Contiguous follicular lymphoma and follicular lymphoma in situ harboring N-glycosylated sites
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Follicular lymphoma in situ (FLIS) is composed of a clonal B-cell population harboring the typical t(14;18) hallmark of follicular lymphoma (FL), forming unconventional BCL2brightCD10− cell foci in an otherwise normal reactive lymph node (LN). The diagnosis of FLIS is made on the fortuitous discovery of unconventional BCL2brightCD10− cell foci. Several studies recently demonstrated that FLIS are already advanced precursors in follicular lymphomagenesis, but not necessarily committed to malignant transformation. However, the relationship between FLIS and FL still remains unclear, as only a minority (<5%) of FLIS patients eventually develop FL. This is in line with the usually indolent progression of the disease, and the genomic instability observed in FLIS cells, which can engage FL precursor cells either in an evolutionary malignant process, or to an evolutionary dead end.

We report the case of a 35-year old male patient who presented with a cervical adenopathy. Histological examination of the excised LN displayed an altered architecture suggestive of FL, consisting of high number of monomorphic large follicles, uniformly spread in the cortical and medullary areas. Most follicles contained a predominant population of small cleaved cells with scant macrophages and mitoses. The mantle zone was reduced or absent. However, in a minor cortical area, a few follicles showed features mimicking residual classical germ cells (GC), including a smaller size, higher cell polymorphism, and a preserved mantle zone (Figure 1A).

The BCL2 immunostaining (clone 100) was negative in follicles displaying a typical FL pattern. In contrast, follicles located in the pseudo-residual area were BCL2bright, i.e. more strongly stained than the surrounding mantle zone and reactive T cells (Figure 1B). Most follicles were only slightly positive for Ki67 (Online Supplementary Figure S1A). Both BCL2 and BCL2+ follicles were CD10 positive (Online Supplementary Figure S1B) and contained a BCL2/JH break-point evidenced by fluorescence in situ hybridization (FISH) (Figure 1C). Taken together these results suggested the diagnosis of simultaneous occurrence of BCL2− FL (grade I/II) and of BCL2+ FLIS in the same LN. We decided to further analyze those two lesions independently, and performed macrodissection in order to proceed with individual molecular analyses when required. Sanger sequencing revealed that both FLIS and FL shared the same BCL2/JH sequence at the t(14;18)+ breakpoint, and thus originated from the same clone (Figure 1D).

We tested two other anti-BCL2 antibodies (E17, SP66) directed against other epitopes, but the staining remained BCL2− in the FL area of the LN, similar to the anti-BCL2 antibody (clone 100) staining (Figure 1E and F). We thus sequenced exons 1 to 3 of the BCL2 gene (B-cell CLL/lymphoma 2, NG_009361.1). Punctual mutations, resulting in amino acid substitutions, were found in the FL component (Online Supplementary Table S1), and were indeed located in the targeted aa41 to aa54 epitope of clone 100 (mutations...
found in aa45-47), in the aa61 to aa76 epitope of clone E17 (mutation found at position aa64) and in the N-terminal region epitope of clone SP66 antibodies (mutation in aa32). None of the registered mutations involved a stop codon. The FLIS and FL sequences were submitted to 3D-molecule Viewer (Vector NTI advanced 5.11.1®), which revealed that the acquired mutations in the FL dissected area resulted in an altered energy profile of the BCL2 protein, probably preventing a proper fixation of most anti-BCL2 antibodies (Figure 1G and Online Supplementary Figure S2).

We thus determined whether the FL cells expressed at least the BCL2 transcript. mRNA was extracted from microdissected FL follicles and qRT-PCR was performed. An approximately 7-fold relative increase in BCL2 transcripts was found from microdissected benign reactive lymph nodes and qRT-PCR was performed. An approximately 7-fold relative increase in BCL2 transcripts was found compared to microdissected benign reactive lymph nodes (Log2[BCL2/GAPDH] was 3.7 in FL follicles compared to 0.5 in benign reactive lymph nodes), indicating that the absence of BCL2 staining was not due to transcriptional downregulation.

Taking into account that the FL follicles were composed of more than 80% of FL B cells, it is unlikely that contaminating T cells could be entirely responsible for this high level of BCL2 transcription, which is thus likely related to FL B cells. Although we cannot exclude that a post-transcriptional mechanism could have induced downregulation of the BCL2 protein, these data are in accordance with the view of the FL component presenting with multiple mutations inducing a conformational change of the BCL2 protein, which may or may not have altered BCL2 function. Sustained activity of activation-induced cytidine deaminase (AID) has been shown to be partly responsible for somatic mutations in FL. Indeed, AID expression was present in both FLIS and FL areas of our sample (Online Supplementary Figure S3A).

Despite the concomitant FL/FLIS localization, these alterations were lacking in the FLIS dissection, suggesting that they were acquired in the FL component after divergence from a common founder clone. To our knowledge, only rare cases of FLIS with concomitant FL or DLBCL have been reported, and are usually observed in distinct LNs. Our case is reminiscent of previously reported cases of FLIS associated with FL, in which the associated FL was often negative for BCL2 protein. This suggests either that FLIS is more easily detected in those cases because of the lack of BCL2 in the FL area, or that BCL2 mutations are frequently associated with progression from FLIS to FL, when compared to de novo or sporadic FL.

To further establish the clonal hierarchy between the FLIS and FL lesions, we investigated the immunoglobulin variable heavy chain (VH) gene region of FL cells, a region frequently mutated in FL. The VH region of the FL clone was identified as IGHV3-48*03/IGHD3-22*01/IGHJ4*02, with approximately 85.4% homology (+/-0.27) among the various FL subclones (n=16 analyzed sequences, correspon-
Oligosaccharides are responsible for lectin binding to lym-1
lectin-like receptors. In fact, lectins are rarely completely
accumulating the hits that will further drive clone fate.
2A). In addition, the intra-clonal variability was higher in
the FLIS than in the FL component, which could be due to
a dynamic trafficking, such as multiple GC re-entries of the
FLIS clones. This is in line with two recent reports showing a
subclonal heterogeneity among genomic alterations
observed in FLIS.13 Overall, our analysis reveals that the
FLIS and the FL clones have evolved through a divergent
evolution model, which postulates the existence of unique
co-existing lesions and subclone selection, in a way similar
to that reported in FL and relapsed FL9 (Figure 2B).

Finally, among the mutations at the VH IGHV3-48*03
sites, we observed that some of them were responsible for
the introduction of a recurrent N-glycosylation motif (N-X-
nose glycans that are characteristic of FL, with an incidence
of nearly 100%. They can occasionally be observed in some
GC-derived tumors other than FL, but are very infre-
quent in normal B cells.10 Notably, a similar “N-I-S” motif
was also found in the CDR2 region of a VH IGHV3-48*03
site sequenced from an FL sample.11

Functionally, added glycans terminate at high mannose,
which might influence the behavior of FLIS or FL cells
through opportunistic interactions between the B-cell
receptor (BCR) with mannose-binding lectin bearing cells.12
The engagement of those lectins with the N-glycosylated
sIg may also contribute to a mucin-like
receptor by tumoral mucins activates an immune suppressive pheno-
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