Lymph Node Stiffness Mimicking Hydrogels Regulate Human B Cell Lymphoma Growth and Cell Surface Receptor Expression in a Molecular Subtype-Specific Manner

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Statement of Purpose: Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma, with multiple molecular subtypes. The activated B cell-like DLBCL subtype accounts for roughly one-third of all the cases and has an inferior prognosis. There is a need to develop better class of therapeutics that could target molecular pathways in resistant DLBCLs; however this requires DLBCLs to be studied in representative tumor microenvironments. The pathogenesis and progression of lymphoma has been mostly studied from the point of view of genetic alterations and intracellular pathway dysregulation. By comparison, the importance of lymphoma microenvironment in which these malignant cells arise and reside has not been studied in as much detail. We have recently elucidated the role of integrin signaling in lymphomas and demonstrated that inhibition of integrin-ligand interactions abrogated the proliferation of malignant cells in vitro and in patient-derived xenograft (1, 2). Here we demonstrate the role of lymph node tissue stiffness on DLBCL in a B cell molecular subtype specific manner.

Methods: To characterize changes in the stiffness of lymphoid tissues during tumorigenesis, we first determined the change in the size of lymph nodes harvested from the Eμ-myc mouse model of human lymphoma (3). Oncogenesis in these mice is driven by the presence of a transgene containing the c-Myc gene under the control of the immunoglobulin heavy chain enhancer (Eμ) and c-Myc promoter. Nearly all Eμ-myc mice eventually develop pre-B or B-cell lymphomas, with approximately 50% of animals developing neoplasms by 15-20 weeks of age. De-identified, freshly isolated primary human follicular B cell lymphoma tissue from lymph node of an untreated patient was used for stiffness measurement. Lymphoid tissue and hydrogel mechanical properties were quantified using our well characterized micropipette aspiration method (4). hydrogels were engineered by reinforcing gelatin with silicate nanoparticles, a hydrogel platform previously reported by us to support the survival, growth, and differentiation of primary B cells (5, 6). All cell analyses were performed using a combination of flow cytometry and microscopy.

Results: Both inguinal and cervical lymph node from the Eμ-myc mouse model showed marked enlargement as compared to a healthy lymph node (**Fig. 1A**; ~10 fold larger size; 0.3 cm with tumor-bearing mice; $p<0.05$). The strain energy density for neoplastic lymph node was a significant two-fold higher than the healthy lymph node ($p<0.5$). We next compared the stiffness of a primary human lymphoma tissue and observed that the stiffness was comparable to neoplastic mouse tissue ($p>0.05$). We engineered tunable bio-artificial hydrogels that mimicked the stiffness of healthy and neoplastic lymph nodes of a transgenic mouse model and primary human lymphoma tumors. Our results demonstrate that molecularly diverse DLBCLs grow differentially in soft and high stiffness microenvironments, which further modulates the integrin and B cell receptor expression level as well as response to two classes of cancer therapeutics (**Fig 1B**), including an epigenetic inhibitor of histone deacetylase. Most importantly, these results were observed as a function of molecular subtypes of DLBCL, with activated B cells subtype being the most responsive to tissue stiffness as compared to germinal center B cell DLBCL (**Fig 1B**).

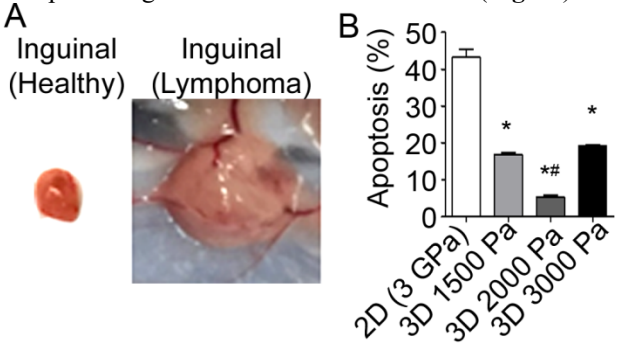

Figure 1. Lymphoid tissue stiffness regulates drug response in lymphomas. **A)** Photographs representing healthy and neoplastic lymph nodes and spleen from Eμ-myc mice. Animals were sacrificed prior to showing clinical signs (healthy) or upon reaching health endpoints (lymphoma). **B)** Lymphoid tissue stiffness modulates drug resistance in mature B cell lymphomas treated with the conventional chemotherapy drug vincristine or histone deacetylase inhibitor Panobinostat. (A) Bar graphs represent % apoptosis in B cell lymphomas exposed to vincristine. Activated B cell DLBCLs were cultured for 3 days in 2D or 3D organoids and exposed to 1 mM vincristine for 48 hr. B cell lymphomas were stained with APC-IgM and Annexin V-FITC for apoptosis analysis (Mean ± S.E.M, n = 3, *$p < 0.05$ compared to all 3D groups and #$p < 0.05$ compared to all other 3D groups).

Conclusions: To the best of our knowledge, our results are the first evidence that lymphoma survival, proliferation, drug response, and BCR signaling is influenced by lymphoid tissue stiffness in a molecular subtype dependent manner. These results emphasize the role of biomaterials based 3D tissues with tumor matched stiffness and the importance of lymphoid tissue stiffness in malignant B cell tumors. In the past, lymphoid tissue stiffness and mechanosensing has been largely ignored in mechanistic study lymphoma progression and its therapeutic evaluation of B cell lymphomas *ex vivo*. We anticipate that our findings will be broadly useful to study lymphoma biology and discover new class of therapeutics that target B cell tumors in physical environments.

References:

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