Molecular pathogenesis of follicular lymphoma

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Follicular lymphoma (FL) accounts for 20%-30% of all lymphoid tumors with the highest incidence in Western countries. Despite recent improvements, FL is still incurable and the clinical course is characterized by repeated relapses and ultimate failure to respond to therapy or by biological and clinical progression to high-grade lymphoma. An interesting report in the current issue of the journal by Gagyi and co-workers[1] sheds light on molecular features of a less well studied subset of FL that lack the classical translocation t(14;18)(q32;q21).

Pathology of follicular lymphoma

Biologically, FL represents the neoplastic equivalent of the normal germinal center (GC) reaction, recapitulating the cellular composition of reactive follicles. Accordingly, neoplastic follicles are composed of constituents of the reactive GC, centroblasts and centrocytes, albeit with varying and abnormal ratios. A lymph node infiltrate of FL consists of closely packed atypical neoplastic follicles that, in contrast to the normal germinal center, are only vaguely separated from the surrounding T-zone area. In some cases, the characteristic follicular pattern is lost either in the early phase of the disease or during progression, and the tumor grows partially or even predominantly diffuse. Even in the latter cases, however, some neoplastic follicles are still discernible allowing for a correct diagnosis. For grading purposes of FL, the number of basophilic blasts that are present in the follicles serves as a means of stratification. FL with 0-5 blasts per high-power field are classified as grade 1, if the blast count is between 6 and 15, as grade 2, and if it exceeds 15, as grade 3. Grade 3 is further subdivided according to the presence (grade 3A) or absence (grade 3B) of typical centrocytes.[2]

The t(14;18) chromosome translocation in follicular lymphoma

The t(14;18)(q32;q21) chromosome translocation represents the defining cytogenetic hallmark of FL and is encountered in 80%-90% of cases. Its molecular consequence is the juxtaposition of the B-cell lymphoma/leukemia 2 (*BCL2*) proto-oncogene with enhancer sequences of the immunoglobulin heavy chain gene (*IGH*) promoter region, thereby deregulating its expression and resulting in an overexpression of the BCL2 protein in the neoplastic follicles.[3,4] What is the biological significance of this event? The *BCL2* proto-oncogene, a potent anti-apoptotic molecule, is expressed in resting B cells in the perifollicular mantle zone and in post-follicular B cells, thereby promoting long-lived follicular precursor and memory B cells. Germinal center B cells, however, physiologically lack BCL2 expression and undergo apoptosis unless they are selected by specific antigens that drive them into processes termed somatic hypermutation and class switching. Due to the lack of BCL2 expression, amongst other factors, the large bulk of B-cells entering the GC microenvironment will be removed by apoptosis. The constitutive overexpression of BCL2 in germinal center B cells inferred by the t(14;18)(q32;q21) leads to an accumulation of inappropriately rescued B cells with a prolonged life span, allowing for the development of additional genetic hits to occur, that are required for the establishment of overt FL. Variant translocations of the t(14;18), such as the t(2;18) or t(18;22), juxtapose *BCL2* to the loci of the immunoglobulin light chains (κ,λ) and, likewise, result in inappropriate and sustained BCL2 expression in GC B cells.

As mentioned above, the presence of the t(14;18) itself is not sufficient to cause neoplastic transformation of B cells, since *BCL2* transgenic mice develop lymphomas only after long latency periods and after secondary chromosome alterations.[5] Moreover, t(14;18)-positive B cells can be identified in the blood and lymphoid tissues of healthy individuals, and the number of t(14;18)-positive cells is influenced by gender, personal lifestyle and exposure to toxic substances.[6]

The early phase of follicular lymphoma

The occurrence of the t(14;18) in a pre-FL B cell can be viewed as a first hit in a multistep process that results in the clonal dysregulation of cell cycle control and apoptosis of the tumor cells. During this multistep process of lymphomagenesis, a number of additional genetic or epigenetic events occur in a non-random fashion that lead to overt FL and/or progression. For example, constitutive expression of activation-induced cytidine deaminase (AID) in the GC environment in B cells overexpressing BCL2 may propagate continuous somatic hypermutation and class switch recombination activity that results in increased genomic instability.[7] This may, in turn, foster the occurrence of secondary oncogenic hits and, finally, result in the malignant transformation to overt FL. Recently, Cong and co-workers described the phenomenon of what they termed *follicular lymphoma in situ* in otherwise reactive, hyperplastic lymph nodes possibly representing the morphological equivalent of early, preinvasive FL.[8]

We have learned from classical cytogenetic studies in FL that the t(14;18) occurs as the sole cytogenetic abberation in less than 10% of cases, whereas in the vast majority at least one, usually more additional alterations are detected. When using higher resolution approaches, such as array-based CGH techniques, the percentage of FL cases showing the t(14;18) as the sole abnormality is even lower reinforcing the concept that alterations in addition to the t(14;18) are required for lymphomagenesis.

