Gene expression analysis provides a potential rationale for revising the histological grading of follicular lymphomas

Pier Paolo Piccaluga,[1,2] Andrea Califano,[3] Ulf Klein,[2] Claudio Agostinelli,[2] Beatriz Bellosillo,[4,5] Eva Gimeno,[6] Sergi Serrano,[4,5] Francisco Solè,[4,5] Yonghui Zang,[7] Brunangelo Falini,[5] Pier Luigi Zinzani,[1] and Stefano A. Pileri[1]

[1]Department of Hematology and Oncology "L. and A. Seràgnoli", Hematopathology and Hematology Units, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy; [2]Institute for Cancer Genetics, [3]Center for Computational Biology and Biochemistry, [7]The Herbert Irving Comprehensive Cancer Center, Columbia University, NY, USA; [4]Unitat de Recerca en Neoplàsies Hematològiques-Parc Recerca Biomèdica Barcelona (URNHE-PRBB), Barcelona, Spain; [5]Laboratori de Citogenètica i Biologia Molecular, Departament de Patologia, [6]Clinical Hematology Department, Hospital del Mar, IMAS, Barcelona, Spain and [6]Hematopathology, Insitute of Hematology, Monteluce Hospital, Perugia University, Perugia, Italy

Acknowledgments: the authors are grateful to Prof. Riccardo Dalla Favera and Dr. Katia Basso for precious advice and discussion and to Vladan Milkjovic for his skilled technical assistance.

Funding: this work was supported by grants from AIRC, BolognAIL, FIRB/RFO (to SAP and PLZ), Progetto Strategico di Ateneo 2006 (to PPP), Fondazione del Monte di Bologna e Ravenna, and Fondazione Cassa di Risparmio in Bologna.
PPP was also supported by a Rotary International-The Rotary Foundation Academic Year Scholarship, and "Vanda Vanini e Sandro Cavagnino" and "Cristina Bassi Association" grants.

Manuscript received January 15, 2008. Revised version arrived on February 14, 2008. Manuscript accepted February 25, 2008.

Correspondence:
Pier Paolo Piccaluga, MD, PhD, Head, Molecular Pathology Laboratory, Unit of Hematopathology, Department of Hematology and Oncology "L. and A. Seràgnoli", S. Orsola Malpighi Hospital, University of Bologna Via Massarenti, 9, 40138 Bologna, Italy.
E-mail: pierpaolo.piccaluga@unibo.it

The online version of this article contains a supplementary appendix.

ABSTRACT

Background

Follicular lymphomas are currently subdivided into grades I, II, IIIa and IIIb. This distinction is, however, questioned.

Design and Methods

We studied the gene expression profile of 43 follicular lymphomas, 50 B-cell non-Hodgkin's lymphomas of different histotype, and 20 samples of normal B-lymphocytes in order to assess: (i) the relationship of follicular lymphoma with normal B cells and other B-cell non-Hodgkin's lymphomas; (ii) whether follicular lymphoma is a unique disease; and (iii) whether follicular lymphoma grade IIIb is closer to follicular lymphoma or diffuse large B-cell lymphoma of the germinal center B-cell type.

Results

First, we found that the molecular profile of follicular lymphoma is intimately related to that of normal germinal center B cells, irrespectively of the histological grade. Secondly, we observed that follicular lymphoma has a relatively homogeneous gene expresion profile that is distinct from that of other B-cell non-Hodgkin's lymphoma and does not include discrete molecular subgroups. However, by further clustering samples according to signatures differentially expressed among follicular lymphomas or in follicular lymphomas versus diffuse large B-cell lymphoma, we showed that grade I-IIIa tumors tend to cluster together, while grade IIIb follicular lymphoma constitutes a distinct subgroup, whose molecular signature is closer to that of the remaining follicular lymphomas than to that of diffuse large B-cell lymphoma of the germinal center B-cell type.

Conclusions

These data support the hypothesis that grade IIIb follicular lymphoma does indeed belong to the group of follicular lymphomas rather than diffuse large B-cell lymphomas, and also suggests a possible revision of the histological grading of follicular lymphomas, with their simple distinction into follicular lymphoma (grade I-IIIa) and follicular lymphoma/large cell (grade IIIb).

