EBV and the systemic EBV-positive T-cell lymphoma of childhood, disease processes that are closely related to EBV-HLH.

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LYMPHOID NEOPLASIA

Comment on Odabashian et al, page 834

Sugar-coated BCR kept during FL clonal evolution

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In this issue of *Blood*, Odabashian et al[1] interrogate by deep sequencing the evolution patterns of N-glycosylation (N-gly) motifs introduced by somatic hypermutation (SHM) into the variable region of the B-cell receptor (BCR) in sequential follicular lymphoma (FL) biopsies taken before and after progression. This phylogenetic study recognizes the maintenance of N-gly motifs from the early stages until disease progression, seemingly independent of the intratumoral (epi)genetic variability, thereby supporting a model for a conserved dependence for this mode of constitutive BCR triggering signal through clonal evolution.

FL is a germinal center (GC)-derived B-cell neoplasia characterized by a long asymptomatic preclinical course followed by remission/relapse cycles becoming eventually refractory to treatment or transforming to a more aggressive lymphoma.[2] Clonal evolution studies, including longitudinal (diagnosis vs relapse/transformation) and spatial profiling, provide insights into the genetic basis of FL and clonal dynamics at progression. By analyzing the hierarchy of mutations, it was inferred that FL progression commonly arises through a divergent evolution process, emerging from a less evolved common precursor cell (CPC) that is responsible for propagating each new FL episode with the acquisition of independent mutations.[3,4] Additional evidence for FL CPCs comes from the detection of precursors in "healthy" individuals years before FL develops[5] and the demonstration that those committed precursors can transfer the disease in bone marrow transplants.[6] As all FL patients ultimately relapse, this indicates that current therapies are unable to eradicate the reservoir of FL CPCs and that targeting key CPC founding alterations may represent an attractive therapeutic strategy to prevent progression and relapse.

Phylogenetic studies have started to unravel the set of molecular alterations contributing to CPC genesis. Besides the founder t(14;18) translocation allowing the BCL2^{pos} cells to recirculate as premalignant precursors years before FL onset, mutations in epigenetic modifiers, such as *KMT2D* or *CREBBP*, have been suggested as cofounding lesions.[3,4] A third key player is the ability of FL cells to coopt BCR signaling pathways and establish a strong dependence toward microenvironment signals. A remarkable feature of FL BCRs, affecting >80% of patients, relies on the introduction during SHM of sequence motifs into the BCR variable region that create novel N-linked glycosylation acceptor sites, a modification rarely seen in normal B cells.[7,8] Loaded with atypical oligomannose glycans, these sugar moieties trigger activation of BCR signaling pathways through interaction with endogenous mannose-binding lectins like DC-SIGN expressed by dendritic cells and macrophages, thereby providing an alternative tumor-supportive mechanism independent from antigen recognition.[7,8] However, it remains largely unexplored when BCR N-gly motifs arise during FL ontogeny, whether they are stable, and how they clonally diverge and evolve during FL clinical course. This study by Odabashian et al. addresses these goals.

To reconstruct the evolutionary patterns of N-gly sites during progression, the authors used deep sequencing of BCR repertoire to profile sequential tumor biopsies paired in space and time from patients with variable clinical course and treatments. By comparing thousands of FL subclones, they identified that N-gly motifs are universally present within the dominant subclones of the first disease event and are retained in >96% of subsequent relapse/transformation subclones, with no further N-gly sites gained that

were not present at diagnosis. As FL cells are characterized by ongoing SHM in the antigen-binding sites, the authors then examine the clonal diversity of the 9-bp region encoding N-gly motifs of each subclone. Despite ongoing SHM, N-gly sites are never lost but replaced by new variants through synonymous and non-synonymous mutations. Thus, although the evolutionary processes are clearly at work within the tumor cells as evidenced by substantial subclonal BCR and N-gly diversification, there is a selective pressure to keep this unique BCR modification during progression. The authors then looked at the outcome of rare N-gly^{neg} subclones emerging from random SHM and showed that they represent a minor component of the tumor bulk (<1%), and when present, they are deleted from subsequent disease events leading the authors to suggest that N-gly^{neg} cells are outcompeted by the clonal expansion of N-gly^{pos} clones. Although we cannot exclude that the genetic landscape of N-gly^{neg} subclones might be different to explain their deletion, this study supports the fact that N-gly^{neg} cells might not receive a similar type of BCR signal induction and survival input from microenvironmental lectins than is received by the N-gly^{pos} one.

Collectively, this and previous reports[7,8] support a model whereby N-gly motifs are early events during lymphomagenesis that must occur by SHM in the first GC transits of t(14;18)^{pos} cells, which may even precede most aberrations acquired by the CPC. Glycan residues might allow the retention/reactivation of t(14;18)^{pos} subclones within the mutagenic GC context leading to a growth/survival advantage and further (epi)genomic modifications. The hierarchy of founding CPC mutations remains to be established by directly purifying those cells, but glycosylated motifs have been found in in situ follicular neoplasia, 1 established premalignant FL stage, corroborating the present conclusions.

Besides BCR N-gly motifs, recent studies have highlighted the link between individual (epi)genetic alterations and the capacity of malignant B cells to interact with and subvert their GC-like microenvironment in both patients and genetically engineered mouse models.[2,4,9] An important perspective of this study would be to identify how the triad of individual mutations, BCL2 translocations, BCR glycans, and epimutations, work together to trigger CPC genesis and impact the crosstalk between tumor cells and their supportive niche and how those cross-dependencies can be exploited therapeutically. Mouse models that mimic the introduction of altered glycans into the BCR are needed.

Regarding clinical applications, precision medicine approaches disrupting glycan-lectin interactions and blocking downstream BCR kinase cascades might represent good candidates to inhibit the repopulating potential of this reservoir of relapse-initiating cells.[7] Whether the addiction for glycan-lectin interactions is preserved in vivo in all relapse settings remains unknown. In vitro functional genomic CRISPR screens in GC-derived lymphomas recently reported a dependency of the tumor cells for a mode of oncogenic BCR signaling that promotes survival in a BCR-PI3K–dependent but microenvironment-independent fashion,[10] suggesting some advanced FL have lost this requirement and necessitate distinct therapeutic approaches.

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MYELOID NEOPLASIA

Comment on Zhang et al, page 845

Cooperating mutations: joint forces, novel vulnerabilities

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In this issue of *Blood*, Zhang et al report on their investigation to elucidate the mechanisms by which loss of function of the DNA methyltransferase 3A (DNMT3A) cooperatively induces hematologic malignancies when cooccurring with IDH1/2 mutations.[1]

Acute myeloid leukemia (AML) is a complex disease that regularly involves mutations in several oncogenes and tumor suppressors. Many mutations have been investigated on the single-gene level. However, cooccurring mutations are likely to alter phenotypes, leukemogenic mechanisms, and potentially even drug responses. To model the scenario of combined mutations in DNMT3A and either IDH1/2 as observed in 5% to 8% of patients with myelodysplastic syndrome/AML,[2,3]