cluster was found in BL and DLBCL, whereas
expression was higher in FL and MZL. There
was a group of SMZL, CLL, and MCL that also
showed increased expression of these genes.
Interestingly, this was the same group that has
high expression of pathways in BCR signature.
It is of note that the presence in this group of a
gene set defined as BCL6 targets, which
probably reflects the existence of close mutual
coregulation between BCL6 and NFκB. BCL6
is a transcriptional repressor whose targets show
increased expression in parallel with other genes
belonging to the NFκB pathway [26].

As a corollary, signatures can be indentified
into therapeutic mapping of pathways. Pathways
recognizing multiple potential therapeutic targets,
such as NFκB, AKT, PI3K, BCR, and PIM show
common expression for multiple lymphoma types
and subclasses (Figure 3).

B-cell receptor and coregulated pathways are associated
with gene expression profiling (GEP) changes in multiple
lymphoma types

One of the three major branches of the GEP data is
dominated by the expression of BCR signaling
pathway genes. At the same time, these BCR genes
define a striking heterogeneity in the most common lymphoma types, singularly in CLL, FL, and MCL. This observation is consistent with what is known on the molecular pathogenesis of these B-cell lymphoma types, where signaling from BCR constitutes an essential survival mechanism for the tumoral cells, with both clinical and biological relevance [2, 27–29]. Thus, we have used the mean expression of the BCR coregulated genes for identifying the pathways enriched in genes coregulated with BCR (SYK, BTK, BLNK, CD79B, and MS4A1), using GSEA (Table II and Supplementary Figure S1).

Mantle cell lymphoma. The analysis was done on a set of 99 previously published MCL cases (http://llmpp.nih.gov/) [22]. The expression of BCR genes was associated with numerous pathways and signatures, including BLIMP1-targets, CREB, BCR signaling pathways, blood pan-B-cells, plasmacytoid dendritic cells, CXC chemokine receptor (CxCR)4, and GC T-helper.

Chronic lymphocytic leukemia. The analysis of 107 cases from this external dataset [23] showed MAPK, mesenchymal–epithelial transition (MET) factor pathways, and BLIMP1 targets to be associated with BCR expression.

Follicular lymphoma. One hundred ninety-one cases of FL [5] (http://llmpp.nih.gov/) were analyzed. The most significant pathways and signatures whose expression was associated with BCR genes were BLIMP1 targets, C-MYC targets, GC B-cells, and blood pan-B cells. Positive correlation with BCR defines a BCR signature whereas negative correlation with BCR follows a stromal signature in FL.

Diffuse large B-cell lymphoma. The set of 176 published cases by Shipp et al. [24] was analyzed. BLIMP1 targets, C-MYC targets, GC B-cells, and blood pan-B cells were the clusters significantly associated with BCR genes.

Figure 3. Signatures recognizing potential therapeutic targets. Multiple targets, such as those represented in the figure, seem to be overexpressed by different lymphoma types. This suggests the existence of common lymphoma oncogenic mechanisms that are relatively independent of the specific histological type, and that could be proposed as potential targets for therapy.
Discussion

In this study, we have defined gene-expression profiling signatures that underlie the biological heterogeneity within major B-cell NHL types in a single heatmap, and presented a holistic view of some of the molecular mechanisms that underlie B-cell lymphoma pathogenesis. This heatmap identifies two major branches: one including gene sets expressed mainly by the tumor cell (cell cycle, GC, MAPK/PIM, BCR, and CD40 signaling, AKT, cytokines) and the other including NFκB and NFκB-associated pathways along with the stroma pathways, these being expressed by both tumoral cells and the microenvironment. It is of particular note that most of the histologically defined B-cell lymphoma types exhibit some degree of molecular heterogeneity, which may be associated with the clinical variability of these tumors. They suggest new opportunities for developing targeted therapeutic approaches to B-cell lymphoma. A cell-cycle signature is overexpressed in BL, DLBCL, and a subset of MCL and SMZL. Deregulation of cell-cycle control genes has been described as being the hallmark of DLBCL and BL cases [30], and has been identified as an important characteristic of a subset of MCL [22] and SMZL [31] cases with increased aggressivity.

GC signatures are also more strongly expressed by BL and a subset of FL, DLBCL, MCL, CLL, and SMZL, which confirms the variability in the level of...
GC differentiation in DLBCL [3], and extends this finding to the major NHL types, hypothetically in relation to somatic hypermutation. No clear overlapping of this DLBCL subclassification was found with the GC/ABC types as defined by Wright et al. [32], although one of the DLBCL subgroups (DLBCLa) expressed a stronger BCL6 targets signature.

Microenvironment signatures comprised of both stroma and inflammatory signatures grouped under the name of NFκB are overexpressed in all FL and NMZL cases, while identifying subsets of MCL, CLL, SMZL, and DLBCL. This confirms previous reports of CLL [13], FL [5], and DLBCL [3,33], and extends these observations to MCL and SMZL. No clear correlation between inflammatory signatures in FL and those defined by Dave et al. was found [5].

These data also identify a cluster of gene sets, including AKT and WNT pathways, clustered with ubiquitins and proteasome genes, which are mainly expressed by DLBCL and several types of small B-cell lymphomas. These are potential therapeutic targets, as recently demonstrated through the use of sirolimus, an AKT inhibitor [34].

BCR signaling and the associated pathways are expressed strongly by subgroups of MCL, CLL, and with lower intensity by DLBCL and FL. This is consistent with the findings of several groups, who have shown that stimulatory and growth signals, delivered by the BCR, allow normal and neoplastic B-cells to avoid apoptosis and proliferate, thus constituting the driving force for B-cell lymphoma survival [27,35–37]. It is of particular note that the intensity of the BCR signaling pathways changes within and among specific histological entities. Variation in this expression of the BCR-signaling pathway has been related to clinical outcome in CLL [27], and identified as a potential therapeutic target in DLBCL [2]. The observations of this study add FL and MCL as B-cell lymphoma types where changes in the expression of BCR signaling pathway could contribute to the biological and clinical variability of these neoplasms.

Interestingly, the BLIMP1 target gene set appears to be one of the most significant pathways that are coregulated with BCR. The study of BLIMP1-deficient B-cells has identified hundreds of genes deregulated in the absence of BLIMP1, including PAX5, BCL6, C-MYC, and SPIB, thus showing that BLIMP1 promotes plasma-cell differentiation by silencing the B-cell program [38].

The clustering of functional pathways yields also information about potential therapeutic targets. For example, CLL expresses genes grouped with AKT, PIM, and MAP kinases; however, only a subset of patients from CLL can express the genes clustered into the BCR and NFκB pathway, thus suggesting both therapeutic targets and gene sets recognizing tumor types that can be used for further therapeutic preclinical studies.

In summary, we have identified functional signatures that distinguish the different lymphoma types, and that recognize cell cycle, apoptosis, cytokine–cytokine-receptor interaction, BCR, NFκB activation, and other functional signatures. These signatures reveal subclasses of diagnosed lymphoma types, illustrating the existence of a distinct functional heterogeneity among CLL, MCL, FL, and DLBCL. Moreover, some of the most relevant signatures (stroma, cell cycle, BCR) are shared by multiple lymphoma types or subclasses. The comparison of these lymphoma clusters yielded pathways, some of which are already known and others are new potential markers and therapeutic targets. We expect the identification of these functional signatures in B-cell lymphomas to lead to a better understanding of common mechanisms, which should help to explain in greater depth the biological basis underlying the clinical variability in B-cell lymphomas. A potential application of this is the identification of multiple therapeutic targets in the different lymphoma types.

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References