Key words: follicular lymphoma, non Hodgkin's lymphoma, histological grading, DNA microarray

Citation: Piccaluga PP, Califano A, Klein U, Agostinelli C, Bellosillo B, Gimeno E, Serrano S, Solè F, Zang Y, Falini B, Zinzani PL, and Pileri SA. Gene expression analysis provides a potential rationale for revising the histological grading of follicular lymphomas. Haematologica 2008; 93:1033-1038. doi: 10.3324/haematol.12754

Introduction

Follicular lymphoma (FL) is the commonest form of indolent lymphoid tumor and the second most frequent histotype of non-Hodgkin's lymphoma (NHL) in western countries.[1] Neoplastic cells resemble centroblasts and centrocytes of normal germinal centers and show a growth pattern that may be follicular (>70%), follicular and diffuse (25%) or diffuse (<5%). Based on the amount of centroblasts, FL is usually classified as grade (G) I, II, IIIa and IIIb.[1] Even though the criterion for this classification appears objective, grading has a notoriously poor reproducibility among community pathologists as well as experienced hematopathologists (agreement 61%-73%).[2] This reflects the variability of the field width among different high power objectives (i.e. x40), as well as the difficulties sometimes encountered in assessing the exact number of centroblasts due to the occurrence of large centrocytes and small centroblasts. Thus, the rationale for FL grading is still matter of debate. Interestingly, according to morphology, phenotype and genetic features (i. e. the presence of ongoing immunoglobulin somatic hypermutations) FL is currently supposed to derive from germinal center B cells, although no data are available in this respect as regards the global molecular profile.

FL typically has an indolent course, with the median survival of affected patients being 7 years, but is virtually incurable with conventional therapies. Furthermore, 25–60% of cases may transform into an aggressive diffuse large B-cell lymphoma (DLBCL), with a very poor prognosis.[1] Though the International Prognostic Index (recently specifically modified for FL) is of some use, to date there are no reliable markers of risk of transformation or factors predictive of response to therapy and survival; furthermore, the molecular mechanisms leading to drug resistance and transformation are not well known.[6] Recently, gene expression studies showed that clinical aggressiveness can be associated with specific molecular signatures partially independent from histological grade[7] and that immune reactive cells can play a major role in determining the outcome.[8] Subsequently, the role of non-neoplastic components was also highlighted by independent tissue microarray analyses.[9-11] However, to date, gene expression studies have shown that clinical aggressiveness is possibly dependent on reactive non-neoplastic components, but have not provided a molecular rationale for histological grading[7,8] and it is still debated whether FL GIIIb should truly be considered a FL or whether it should be included within DLBCL.

In this study, we performed a gene expression analysis of 43 FL, 50 B-NHL of different types, and 20 samples of normal B-lymphocytes in order to assess: (i) the relationship of FL with normal B cells and other B-cell malignancies; (ii) whether FL is a unique disease; and (iii) whether FL GIIIb should be considered within the frame of FL or DLBCL.

Design and Methods

Case selection

Forty-three cases of FL were studied. In 37 cases, cryopreserved tissue blocks were used and in the other six cases enriched neoplastic cells.[12] The diagnosis and grading of FL was made according to the World Health Organization (WHO) classification by four experienced hematopathologists (*CA, SS, BF* and *SAP*). Notably, the four examples of GIIIb FL showed a pure follicular growth pattern and positivity for BCL2. All the samples were obtained at the time of diagnosis, before administration of treatment. The clinical and histopathological characteristics of the 37 FL cases for which whole genome profiling was performed are summarized in Table 1. In addition, samples of normal B-cell subpopulations including centroblasts (n=5), centrocytes (n=5), naïve cells (n=5), and memory cells (n=5) were studied.[13] Finally, a panel of B-NHL including Burkitt's lymphoma (BL, n=4), DLBCL (n=16), mantle cell lymphoma (MCL, n=10), hairy cell leukemia (HCL, n=10), and B-cell chronic lymphocytic leukemia (B-CLL, n=10) were studied. Of note, the samples of these cases (excluding the HCL) consisted of enriched neoplastic cells. Details on their characteristics have been reported previously.[12, 14] Finally, *in silico* data concerning 37 cases of germinal center B-cell type (GCB) DLBCL were retrieved from URL *http://www.ncbi.nlm.nih.gov/projects/geo/*.[15] These cases were randomly selected from among those with a diagnosis of GCB-DLBCL (as we could show a consistent relation of FL with germinal center cells) and no *MYC* rearrangements or the molecular signature of BL. For proper comparison with our samples, gene expression values were normalized *per gene* and *per chip*. In addition, analysis of control genes revealed no significant differences between the two series.