Secondary chromosomal aberrations in follicular lymphoma

A number of secondary chromosomal alterations have been described in FL including structural and numerical changes. The complexity of the secondary alterations correlates with the grade – the higher the grade, the more complex aberrations are usually encountered.[9,10] Of note, it has long been recognized that these alterations occur in a non-random fashion. Partial trisomies of chromosomes 1q, 7, 8 and 18q, and deletions in 1p and 6q have been described as the most common secondary alterations, and deletions in the long arm of chromosomes 1 and 6 and in the short arm of chromosome 17 have been associated with a worse prognosis.[11-15] Of pivotal importance is the recent insight that there is a temporal order in the appearance of these aberrations. Some of these may occur early in the course of the disease, whereas others might represent late genetic events. In addition, some of the alterations are mutually exclusive, while alterations of other chromosomal regions frequently appear together possibly leading to a co-ordinated deregulation of genetic pathways.[16] For example, one of the proposed pathways frequently starts with a +18q followed by an acquisition of the trisomies +7 and +8, while another scenario involves deletions in 6q followed by deletions in 1p.[16] Some of the secondary chromosomal alterations may abrogate the effect of the t(14;18) that initially forms a low-grade neoplasia with a follicular growth pattern and subsequently enable the transformation to high-grade lymphoma. This latter process has been associated with three distinct secondary genetic alterations in FL that have a profound impact on the biological program and the clinical course in FL. These include an additional introduction of a t(8;14)/*MYC* rearrangement in the tumor cells,[17,18] the inactivation of *TP53* by mutation and deletion and, finally, the inactivation of p16, frequently occurring by biallelic deletion.[19-21] The occurrence of a secondary *MYC* rearrangement in FL deserves particular attention, because these cases frequently demonstrate a *Burkitt-like* appearance and may be detectable by virtue of this specific morphology in combination with an overexpression of the BCL2 protein caused by the t(14;18) that is usually not encountered in classical Burkitt's lymphomas. A recent study suggests that the detection of *TP53* mutations in primary diagnostic specimens of FL without signs of transformation also characterizes a patient subgroup with worse prognosis.[22]

BCL2-negative follicular lymphoma

Given the importance of the t(14;18) as the initiating event in the lymphomagenesis of FL, it is surprising that roughly 10-15% of FL stain negatively for BCL2 at the immunohistochemical level. Two reasons account for this phenomenon. In approximately 50% of BCL2 protein negative cases, the t(14;18) is nevertheless present in the tumor cells, but BCL2 is undetectable by at least some of the presently used BCL2 antibodies due to somatic mutations in the BCL2 epitopes that are recognized by these antibodies. In other words, BCL2 is expressed in these cases as a consequence of the t(14;18), albeit in a mutated form.[23] The remainder of the BCL2-negative FL, however, indeed lack the t(14;18). On the other hand, t(14;18)-negative FL may or may not express BCL2, arguing for the existence of other mechanisms of the tumor cells to up-regulate BCL2, e.g. by a gain or amplification of the *BCL2* locus in 18q21 that is present in some of these cases.[24] There is also evidence that in BCL2-negative FL alternative mechanisms may provide anti-apoptotic stimuli, such as BCL-X(L).[25]

Follicular lymphomas negative for the t(14;18) generally fall into different categories. First, the occurrence of the translocation is related to the tumor grade. While approximately 90% of FL grade 1 and 2 harbor the t(14;18), it is detectable in FL grade 3A in only 60-70%.[9,26] Conversely, FL grade 3B show *BCL2* rearrangements in only 15-30%. Second, absence of the t(14;18) is generally observed in FL arising at extranodal sites such as the skin or the testis, or in pediatric FL that are predominantly of grade 3.[27,28] Taken together, the t(14;18) chromosome translocation is the cytogenetic hallmark in low-grade (grades 1 and 2) nodal follicular lymphomas in particular. Yet, even in this group, roughly 10% of the cases remain t(14;18) and/or BCL2-negative. In a detailed classical banding and FISH analysis of 50 cases, Horsman and colleagues identified two subgroups within t(14;18)-negative FL, namely one with supernumerary chromosomes 18 and overexpression of BCL2 and another group with the frequent presence of the t(3;14)(q27;q32) involving *BCL6* and no BCL2 expression.[24]

