Somatic mutations encoding germline-like motifs in germinal center B cells are associated with clonal expansion and differentiation into memory compartments

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Abstract

Humoral immunity is critically dependent on the acquisition of somatic mutations to refine the specificity of cognate antibodies. Broadly, somatic mutations are classified into two categories: canonical somatic hypermutation (SHM), and gene conversion (GC). These differ in that mutations introduced by SHM are unrelated to preexisting sequences in the genetic repertoire, whereas those introduced by GC are copied from preexisting motifs. We recently reported a computational pipeline, TRACE, that allows for detection of such events at the genome scale. Here, we have tuned the pipeline for specificity and sensitivity at both the genomic and local (IgH locus only) scale resulting in the new TRACE_SVM pipeline. This approach was combined with machine learning to effectively limit false positives while retaining true signal (ROC AUC - Genome: 0.83, Local – 0.98). We then examined Influenza and SARS-CoV2 human vaccination datasets that were obtained by fine needle aspiration of draining lymph nodes followed by 10x single cell sequencing to dissect the role of GC in germinal center B cell dynamics. In our analysis we find that GC accounts for 1.5% of mutations and occurs in ~7% of cells and clones. We find that GC is limited to antigen experienced cells ($p=0.002$). Clonal analysis in both datasets reveals that clones that undergo GC are significantly larger than clones that do not ($p<0.05$), even when adjusted for clone mutation loads ($p<0.05$). Stratification of clones by mutation load reveals that at low mutation frequencies, clones that acquire GC differentiate preferentially into lymph node resident plasma cells as compared to similar clones without GC ($p<0.001$). Quantitation of the relative amounts of these clones in post-germinal center compartments reveals that the largest clones originate from clones that undergo GC suggesting that high levels of differentiation into these compartments are associated with the presence of GC in the clone.