Informed consent to these studies was obtained from all the enrolled patients and the tissue collection was approved by each institutional ethical committee.

Gene expression analysis

Gene expression profiles were generated and analyzed as previously reported[12,14,16] (*for details, see the online Supplementary appendix*). Fragmented cRNA was hybridized to HG-U133 2.0 plus (samples from 37 FL and 20 normal B cells) or to HG-U95Av2 (samples from 4 BL, 16 DLBCL, 10 B-CLL, 10 MCL, 10 HCL, 6 FL, and the same above mentioned 20 normal B-cell samples) microarrays (Affymetrix, Inc. available at URL *http://www.affymetrix.com/support/index.affx*). The gene expression values were determined by the MAS 5 algorithm in GCOS 1.2 (Affymetrix, Inc.).

Gene expression studies were conduced according to MIAMI guidelines. Raw gene expression data will be available at *http://www.ncbi.nlm.nih.gov/projects/geo/* (accession number GSE6338).

Results

Relationship between follicular lymphoma, normal B cells and other B-cell malignancies

We first performed an unsupervised analysis and studied normal B cells (n=20) and cases of FL (n=6), BL (n=4), DLBCL (n=16), MCL (n=10), B-CLL (n=10) and HCL (n=10). According to the global gene expression profile FL turned out to be clearly distinct from other B-cell derived malignancies (*Online Supplementary Figure S1*). As expected, FL was closer to germinal center related B-NHL (BL and DLBCL) than to tumors more related to naive or memory B cells (MCL, B-CLL and HCL).

Secondly, in order to evaluate the relationship between FL and normal B cells we applied a molecular classifier previously used to identify possible counterparts of lymphoid neoplasia.[12,13,16] As expected, we found that FL has a gene expression profile closer to that of GC cells rather than to naive and memory B-cells (Figure 1). However, according to the indolent behavior of the tumor, different from that of the other GC B-cell derived lymphomas (i.e. BL and GCB-DLBCL) and more similar to the one of post-GCB tumors (such as HCL), we found that many cellular programs are regulated in FL as in memory B cells (Figure 1C, *Online Supplementary Table S1*). Comparable results were obtained when analyzing enriched neoplastic cells from patients with FL (Figure 1) or frozen lymph-nodes (*not shown*). As a clear differentiation between centroblasts and centrocytes is still problematic,[13] it was not possible to definitely establish the origin of FL from these cells. Nevertheless, our analysis showed that FL is possibly closer to centrocytes rather than to centroblasts, independently of the grade (*Online Supplementary Figure S2*). Of note, even GIIIb cases, by definition constituted by a large cell population, appeared to be closer to centrocytes (*Online Supplementary Figure S2*).

Details on gene profiling of normal B cells have been already reported.[13]

Follicular lymphoma is a single disease

We investigated whether FL is a unique entity or comprises discrete molecular subgroups. To address this issue, we used unsupervised hierarchical clustering to study the 37 cases of FL for which whole genome profiling was performed. We found that FL had a relatively homogeneous gene expression profile, independently of their grade (Figure 2A). In particular, the GIIIb cases did not constitute a separate cluster but were scattered within the other cases (Figure 2A). However, when

Table 1. Main clinical and histopathological characteristics of the 37 follicular lymphoma for which whole genome profiling was performed.

	All grades	*Grade I-IIIa*	*Grade IIIb*
Cases	37	33	4
Median age, years (range)	44 (26-78)		
Male/female	20/17	18/15	2/2
FLIPI			
0-1	12	10	2
2	6	4	2
3	11	11	-
4-5	8	8	-
Stage			
I	1	1	-
II	5	3	2
III	5	3	2
IV	26	26	-
BCL2 positivity at immunohistochemistry	36/37	32/33	4/4
Median PFS, months	19	19	37
Median OS, months	46	45	38

FLIPI: follicular Lymphoma International Prognostic Index; PFS: progression-free survival; OS: overall survival.

