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Detection of occurrent somatic hypermutation and associated gene expression profile in single follicular lymphoma B cells

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Somatic Hypermutation (SHM) in B lymphocytes is essential for antibody affinity maturation. SHM has been quantified only as its cumulative outcome of sequence alterations of immunoglobulin (Ig) genes at the population level of selected B cells. To detect and to quantify the actual occurrence of single SHM events, we performed single-cell RNA and B-cell receptor (BCR) sequencing of viable tumor cells from 12 follicular lymphoma (FL) biopsies. FL is a B-cell neoplasia arrested at the germinal center stage with constitutive expression of activation-induced deaminase (AID) and ongoing SHM. Based on assembly of cell-individual V(D)J sequences and realigning sequence reads characterized by their unique molecular identifiers (UMI), we searched with particularly rigid filtering for individual FL cells expressing two populations of Ig transcripts from the same Ig heavy or light chain locus that differed by only one nucleotide (or at most few).
Essential aspects to ensure data quality included base quality scores, exclusion of cell doublets, Ig productivity, position within the V(D)J region, and proportion of cell-level variants.
1239 alternative V(D)J nucleotides were identified in 364 of 42,985 cells (0.84%) in 7 of the 12 FL biopsies. 268 BCR transcripts had two or more alternative nucleotides created in tandem. In 93.9% of these instances of co-occurrent SHM events on the same Ig chain of an individual cell, >90% of all V(D)J sequences carried either the most abundant combination of variants or the respective alternative nucleotide at all positions (i.e. the “mirroring” combination of variants). No V(D)J neovariant was detected in 4439 CLL cells from 5 patients ($p<0.0001$).
Based on identity to the corresponding germ-line sequence, we could distinguish *bona fide* original and neovariant alternatives for 1055 of the 1239 occurrent SHM variants (85.2%). The original variant of 50.1% of occurrent SHM events was positioned within known AID motifs. Neovariant-carrying FL cells expressed increased levels of AID. KEGG pathway analysis identified mismatch repair, base excision repair, and DNA replication as the three most prominently upregulated pathways in FL cells with occurrent SHM events. Our data provide the first quantitative evidence of ongoing SHM in single cells, demonstrate a high incidence of tandem mutations during SHM, and support the functional cooperation between AID and DNA repair pathways for initiation and processing of SHM in GC B lymphocytes.