In the current issue of the journal, Gagyi and co-workers asked the question whether t(14;18)-negative FL differed from t(14;18)-positive FL with respect to classical features of germinal center-associated neoplasias, i.e. presence of ongoing somatic mutations, aberrant somatic mutations and expression of AID.[1] These characteristics are well described in t(14;18)-positive FL and provide molecular evidence that FL are indeed the neoplastic equivalent of the normal germinal center reaction besides typical histological and immunophenotypic features. Since the presence of the t(14;18) may, at least in part, be responsible for preventing tumor cell maturation into plasma cells, the authors wondered whether t(14;18)-negative FL may arise from B-cells at a later stage of development (*follicle exit population*) in which some of the above mentioned hallmark features of GC B-cells are no longer operative. To this end, they extensively characterized 11 t(14;18)-negative FL without BCL2 expression for the occurrence of somatic mutations in the immunoglobulin heavy chain (IGVH) genes, the occurrence of aberrant mutations in the *MYC*, *PAX-5* and *RhoH* genes as well as for the expression of AID and compared the results with a control group of 7 t(14;18)-positive tumors. In keeping with previous reports, the t(14;18)-negative FL were of higher grade (two grade 2 FL and 9 grade 3A FL) again emphasizing that the t(14;18) is more common in FL of low grade. The results of the study by Gagyi *et al.* are remarkably clear. FL with and without the t(14;18) show no difference in the occurrence of somatic hypermutations of the *IGHV* genes and in the expression levels of AID thus

providing evidence for a germinal center origin and differentiation in both groups. Likewise, no differences in the quality or quantity of aberrant somatic hypermutation of the *MYC*, *RhoH* and *PAX-5* genes were observed confirming that t(14;18)-positive and negative FL are equally targeted by this molecular feature. Taken together, the authors rightly conclude that t(14;18)-positive and -negative FL should still be viewed as a single entity in which divergent oncogenic pathways lead to highly similar morphological, immunophenotypic and also molecular features.

What are the next steps? It appears obvious from published data and the current study by Gagyi and colleagues that FL can develop without the presence of the t(14;18) chromosomal translocation. In a subset of these cases, BCL2 protein may be over-expressed by other genetic events, such as gains or amplifications of the *BCL2* gene locus. In truly BCL2-negative FL, rearrangements of the *BCL6* gene in 3q27 may provide an alternative molecular mechanism that can substitute for the lack of BCL2 expression. Nevertheless, the search for genetic or epigenetic hallmark alterations in t(14;18)-negative FL will have to continue. This question could be tackled by molecular studies involving high-resolution genomic and gene expression profiling techniques. On the other hand, the composition of the microenvironment has gained considerable interest in FL in recent years, and the interaction between the malignant tumor cells and bystander cells (T cells, dendritic cells, fibroblasts, histiocytes) in lymph node or bone marrow infiltrates of FL may have important biological and clinical implications. Specifically, gene expression profiling defined two signatures termed immune response signature 1 (IR1) and immune response signature 2 (IR2) that predict survival of FL patients at the time of diagnosis.[29] Although the more favorable expression signature IR1 is enriched for genes expressed in various T-cell subsets, whereas IR2 – associated with inferior outcome – harbors genes expressed in macrophages and dendritic cells, the biological conclusions are not straightforward. These signatures appear to capture a snapshot of an intricate meshwork of interactions between the neoplastic B cells with their microenvironment rather than the presence of particular cell types in the lymphoma infiltrate. As a consequence, subsequent studies correlating the number or distribution of various cells of the microenvironment, such as histiocytes (CD68) or T-cell subsets (CD4, CD8 and T_{reg}) with the clinical course of the disease were rather inconclusive between different groups.[30-34] With regard to the FL subset lacking the t(14;18) it will – besides deciphering the genetic, or epigenetic, background – be of equal importance to study differences in their microenvironment as compared to classical, t(14;18)-positive FL and to determine their clinical behavior in large prospective trials.