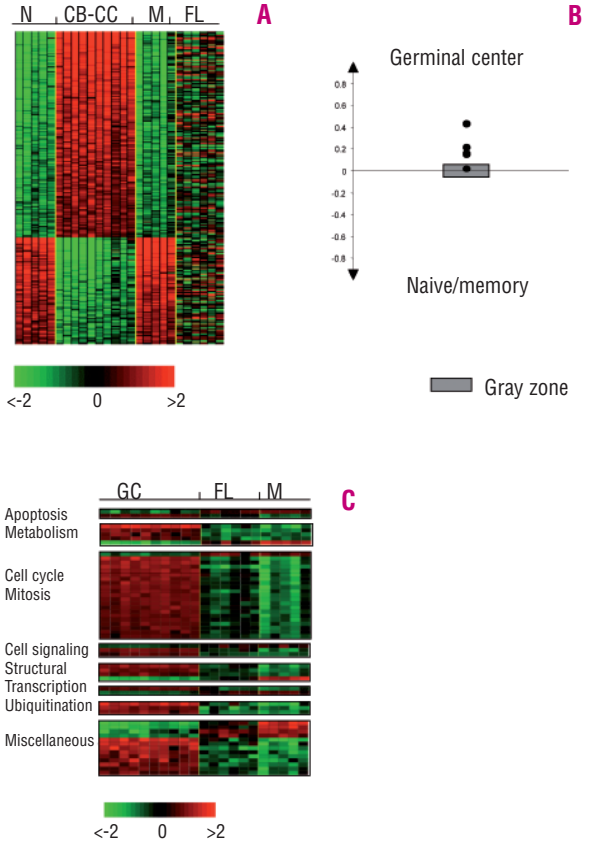

Figure 1. Relatedness of the gene expression profile of follicular lymphoma (FL) to that of normal B-cell populations. Analysis in FL of genes associated in normal B-cells to the GC transitions. The genes that are differentially expressed in naive (N), memory (M), and GC B-cells during the GC transit were identified by supervised analysis. The expression of the transition genes was investigated in FL. FL is more related to GC B cells than to naive or memory B cells. Centroblasts (CB); centrocytes (CC). (A) The expression of the selected genes was investigated in enriched FL cases represented on the right side of the matrix. (B) A cell-type classification was used to measure the relatedness of FL to naïve/memory and GC B-cells. The gray area marks 95% of confidence: the *p*-value decreases with increasing distance from the x axis. (C) Some genes, differentially expressed in GC *vs.* memory cells, are regulated in FL as in memory B cells.

