Abstract #6 | Poster

B Cell Differentiation in the Germinal Centre Reaction

A.-K. Schnormeier[1], B. Budeus[1], B. Höing[3], M. A. Weniger[1], D. Hoffmann[2], R. Küppers[1]

[1] *University Duisburg-Essen, Institute of Cell Biology (Tumour Research), Essen, North Rhine-Westphalia, Germany*
[2] *University Duisburg-Essen, Bioinformatics and Computational Biophysics, Essen, North Rhine-Westphalia, Germany*
[3] *University Medical Centre Essen, Otolaryngology, Essen, North Rhine-Westphalia, Germany*

During humoral immune responses, B cells undergo multifaceted differentiation and selection processes in germinal centres (GCs), generating long-lived plasma cells and memory B cells with higher affine immunoglobulins (Igs). Basic principles of GC reactions are well known, such as rapid proliferation and somatic hypermutation (SHM) of Ig V genes in dark zone (DZ) B cells and positive selection and differentiation of light zone (LZ) B cells. SHM is crucial for adaption to antigens and production of high affinity antibodies. B cells can further switch the isotype of their B cell receptors, impacting antibody effector function.

DNA damage during SHM and class switching, and the high proliferative capacity of DZ B cells, are not only mechanisms involved in normal B cell differentiation, but also form the basis for possible malignant transformations and tumorigenesis. This involves off-target mutations and failure of selection mechanisms. Heterogeneity of GC B cell-derived lymphomas is reflected by complex dynamics of normal GC B cells, including a switch from highly proliferative to resting cells, interaction with a complex environment and distinct fate decisions, concomitant with divergent transcriptional programs.

Detailed knowledge of differentiation stages, selection mechanisms and fate decisions is limited, especially at single cell resolution. Understanding normal B cell differentiation on single cell level may also improve our understanding of lymphoma development from GC B cells and aberrations of lymphoma cells.

To gain insights into GC B cell differentiation, we subjected FACS-purified GC B cells from human tonsils to 10x Genomics single cell sequencing, receiving a combined data set of RNA-, Ig- and cell surface protein expression for 8 donors. Ig sequences and cell surface molecule expression are included for an even more in-depth characterisation.

Initial transcriptome-based clustering identified known subsets and differentiation stages, such as DZ and LZ B cells, and precursors of plasma cells and memory B cells, as well as previously not categorised subgroups. By characteristic gene expression and gene set enrichment analysis of annotated LZ clusters, we found signatures of different stages of affinity-based selection. We could track cells re-entering the DZ for further proliferation and affinity maturation, representing one fate of positively selected GC B cells. Covering these subsets in our data set enables more profound investigations.