Bio-inspired lymphoid tissues for immune cell cancers

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Introduction: B- and T- cell lymphomas arise from cells that depend on highly orchestrated interactions within immune organs in the course of normal development and immune response.[1] B-cell lymphomas are tumors of antibody-producing cells that undergo unwanted mutations as a mistake during the natural process of gene rearrangement in germinal centers of secondary immune organs (e.g. lymph nodes). Despite our improved understanding of the healthy and diseased B-cell biology from mutational standpoint, many B-cell lymphomas cannot be cultured ex vivo as no relevant 3D models recapitulating lymphoid tissue microenvironment exist. As a consequence, attempts to establish primary cultures of B- and T-cell lymphomas have failed. We hypothesize that engineering lymphoid-mimicking microenvironments presenting signals that promote malignant B-cell survival and growth will enable culture of primary B-cell lymphomas allowing a better understanding of its pathogenesis and response to therapeutics. In this study, we present a modular and tunable 3D organoid of PEG-Maleimide (PEG-MAL) recapitulating the key pro-survival signals from a lymphoid extracellular matrix and those presented by follicular dendritic cells. We demonstrate that B- and T-cell lymphomas respond differentially to extracellular stromal signals and the modular nature of our immune organoids support their survival, growth, and clustering.

Methods: 3D-organoids of 4-arm PEG-MAL (MW:20,000) were engineered by allowing Michael-type addition reaction between Maleimide groups and thiolated crosslinkers.[2,3,4] Peptides targeting αvβ3 and α4β1 specificities were incorporated in 3D organoids. Primary human B-cell lymphoma cell line HBL-1 and T-cell lymphoma OCI-Ly12 were encapsulated in organoids along with human tonsil tissue derived follicular dendritic cells (HK). The survival, proliferation, clustering, and cell-cell interactions between B- and T-cell lymphomas and follicular dendritic cells (FDCs) was confirmed with confocal microscopy and biochemical assays.

Results: We have identified that αvβ3 integrins, that bind RGD motifs, are critical for survival of human T-cell lymphomas and human T-cell lymphoblastic leukemia. Since *Arg-Gly-Asp* (RGD) motifs are abundant in many extracellular matrix (ECM) proteins, we first investigated the response of RGD signaling in organoids on B- and T-cell lymphomas as well the FDCs. We observed enhanced clustering of follicular dendritic cells in absence of RGD and degradable crosslinkers. In presence of RGD and enzymatically degradable crosslinkers, follicular dendritic cells demonstrated enhanced spreading and superior viability. After encapsulation, more than 95% survival of B- and T-cell lymphomas was observed. When presented with RGD and FDCs in 3D lymphoid mimicking tissues, B-cell tumors were dependent on HK FDCs. In contrast,

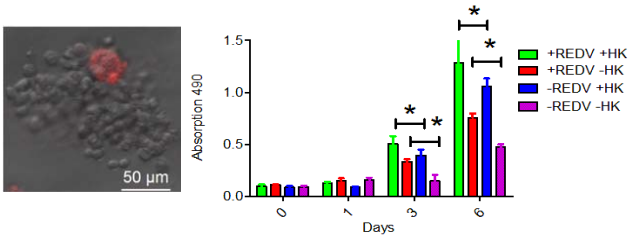

Fig. 1 Primary B-cell lymphoma lines were encapsulated in PEG-MAL hydrogel and the presence of both stromal FDCs (Red, left image) and adhesive ligand (REDV) additively promoted proliferation of lymphomas over 6 days (right image).

proliferation of T-cell lymphomas were synergistically dependent on RGD and FDCs. We next examined if presentation of adhesive ligand *Arg-Glu-Asp-Val* (REDV) that binds Specifically to B-cell specific integrin α4β1 (VLA-4) would show differential response than RGD. As indicated in **Fig 1**, presence of REDV & HK synergistically promoted B-cell lymphoma proliferation. These findings suggest that B- and T-cell tumors have different pro-survival signaling requirements and the presence of FDCs is critical for long term proliferation. We also observed that while RGD signaling does not play an important role in B-cell lymphoma proliferation, it does promote enhanced clustering of cancerous B-cells **(Fig. 2).**

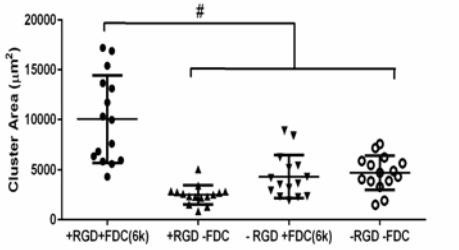

Fig. 2 Presence of both FDCs and adhesive ligand additively promote cell clustering of B-cell lymphomas.

Conclusion: We have engineered 3D microenvironment recapitulating key ECM and stromal aspects of a lymphoid tissue and demonstrated its role in promoting growth of B- and T- cell lymphomas. Till date no such organoids exist for immune cell malignancies and therefore we foresee that such 3D lymphoid niches should enable better mechanistic understanding of lymphomas and promote development of newer, more targeted therapeutics.

Reference: 1. Scott and Gascoyne. *Nature Reviews Cancer* 2014, 517-534

2. Phelps et. al. *Advanced Materials* 2012, 64-70

3. Patel et. al. *Cellular and Molecular Bioengineering* 2014 394-408

4. Lee et al. *Nature Materials*. (Accepted, 2014)