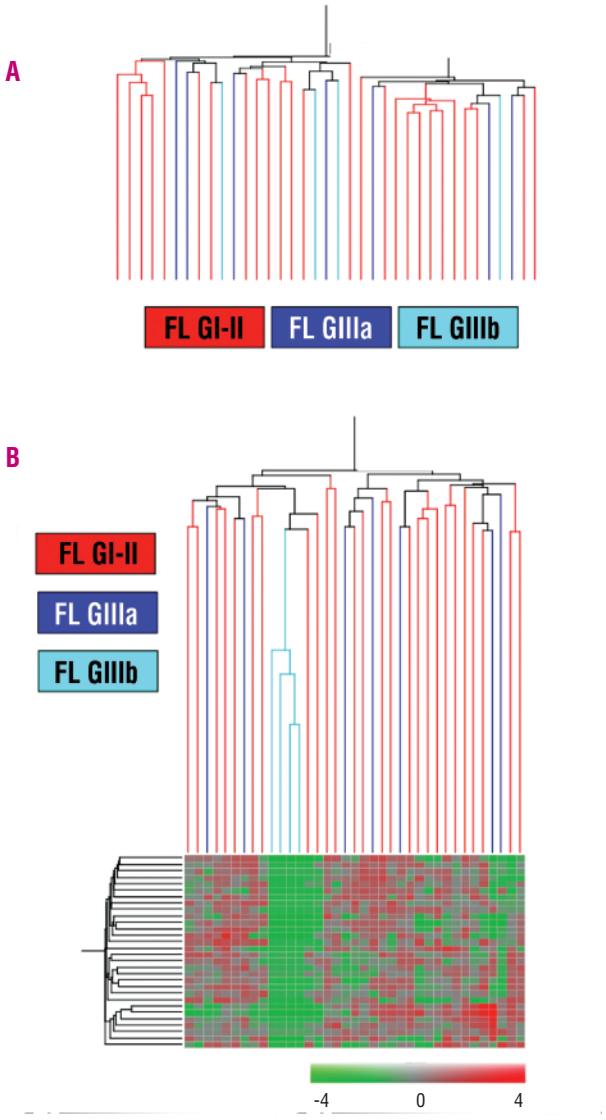

Figure 2. Hierarchical clustering of follicular lymphoma (FL) (A) Unsupervised analysis performed on 37 FL samples. The samples are clustered according to the expression of 354 genes. The samples do not cluster together based on the histological grade. (B) Hierarchical clustering of 37 FL cases. The samples are clustered according to the expression of 30 genes which emerged from supervised analysis comparing GI-II *vs.* GIIIa *vs.* GIIIb (ANOVA test, one-way test with Bonferroni's correction for multiple testing and a family-wise error rate of 0.01). The dendrograms were generated using a hierarchical clustering algorithm based on the average-linkage method. In the matrix, each column represents a sample and each row represents a gene. The color scale bar shows the relative gene expression changes normalized by the standard deviation (0 is the mean expression level of a given gene). GIIIb constitute a discrete subgroup while GIIIa are scattered within GI-II cases.

supervised analysis (ANOVA test) - a stronger methodology for identifying molecular signatures of specific subgroups of samples - was applied, comparing FL of different histological grades (GI-II *vs.* GIIIa *vs.* GIIIb), a signature (30 genes) able to discriminate GIIIb forms was identified, whereas GI-II and GIIIa cases were still mixed up (Figure 2B) (*Online Supplementary Table 2S*).

Grade IIIb folicular lymphoma is closer to folicular lymphoma than to diffuse large B-cell lymphoma

There have been some suggestions that GIIIb FL should be reclassified as DLBCL with follicular morphology.[17] We, therefore, investigated whether GIIIb FL is more related to GI-IIIa FL or to DLBCL. In order to address this issue, we first applied unsupervised hierarchical clustering to 37 FL and 37 GCB-DLBCL cases. We found that GIIIb FL are closer to other FL than to GCB-DLBCL, according to the global gene expression profiling as all the cases clustered within the FL group (Figure 3A). We then clustered the same cases on the basis of genes differentially expressed in FL (GIIIb excluded) and GCB-DLBCL - using either tissue samples or enriched cases - and previously identified by supervised comparison (*Online Supplementary Tables S3 and S4*). Again, GIIIb FL clustered within the other FL (Figure 3B and *Online Supplementary Figure S3A*). Finally, we generated a molecular signature distinguishing GIIIa FL and GCB-DLBCL by supervised analysis (268 genes, *Online Supplementary Table S5*). According to the expression of these genes, GIIIb FL again appeared to be closer to FL rather than to DLBCL (*Online Supplementary Figure S3B*).

Grade IIIa folicular lymphoma are closer to grade I-II rather than to grade IIIb folicular lymphoma

Whether GIIIa FL should be classified within GI-II FL or GIIIb FL is currently under debate. We, therefore, investigated at a molecular level whether GIIIa are closer to the former or to the latter based on gene expression profiling. To this end, we performed a hierarchical clustering of FL using a gene list obtained by supervised comparison of GI-II *vs.* GIIIb cases (*Online Supplementary Table S6*). We found that GIIIa tumors were definitely closer to the indolent forms, being scattered among them within the same cluster (*Online Supplementary Figure S4A*). In addition, we applied a cell type classification method and confirmed that GIIIa FL are closer to GI-II FL than to GIIIb FL (*Online Supplementary Figure S4B*).

