Transformation of Indolent Lymphomas and Chronic Lymphocytic Leukemia (CLL)

New Insights into the Transformation of Indolent Lymphomas and CLL From in Vivo Models

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Take-home messages:
- Mouse models that faithfully recapitulate human indolent lymphomas have extended knowledge in elucidating complex disease mechanisms.
- This knowledge has implications for personalized medicine harnessing target pathways and mutations which contribute to lymphomagenesis.

Introduction
Over the past several years, next-generation sequencing efforts have identified a number of previously unrecognized, recurrently mutated genes with roles in the pathogenesis of indolent lymphomas, including follicular lymphoma (FL), and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). In many cases it remained elusive as to how the specific gene mutations drive lymphomagenesis. Studies on human tumor-derived cell lines could provide initial insights into the pathogenetic mechanism of those genes. However, it became clear that the oncogenic function of a mutated gene can be fully elucidated only using an in vivo model. Consequently, the establishment of genetically engineered mouse models that faithfully recapitulate human indolent lymphomas has extended knowledge in elucidating complex disease mechanisms.

Current state of the art

FL is characterized by the t (14;18) translocation causing constitutive expression of the anti-apoptotic BCL2 protein, and by recurrent mutations in the genes encoding the histone-modifying enzymes KMT2D methyltransferase (70% of cases), CREBBP acetyltransferase (65%), and EZH2 methyltransferase (22%), in addition to several linker-histone family members (>44%). Thus, pro-survival signals and altered epigenetic regulation seem to jointly contribute to the malignant transformation of FL. Compared to most other non-Hodgkin lymphomas, CLL is characterized by a largely different spectrum of genetic aberrations that comprise amplifications (trisomy 12) and chromosomal deletions, including 13q14, TP53 and ataxia telangiectasia mutated (ATM). We here focus on genes mutated in FL or CLL whose function in malignant transformation has remained elusive.

TNFRSF14

TNFRSF14 encodes the herpesvirus entry-mediator (HVEM) B-cell surface receptor. Mutations and deletions in TNFRSF14 causing loss of function of HVEM were reported in up to 40% of FL cases, and its proposed role as a tumor suppressor was established in a FL mouse model. Recent work revealed that, mechanistically, HVEM is activated through ligation with B and T-lymphocyte attenuator (BTLA), a surface protein on T-follicular helper cells (Tfh), in the germinal center (GC) microenvironment. Biologically, this interaction restrains T-cell help available to GC B cells; hence, loss of HVEM likely promotes B-cell proliferation and lymphomagenesis by exaggerated T-cell help. This may be achieved by increased CD40L expression on Tfh cells, since in the wild-type context, it was found that BTLA signaling restrains the mobilization of CD40L to the immunological synapse, thus restraining T-cell help to GC B cells with the highest antigen affinities. Importantly, this study showed that HVEM + Bcl2-transgene GC B cells maintained the growth advantage over the Bcl2-transgene wild-type HVEM counterparts in a competitive setting (ie, HVEM-deficient and wild-type cells were transferred at equal portions into recipient mice), indicating that the pro-survival pathways are distinct, but can cooperate with high BCL2 expression. The findings identify soluble HVEM as a conceivable therapeutic strategy in TNFRSF14-mutated lymphomas.
RRAGC

The RAGC GTPase gene (RRAGC) shows missense mutations in 17% of FL cases. RAGC GTPase functions with RAGA GTPase in the cellular nutrient-sensing pathway. In the case of sufficient nutrients, the mammalian target of rapamycin complex 1 (mTORC1) is activated, resulting in cellular anabolism and growth. Recent work has recapitulated the most common human FL-associated RRAGC mutations using CRISPR-Cas9 technology to generate Rragc-mutant knock-in mouse models. In a reduced nutrient environment, these models showed increased mTORC1 activity compared to wild-type counterparts; hence, Rragc mutations were proposed to increase GC B-cell fitness. Critically these observations were recapitulated on a Bcl2-transgenic background, accelerating tumorigenesis. Following T-cell dependent immunization, GC B cells in Rragc mutants showed dramatically higher abundance than wild-type counterparts, an observation that was exacerbated in a competitive setting. This increase is likely due to suppression of cell death and a decreased dependency on microenvironmental signals, critically including T-cell help. This competitive advantage permits Rragc-mutant cells to undergo iterative cycles within the GC where additional aberrations may be acquired that promote lymphomagenesis. It is conceivable that such potential tumor metabolic vulnerability can be exploited for therapy of RRAGC-mutated malignancies.