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References

1. Gagyi É, Balogh Z, Bödör C, Timár B, Reiniger L, Deák L, et al. Somatic hypermutation of IGVH genes and aberrant somatic hypermutation in follicular lymphoma without BCL-2 gene rearrangement and expression. Haematologica 2008; 93:1822-8.
2. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J. WHO classification of tumors of haematopoietic and lymphoid tissues. Geneva: WHO Press; 2008.
3. Bakhshi A, Jensen JP, Goldman P, Wright JJ, McBride OW, Epstein AL, Korsmeyer SJ. Cloning the chromosomal breakpoint of t(14;18) human lymphomas: clustering around JH on chromosome 14 and near a transcriptional unit on 18. Cell 1985;41:899-906.
4. Tsujimoto Y, Gorham J, Cossman J, Jaffe E, Croce CM. The t(14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining. Science 1985; 229:1390-3.
5. McDonnell TJ, Deane N, Platt FM, Nunez G, Jaeger U, McKearn JP, Korsmeyer SJ. bcl-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. Cell 1989;57:79-88.
6. de Jong D. Molecular pathogenesis of follicular lymphoma: a cross talk of genetic and immunologic factors. J Clin Oncol 2005;23:6358-63.
7. Pasqualucci L, Bhagat G, Jankovic M, Compagno M, Smith P, Muramatsu M, et al. AID is required for germinal center-derived lymphomagenesis. Nat Genet 2008;40:108-12.
8. Cong P, Raffeld M, Teruya-Feldstein J, Sorbara L, Pittaluga S, Jaffe ES. In situ localization of follicular lymphoma: description and analysis by laser capture microdissection. Blood 2002;99:3376-82.
9. Ott G, Katzenberger T, Lohr A, Kindelberger S, Rudiger T, Wilhelm M, et al. Cytomorphologic, immunohistochemical, and cytogenetic profiles of follicular lymphoma: 2 types of follicular lymphoma grade 3. Blood 2002;99:3806-12.
10. Yunis JJ, Frizzera G, Oken MM, McKenna J, Theologides A, Arnesen M. Multiple recurrent genomic defects in follicular lymphoma. A possible model for cancer. N Engl J Med 1987;316:79-84.
11. Cheung KJ, Shah SP, Steidl C, Johnson N, Relander T, Telenius A, et al. Genome-wide profiling of follicular lymphoma by array comparative genomic hybridization reveals prognostically significant DNA copy number imbalances. Blood 2008 Aug 14. [Epub ahead of print]
12. Tilly H, Rossi A, Stamatoullas A, Lenormand B, Bigorgne C, Kunlin A, et al. Prognostic value of chromosomal abnormalities in follicular lymphoma. Blood 1994;84:1043-9.
13. Bentz M, Werner CA, Dohner H, Joos S, Barth TF, Siebert R, et al. High incidence of chromosomal imbalances and gene amplifications in the classical follicular variant of follicle center lymphoma. Blood 1996;88:1437-44.
14. Viardot A, Moller P, Hogel J, Werner K, Mechtersheimer G, Ho AD, et al. Clinicopathologic correlations of genomic gains and losses in follicular lymphoma. J Clin Oncol 2002; 20:4523-30.
15. Avet-Loiseau H, Vigier M, Moreau A, Mellerin MP, Gaillard F, Harousseau JL, et al. Comparative genomic hybridization detects genomic abnormalities in 80% of follicular lymphomas. Br J Haematol 1997;97:119-22.
16. Hoglund M, Sehn L, Connors JM, Gascoyne RD, Siebert R, Sall T, et al. Identification of cytogenetic subgroups and karyotypic pathways of clonal evolution in follicular lymphomas. Genes Chromosomes Cancer 2004;39:195-204.
17. Macpherson N, Lesack D, Klasa R, Horsman D, Connors JM, Barnett M, Gascoyne RD. Small noncleaved, non-Burkitt's (Burkit-Like) lymphoma: cytogenetics predict outcome and reflect clinical presentation. J Clin Oncol 1999;17:1558-67.
18. De Jong D, Voetdijk BM, Beverstock GC, van Ommen GJ, Willemze R, Kluin PM. Activation of the c-myc oncogene in a precursor-B-cell blast crisis of follicular lymphoma, presenting as composite lymphoma. N Engl J Med 1988; 318:1373-8.
19. Lo Coco F, Gaidano G, Louie DC, Offit K, Chaganti RS, Dalla-Favera R. p53 mutations are associated with histologic transformation of follicular lymphoma. Blood 1993;