Discussion

In this study, we performed a gene expression analysis of FL, normal B-cell subpopulations and a consistent panel of other B-NHL, and addressed specific issues of FL biology. First of all, we showed that although the global profile confirmed the relation of FL with germinal center B cells, many relevant programs were regulated as in resting (memory) B cells (Figure 1C), consistently with an indolent clinical behavior. To understand whether this phenomenon corresponds to an initial differentiation into memory cells or an aberrant malignant phenotype, further studies are needed. Secondly, we provide relevant information which could be useful for a future classification of FL. Cases of FL are currently subdivided into grades I, II, and III according to the number of large cells.[1] Different therapeutic approaches are sometimes applied to the *indolent* (GI-II) and *aggressive* (GIII) forms.[18] Furthermore, GIII FL are divided into GIIIa and GIIIb forms, although some authors consider

that the latter should be reclassified as DLBCL with follicular morphology.[17] Notably, GIIIb cases have also been reported to have peculiar clinical features.[19]

In the last few years, FL have been studied by gene expression profiling by different groups. For example, de Jong *et al.* analyzed the molecular basis of clinical aggressiveness and an expression profile of 81 genes was established that could, with an accuracy of 100%, distinguish low-grade from high-grade disease.[10] In another study it was shown that non-neoplastic components are probably relevant to the clinical outcome, but the roles of single cellular populations could not be clearly defined even though macrophages appeared to be significantly involved.[8-10,20,21] In addition, the molecular basis of transformation to DLBCL has been extensively analyzed.[22-24] However, although important new elements for prognostication have been discovered and are currently being evaluated in a prospective manner, a molecular rationale for a FL classification had not been identified yet.

Our molecular analysis shows, for the first time, that the gene expression profile of FL is relatively homogeneous, independently of the histological grade (Figure 2). However, we also found that GIIIb cases constitute a clearly distinct subgroup among FL (Figure 2B) and, most importantly, showed that they should be still considered as FL since they appeared to be closer to FL than to DLBCL (Figure 3 and *Online Supplementary Figure S3*). Nevertheless, two out of four GIIIb cases clustered close to GCB-DLBCL, indicating the potential existence of similarities. Certainly, our observations warrant further validation in an independent context, given the limited number of GIIIb FL cases analyzed.

Interestingly, when looking for biological processes possibly regulated by genes belonging to the molecular signatures able to distinguish among FL with different histological grade (*Online Supplementary Table S2*), it appears that some categories, such as protein/macromolecule biosynthesis, metabolism, cellular morphogenesis, cell cycling and cell-growth and maintenance, are differentially expressed in GI-II *vs.* GIII tumors. However, no specific functional category can be found to be significantly over-represented in those signatures. On the other hand, at present, we cannot discriminate between FL GI-IIIa and FL GIIIb based on immunohistochemical markers.

