Integrin Ligand-specific, Lymphoid Niches to Study Lymphoma Tumors

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Statement of Purpose: Non-Hodgkin lymphomas (NHL) are a heterogeneous group of lymphoproliferative disorders with the most common form being Diffuse Large B cell lymphoma (DLBCL). Still, most mature B cell lymphomas remain incurable. New classes of drugs have emerged, but only a fraction of patients are responsive. Discrepancies in response are in part due to the genetic heterogeneity of the B cell lymphomas and the complex growth and survival signaling provided by the tumor microenvironment (TME)[1]. Within the TME, integrins, particularly αvβ3 and α4β1, have shown to be variably expressed in different lymphomas and heavily contribute to resistant phenotypes[2,3]. Our understanding of what regulates the drug resistance phenotype and establishing a predictive indicator is limited, in great part, by the lack of adequate ex vivo lymphoma microenvironment models. We have previously designed a biomaterials-based modular lymphoma organoid that presents specific integrin ligands at controlled densities[3]. Here we use this platform to understand the role of lymphoid ECM signaling on proliferation, clustering, and drug response of molecular subtypes of highly heterogeneous DLBCL tumors including those affected by the Epstein-Barr Virus (EBV).

Methods: Lymphoma organoids were synthesized as outlined in Fig 1A. A maleimide-functionalized 4-arm polyethyleneglycol (PEG-MAL) was functionalized with an integrin ligand of interest (peptide REDV for α4β1 and RGD for αvβ3) at specific densities and crosslinked with both MMP-9 degradable (VPM) and non-degradable linkers (DTT). These hydrogels encapsulated human B cell lymphoma cell lines and stromal cells (tonsil derived follicular dendritic cells, HK). For comparison, standard 2D cultures were maintained. In all studies, proliferation and clustering were studied with flow cytometry and microscopy. For drug-response analysis, organoids were cultured for 2 or 4 days before the 24 hours administration of Doxorubicin, Panobinostat, or PU-H71 Cell viability and apoptosis or downstream biochemical signaling were then respectively measured with luminescence plate readings and flow cytometry.

Results: Organoid cultures facilitated the formation of clusters, reflecting native pathological morphologies. These clusters were not seen in 2D culture (Fig 1B). When cultured in organoids, DLBCL cells also demonstrated drug-resistance to both chemotherapy Doxorubicin and the histone deacetylase inhibitor Panobinostat (Fig 1C). We discovered that B lymphoma exhibited upregulation of BCR in the organoid culture (Fig 1D) and believed that this upregulation contributed to the lymphomas' drug resistance. We therefore applied Syk inhibitor R406 to the culture and found that blocking the BCR pathway caused more apoptosis in the organoid culture when compared to 2D culture (Fig 1D). Lastly, in comparison to 2D culture, we found that the presence of EBV made DLBCL more responsive to PU-H71 (Fig 1E). These results are in contrast to non-EBV infected tumors and provide early evidence that the presence of EBV can potentially alter the TME and affect therapeutic response, a phenomenon that remains understudied to date.

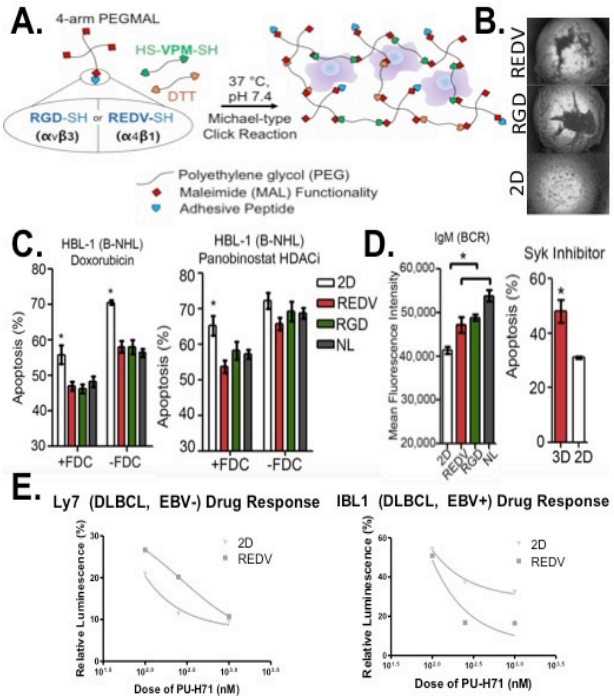

Figure 1. A.) *Schematic of PEG-MAL organoid synthesis and underlying Michael-addition chemistry with thiolated integrin ligands (RGD & REDV) and di-thiolated crosslinkers (VPM sequence and non-degradable DTT).* ***B.)*** *Brightfield images of Ly7 (DLBCL, EBV-) clustering in 2D cultures, RGD-functionalized, and REDV-functionalized organoids.* ***C.)*** *Quantitative analysis of apoptosis (%) in HBL-1 (DLBCL) response to chemotherapy (Doxorubicin) and epigenetic drug (Pabinostat).* ***D.)*** *Quantitative analysis of B cell receptor (BCR) surface expression (left) and ensuing apoptosis (%) after Syk inhibitor treatment (right) in 2D and 3D cultures.* ***E.)*** *Response to PU-H71 in 2D culture and REDV-functionalized organoids for Ly7 cells (DLBCL, EBV-) and IBL-1 cells (DLBCL, EBV+).*

Conclusions: We have engineered a 3D-microenvironment recapitulating key ECM and stromal aspects of lymphoid tissue and demonstrated its role in promoting DLBCL clustering and rendering them drug-resistance. We've also displayed that the presence of EBV further affects microenvironmental signaling and the ensuing therapeutic response. This organoid system will contribute to the study of lymphoma pathology, viral pathogenesis in lymphoma, screening for more efficient regimens, and personalized medicine as a whole.

References: 1. (Scott DW. Nat Rev Cancer, 2014; 14:517-24) 2. (Cayrol F. Blood, 2015;125:841-851.) 3. (Tian YF. Biomaterials, 2015;73:110-119.)