Lymphoma-associated mutations in autoreactive memory B cells of patients with Sjögren's syndrome

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Supplementary Text S1

Text S1. Detailed explanatory text for Table 3

Reference numbers refer to the main text.

The most prominent lymphoma-associated gene mutations in RF B cells

The SG22 RF clone harbors an oncogenic D357V mutation in the coiled-coil domain of *CARD11*, which is known to enhance basal CARD11 signaling 80- to 160- fold independent of BCR engagement, resulting in induction of pro-proliferative and anti-apoptotic canonical NF-κB gene targets [33,34]. D357V has been detected before in four DLBCLs and in a splenic marginal zone B-cell lymphoma [27,28,51–53].

The SG22 (group 1), SG39 CT5, and S39 CT12 RF clones carry missense mutations in IG lambda-like 5 (*IGLL5*). In addition, the SG22 group 1 RF clone harbors two intron 1 *IGLL5* mutations. IGLL5 resembles IGLL1, which is expressed in pre-B cells together with VpreB and membrane-bound IgM to form the pre-BCR. IGLL5 is expressed in mature B cells and in plasma cells [24] and was found to be mutated in various B-cell lymphomas, particularly DLBCL and CLL. The function of IGLL5 is not known, nor its possible role in lymphomagenesis. Interestingly, about 70% of the *IGLL5* mutations are within the classical AID hotspots (WR<u>C</u>) [30–32,54] and *IGLL5* is a target for AID-mediated kataegis in a subset of DLBCLs [45].

In the SG39 CT5 RF clone, a K308N mutation was detected in *ZEB2*, a transcription factor that can repress gene transcription through recruitment of the CtBP co-repressor complex [35]. In DLBCL, missense mutations in *ZEB2* are observed across the molecule, strongly suggesting a tumor-suppressor function [52]. The recurrent loss of the 2q22.3 region, containing the *ZEB2* locus, in HCV-associated lymphomas supports this notion [55].

SG39 CT12 has an A311P mutation in *TBL1XR1*, which is a component of the NCOR1/SMRT co-repressor complex. *TBL1XR1* mutations have been found in DLBCL and in MALT lymphomas, including those of the salivary gland and ocular adnexa [25,26,32,52,55]. Mutations in MALT lymphomas are in the majority in WD40 domains. The A311P is located in the fourth WD40 domain, next to the predicted top-facing leucine at position 312 [26,42]. In GC B cells, in which the NCOR1/SMRT complex is recruited to chromatin by BCL6, mutated *TBL1XR1* leads to binding of the NCOR1/SMRT complex to chromatin via BACH2. This leads to transcriptional reprogramming and suppression of the plasma cell differentiation of GC B cells [36]. Strikingly, in mice expressing mutated *TBL1XR1*, memory B cells show an increased tendency to re-enter GCs [36].

Re-entry of memory B cells in GCs has previously been proposed to play a role in lymphomagenesis [56,57].

The SG39 CT12 RF clone also carries a Y188N mutation in *CD79A*. CD79A forms a heterodimer with its homologue CD79B and associates with membrane Ig to form the BCR complex [58]. *CD79B* is frequently mutated in ABC-type DLBCL, particularly at the first tyrosine (Y196) located in the immunoreceptor tyrosine-based activation motif (ITAM) [27,29,31,59]. *CD79A* mutations occur much less frequently and typically remove the complete ITAM region [59]. Interestingly, the Y188N mutation of SG39 CT12 concerns the first tyrosine in the ITAM of *CD79A*. It seems paradoxical that mutations altering tyrosine residues that are critical for BCR signaling are oncogenic. However, these mutations increase BCR expression and Y196-mutated *CD79B* activates LYN kinase less efficiently than wildtype CD79B, which is a potent negative regulator of BCR signaling [49,59]. Altogether, these data support a model in which oncogenic *CD79A/B* mutants counteract negative feedback of BCR signaling [49].

A *BTG1* S43N mutation was found in the SG39 CT13 RF clone. BTG1 overexpression leads to cessation of growth by negatively regulating the cell cycle [37]. The S43N mutation is next to L-44 at the start of the LxxLL motif, which binds and modulates various nuclear receptors [60]. In studies by Reddy *et al* [52] and Chapuy *et al* [32], respectively in 3 of 35 (9%) and in 11 of 68 (16%) DLBCLs, *BTG1* mutations were in this stretch of six amino acids (S43-L48), including one identical S43N mutation. *BTG1* mutations have been correlated with poor survival in ABC-type DLBCL [52].

As many as three RF clones displayed a histone gene mutation, i.e. SG12 (L61V in *HIST1H3G*), SG13 (E94D in *HIST2H2BF*), and SG39 CT5 (A102T in *HIST1H1D*). Linker histone genes (*HIST1H1 B–E*) are frequently mutated in follicular lymphoma [61,62], DLBCL [32], and MALT lymphoma [25]. As in lymphomas, the *HIST1H1D* mutation of SG39 CT5 was in the C-terminal portion [61,62]. As HIST1H1 B–E proteins bind directly to the DNA methyltransferases DNMT1 and DNMT3B, mutations in *HIST1H1 B–E* likely affect epigenetic regulation, similarly to *KMT2D*, *EZH2*, and *ARID1A* mutations in lymphomas [62]. Of the other histone genes, mutations in the core H2B and H3 genes, such as *HIST2H2BF* and *HIST1H3G*, have also been found in follicular lymphoma, DLBCL, and various other cancers [32,38,39,61]. The L61V *HIST1H3G* mutation in SG12 and the E94D *HIST2H2BF* mutation in SG13 are both in the globular domains. Globular domain mutations in H2A and H2B preclude the need for SWI/SNF chromatin remodeling complexes [38]. Interestingly, *SMARCC1*, another SWI/SNF complex member, is also mutated in SG12 (Table 3) [61,63]. Finally, the SG12, SG13, and SG39 CT5 clones harbored non-synonymous mutations in *GLI1, WWOX, HERC4*, and *FLI1*, respectively, genes reported to potentially have a role in lymphomagenesis (Table 3) [64–67].

The TT3 memory A(36) clone harbored an R16S mutation in isoform 1 of *SGK1*. As SGK1 is an AKT-family kinase, it may regulate PI3K signaling [44]. Mutations in *SGK1* occur frequently in GC-type DLBCL and are associated with favorable prognosis. *SGK1* mutations in DLBCL are found across the molecule and include truncating mutations, suggesting a tumor-suppressor function. SGK1 has been shown to be a target of aberrant AID activity and/or AID-mediated kataegis [32,45].