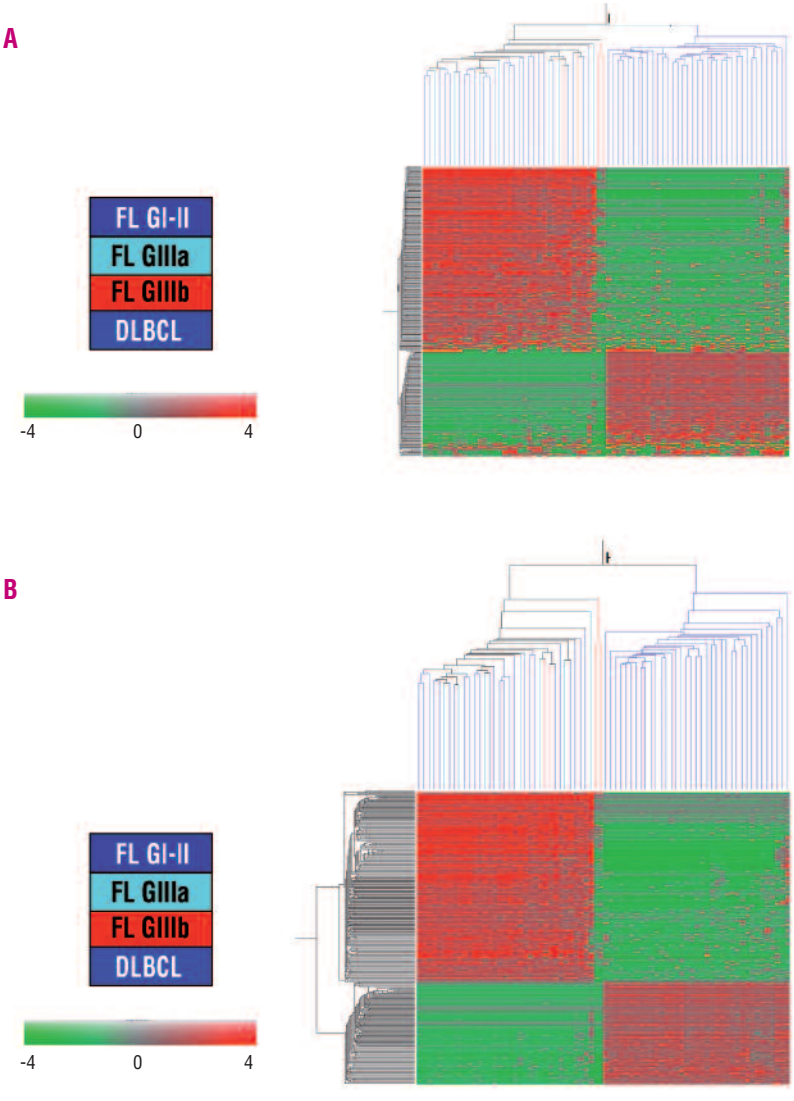

Figure 3. Follicular lymphoma (FL) GIIIb is indeed closer to FL than to diffuse large B-cell lymphoma based on gene expression profiling. (A) Unsupervised analysis performed on 37 FL and 37 GCB-DLBCL samples. The samples are clustered according to the expression of 544 genes. FL GIIIb actually cluster together with the other FL rather than with DLBCL. (B) Hierarchical clustering of 37 FL and 37 GCB-DLBCL cases. The samples are clustered according to the expression of 500 genes that emerged from supervised analysis comparing GI, GII and GIIIa FL *vs.* GCB-DLBCL (ANOVA test, one-way test with Bonferroni's correction for multiple testing and a family-wise error rate of 0.01). As in the previous analysis, FL GIIIb actually cluster together with the other FL rather than with DLBCL.

In conclusion, our molecular analysis showed that: (i) FL has a molecular profile distinct from other B-cell malignancies and consistently related to germinal center B cells; (ii) FL is a single disease; in particular, GI-IIIa FL tend to cluster together, with GIIIa being closer to GI-II FL than to GIIIb FL; and (iii) GIIIb FL cases constitute a distinct subgroup whose molecular signature is, nevertheless, more similar to that of the other FL than to that of GCB-DLBCL. Taken together, these data do not justify lumping FL GIIIb with DLBCL and support a possible revision of histological grading of FL, which might simply have two categories: FL and FL/large cell, corresponding to the present grades I-IIIa and IIIb, respectively. Further analyses on larger independent panels of cases are warranted.

Authorship and Disclosures

PP was responsible for the study design, gene expression analysis and drafting the article; UK, AC and YZ were responsible for gene expression generation and analysis; CA, BB, SS and FS were responsible for data and sample collection; EG was responsible for patients' care; BF took part in case selection and evaluation; PLZ was responsible for patients' care and funding; SAP was responsible for study design, drafting the article, and funding. The authors have no conflicting financial interests to declare.

