INVESTIGATING LYMPHOMAGENESIS USING TRANSFORMED GERMINAL CENTRE B CELLS AS A MODEL OF EBV⁺ DIFFUSE LARGE B CELL LYMPHOMA

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Topic: Diffuse Large B cell Lymphoma

Diffuse large B cell lymphoma (DLBCL) accounts for over one-third of lymphomas [1,2]. Despite highly toxic immunochemotherapy using rituximab, cyclophosphamide, hydroxy-daunorubicin, vincristine, and prednisone (R-CHOP), 30-40% of patients have refractory or relapsed (R/R) disease which is eventually fatal [3,4]. Particularly poor survival is associated with DLBCL subtypes that over-express MYC, BCL-2 and/or BCL-6, referred to as double or triple hit lymphomas (DHL/THL) [5-7]. Similarly poor outcomes occur in a subset of aggressive DLBCL containing the oncogenic Epstein-Barr virus (EBV) [8]. EBV⁺ tumours are associated with adverse outcomes, even after adjustment for confounding factors [10,11], and express viral nuclear antigens and latent membrane proteins [12]. In these EBV⁺ DLBCL tumours, the virus may provide an additional oncogenic hit driving lymphomagenesis. However, models of B cell lymphomagenesis are poorly developed and overly reliant on cell lines generated from late-stage tumours which represent a poor model to study lymphomagenesis.

To explore the role of EBV in the progression of DLBCL, we utilize DHL models over-expressing MYC/BCL-2 or BCL-2/BCL-6. Germinal centre (GC) B cells, extracted from tonsils, are retrovirally transformed using a combination of these oncogenic hits, as described by Caesar et al. (2019) [13]. These cells are then infected with the M81 strain of EBV. The EBV-infected BCL-2/BCL-6 cells retain markers for GC B cell phenotype (CD10+, CD19+, CD20+, CD85+, CD90+, CD184+) while the expression of markers in the BCL-2/MYC transformed cells and untransformed GC B cells indicate differentiation towards plasmablasts. All cells also show an increase in the expression of the EBV receptor, CD21, up to 7 days post-infection.

7 days post-EBV infection, RT-qPCR shows that both cell types express EBV genes namely, EBNA-1,-2,-3A,-3B, and LMP-2A. Differences in EBV gene expression are observed between MYC/BCL-2 and BCL-2/BCL-6 over-expressing cells. In particular, the levels of LMP-2A and EBNA2 are higher in the BCL-2/BCL-6 DHL model. Taken together, these preliminary results indicate that the role of EBV in the progression of DLBCL may be impacted by the underlying oncogenic transformations. This will likely have implications for patient stratification and treatment strategies.

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