MEF2B

Gain-of-function mutations in MEF2B protein occur in around 15% of FL cases. For example, amino acid substitutions frequently occur in the amino-terminal DNA-binding domain, which prevents MEF2B from interacting with negative modulators of its activity (histone chaperone complex HUCA, and several class IIa-histone deacetylases; HDACs). A conditional knock-in mouse model mimicking the most commonly identified amino-terminal mutation D83V was generated and specifically activated in mature B cells by crossing to CD21-Cre mice. Compared to the unmutated controls, these mice showed a significant increase in GC B cells upon immunization, and over time developed clonal FL and diffuse large B-cell lymphomas in around 20% of cases. Crossing the mutated allele to Bcl2-transgenic mice resulted in a fully penetrant lymphoma phenotype. Evidence suggests that the underlying pathogenic mechanism is the inability of the mutated MEF2B to bind to the HUCA complex, thus escaping repression. This lack of negative regulation may lead to the establishment of an activated phenotype in GC B cells, leading to the acquisition of additional oncogenic mutations.

SF3B1

Missense mutations in the splicing factor SF3B1 occur in around 10% of CLL cases, with the recurrent SF3B1-K700E variant accounting for ~50% of mutation events. In order to elucidate the functional consequences of SF3B1 mutations, a conditional knock-in allele (Sf3b1-K700E) was created and crossed to CD19-Cre mice to achieve B-cell specific deletion. The B cells of the mutant Sf3b1 mice were associated with disruption of pre-mRNA splicing and cellular senescence; however, the mutation did not lead to CLL development. When bred on an ataxia telangiectasia mutated (ATM)-deficient background, approximately 50% of the animals developed a clonal CLL-like disease late in life and phenotypically resembled human CLL cells in terms of genome instability and dysregulated B-cell receptor (BCR) signalling. Of note, SF3B1 mutations and chromosomal deletions encompassing the ATM gene frequently co-occur in CLL. Interestingly, the mean expression of BCR-signaling genes was lower in SF3B1 CLLs compared to controls, which is in agreement with the finding in the Sf3b1-mutant mouse model that the mutation alters BCR-signaling gene expression in vivo.
Future perspectives

These works that faithfully model human FL or CLL gene mutations in mice all report oncogenic affects that were relatively minor. For example, the biological consequences of the Tnfrsf14 and Rragc mutations could be conclusively detected only in a competitive setting where mutated and wild-type B cells occurred at equal proportions. Thus, the effects of mutated TNFR514, RAGC and MEF2B proteins may accumulate over a longer timespan during which GC B cells undergo repeated rounds of mutation and selection to improve antigen affinity. In this scenario, GC B cells with gene mutations would have a competitive advantage, outgrowing their unmutated counterparts. This is further highlighted by the observation that in all three cases, the simultaneous expression of a Bcl2-transgene markedly enhanced lymphoma development. Importantly, the lymphoproliferations developing in those compound mice closely resembled the lymphomas in humans. Thus, these mouse models represent useful tools for studying oncogenic mechanisms and provide preclinical models for testing new therapies. With regard to the finding that mutated SF3B1 was found to be associated with down-modulated BCR-signaling gene expression, it was noted this phenotype may be exploited for a personalized therapy of SF3B1-mutated CLL patients, as they would be expected to be more sensitive to inhibitors targeting the BCR-signaling pathway.

References

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