References

1. Nathwani BN, Harris NL, Weisenburger D, Isaacson PG, Muller-Hermelink HK, Gerger F, et al. Follicular Lymphoma. Lyon: IARC Press. 2001.
2. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. Blood 1997;89:3909-18.
3. Tiesinga JJ, Wu CD, Inghirami G. CD5+ follicle center lymphoma. Immunophenotyping detects a unique subset of "floral" follicular lymphoma. Am J Clin Pathol 2000; 114:912-21.
4. Rowley JD. Chromosome studies in the non-Hodgkin's lymphomas: the role of the 14;18 translocation. J Clin Oncol 1988;6:919-25.
5. Aster JC, Longtine JA. Detection of BCL2 rearrangements in follicular lymphoma. Am J Pathol 2002;160: 759-63.
6. Solal-Celigny P, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, et al. Follicular lymphoma international prognostic index. Blood 2004; 104:1258-65.
7. Glas AM, Kersten MJ, Delahaye LJ, Witteveen AT, Kibbelaar RE, Velds A, et al. Gene expression profiling in follicular lymphoma to assess clinical aggressiveness and to guide the choice of treatment. Blood 2005;105: 301-7.
8. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med 2004; 351:2159-69.
9. Farinha P, Masoudi H, Skinnider BF, Shumansky K, Spinelli JJ, Gill K, et al. Analysis of multiple biomarkers shows that lymphoma-associated macrophage (LAM) content is an independent predictor of survival in follicular lymphoma (FL). Blood 2005; 106:2169-74.
10. de Jong D. Molecular pathogenesis of follicular lymphoma: a cross talk of genetic and immunologic factors. J Clin Oncol 2005;23:6358-63.
11. Lee AM, Clear AJ, Calaminici M, Davies AJ, Jordan S, MacDougall F, et al. Number of CD4+ cells and location of forkhead box protein P3-positive cells in diagnostic follicular lymphoma tissue microarrays correlates with outcome. J Clin Oncol 2006;24: 5052-9.
12. Basso K, Liso A, Tiacci E, Benedetti R, Pulsoni A, Foa R, et al. Gene expression profiling of hairy cell leukemia reveals a phenotype related to memory B cells with altered expression of chemokine and adhesion receptors. J Exp Med 2004;199:59-68.
13. Klein U, Tu Y, Stolovitzky GA, Keller JL, Haddad J Jr, Miljkovic V, et al. Transcriptional analysis of the B cell germinal center reaction. Proc Natl Acad Sci USA 2003;100:2639-44.
14. Klein U, Tu Y, Stolovitzky GA, Mattioli M, Cattoretti G, Husson H, et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. J Exp Med 2001;194:1625-38.
15. Hummel M, Bentink S, Berger H, Klapper W, Wessendorf S, Barth TF, et al. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. N Engl J Med 2006; 354:2419-30.
16. Piccaluga PP, Agostinelli C, Califano A, Rossi M, Basso K, Zupo S, et al. Gene expression analysis of peripheral T cell lymphoma, unspecified, reveals distinct profiles and new potential therapeutic targets. J Clin Invest 2007;117:823-34.
17. Bosga-Bouwer AG, van den Berg A, Haralambieva E, de Jong D, Boonstra R, Kluin P, et al. Molecular, cytogenetic, and immunophenotypic characterization of follicular lymphoma grade 3B; a separate entity or part of the spectrum of diffuse large B-cell lymphoma or follicular lymphoma? Hum Pathol 2006;37:528-33.
18. Ganti AK, Weisenburger DD, Smith LM, Hans CP, Bociek RG, Bierman PJ, et al. Patients with grade 3 follicular lymphoma have prolonged relapse-free survival following anthracycline-based chemotherapy: the Nebraska Lymphoma Study Group experience. Ann Oncol 2006;17:920-7.
19. Chau I, Jones R, Cunningham D, Wotherspoon A, Maisey N, Norman AR, et al. Outcome of follicular lymphoma grade 3: is anthracycline necessary as front-line therapy? Br J Cancer 2003;89:36-42.
20. Alvaro T, Lejeune M, Camacho FI, Salvado MT, Sanchez L, Garcia JF, et al. The presence of STAT1-positive tumor-associated macrophages and their relation to outcome in patients with follicular lymphoma. Haematologica 2006;91:1605-12.
21. Alvaro T, Lejeune M, Salvado MT, Lopez C, Jaen J, Bosch R, et al. Immunohistochemical patterns of reactive microenvironment are associated with clinicobiologic behavior in follicular lymphoma patients. J Clin Oncol 2006;24:5350-7.
22. Lossos IS, Alizadeh AA, Diehn M, Warnke R, Thorstenson Y, Oefner PJ, et al. Transformation of follicular lymphoma to diffuse large-cell lymphoma: alternative patterns with increased or decreased expression of c-myc and its regulated genes. Proc Natl Acad Sci USA 2002;99:8886-91.
23. Martinez-Climent JA, Alizadeh AA, Segraves R, Blesa D, Rubio-Moscardo F, Albertson DG, et al. Transformation of follicular lymphoma to diffuse large cell lymphoma is associated with a heterogeneous set of DNA copy number and gene expression alterations. Blood 2003;101:3109-17.
24. de Vos S, Hofmann WK, Grogan TM, Krug U, Schrage M, Miller TP, et al. Gene expression profile of serial samples of transformed B-cell lymphomas. Lab Invest 2003;83:271-85.