82:2289-95.
20. Sander CA, Yano T, Clark HM, Harris C, Longo DL, Jaffe ES, Raffeld M. p53 mutation is associated with progression in follicular lymphomas. Blood 1993;82:1994-2004.
21. Elenitoba-Johnson KS, Gascoyne RD, Lim MS, Chhanabai M, Jaffe ES, Raffeld M. Homozygous deletions at chromosome 9p21 involving p16 and p15 are associated with histologic progression in follicle center lymphoma. Blood 1998;91:4677-85.
22. O'Shea D, O'Riain C, Taylor C, Waters R, Carlotti E, Macdougall F, et al. The presence of TP53 mutation at diagnosis of follicular lymphoma identifies a high-risk group of patients with shortened time to disease progression and a poorer overall survival. Blood 2008;112:3126-9.
23. Schraders M, de Jong D, Kluin P, Groenen P, van Krieken H. Lack of Bcl-2 expression in follicular lymphoma may be caused by mutations in the BCL2 gene or by absence of the t(14;18) translocation. J Pathol 2005;205:329-35.
24. Horsman DE, Okamoto I, Ludkovski O, Le N, Harder L, Gesk S, et al. Follicular lymphoma lacking the t(14;18)(q32;q21): identification of two disease subtypes. Br J Haematol 2003;120:424-33.
25. Zha H, Raffeld M, Charboneau L, Pittaluga S, Kwak LW, Petricoin E 3rd, et al. Similarities of prosurvival signals in Bcl-2-positive and Bcl-2-negative follicular lymphomas identified by reverse phase protein microarray. Lab Invest 2004;84:235-44.
26. Katzenberger T, Ott G, Klein T, Kalla J, Muller-Hermelink HK, Ott MM. Cytogenetic alterations affecting BCL6 are predominantly found in follicular lymphomas grade 3B with a diffuse large B-cell component. Am J Pathol 2004; 165:481-90.
27. Finn LS, Viswanatha DS, Belasco JB, Snyder H, Huebner D, Sorbara L, et al. Primary follicular lymphoma of the testis in childhood. Cancer 1999;85:1626-35.
28. Lorsbach RB, Shay-Seymore D, Moore J, Banks PM, Hasserjian RP, Sandlund JT, Behm FG. Clinicopathologic analysis of follicular lymphoma occurring in children. Blood 2002;99:1959-64.
29. 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.
30. Glas AM, Knoops L, Delahaye L, Kersten MJ, Kibbelaar RE, Wessels LA, et al. Gene-expression and immunohistochemical study of specific T-cell subsets and accessory cell types in the transformation and prognosis of follicular lymphoma. J Clin Oncol 2007;25:390-8.
31. 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.
32. 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.
33. Carreras J, Lopez-Guillermo A, Fox BC, Colomo L, Martinez A, Roncador G, et al. High numbers of tumor-infiltrating FOXP3-positive regulatory T cells are associated with improved overall survival in follicular lymphoma. Blood 2006;108:2957-64.
34. Wahlin BE, Sander B, Christensson B, Kimby E. CD8+ T-cell content in diagnostic lymph nodes measured by flow cytometry is a predictor of survival in follicular lymphoma. Clin Cancer Res 2007;13:388-97.

Salvage therapy for relapsed or refractory diffuse large B-cell lymphoma: impact of prior rituximab

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Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma in the developed world.[1] Anthracycline based combination chemotherapy regimens became standard of care in the 1970s after a series of studies showed long-term disease free survival with this approach. It was the pivotal study by the Group d'Etude des Lymphomas de l'Adulte (GELA), comparing CHOP versus the same regimen plus rituximab that changed practice. The use of rituximab, a monoclonal antibody targeting CD20 along with combination chemotherapy, CHOP or equivalent regimens led to complete remission (CR) rates of 75-80% and 3-5 year progression free survival (PFS) of 50-60%.[2] Retrospective analysis of this strategy demonstrated that in adult DLBCL patients of all ages, 2-year OS increased from 52% with anthracycline based chemotherapy to 78% in the post-rituximab era.[3]

Despite this major advance, a significant proportion of patients will either experience early treatment failure, partial response or relapse after the initial chemotherapy. The initial approach to relapsed DLBCL management is to determine if the patient is a candidate for high-dose chemotherapy and autologous stem cell transplant (ASCT). In the PARMA trial, chemotherapy-sensitive relapsed DLBCL patients were randomized to salvage chemotherapy with platinum and cytarabine based regimen alone or in combination with ASCT. Both EFS and OS were significantly superior in the transplant group versus the chemotherapy alone group (46% and 53% *vs.* 12% and 32% respectively). Chemotherapy sensitive patients did significantly better than those who were chemotherapy-resistant (5-year PFS 43% *vs.* 1-year survival of 22%).[4] Based on these results, HDT/ASCT has become the standard of care in younger patients with chemosensitive relapsed or primary refractory aggressive lymphoma.

Role of rituximab in salvage therapy

In this issue of the journal, Martin and colleagues report the GEL/TAMO study, which evaluated the influence of prior rituximab use in response rates of R-ESHAP as salvage therapy for patients with relapsed or refractory diffuse large B-cell lymphoma.[5] The efficacy of rituximab-containing salvage after induction treatment with rituximab containing chemotherapy has not been well established. In this retrospective analysis, 163 patients with relapsed or refractory DLBCL, who received R-ESHAP with curative intent, were analyzed. Patients were stratified according to whether rituximab