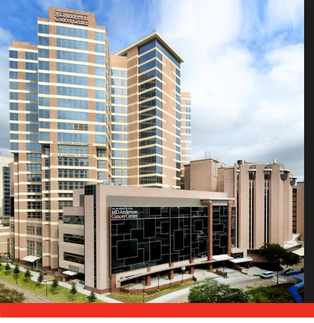

Tumor Stiffness is Associated with Reduced CD45+ Immune Cell Penetration of Tumors in a Rat Hepatocellular Carcinoma Model

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Introduction –

Tumor stromal heterogeneity can negatively impact immune surveillance and treatment outcomes. Current cross-sectional imaging tools rely solely on tumor size and perfusion to monitor therapy and fail to characterize tumor biomechanics. Shear wave elastography (SWE) has emerged as a non-invasive technique that can quantify stromal elasticity. The present study aims to characterize the molecular underpinnings associated with SWE in liver cancer, as well as identifying the differences in immune infiltration of tumors of different phenotypes.

Methods –

- Orthotopic implantation of Rat hepatoma cell line McA-RH777 with green fluorescent protein (GFP)
- Post 3- week ultrasound and 2D shear wave elastography to verify engraftment
- Tumors then collected, fixed, and sectioned by histology dept.
- Multiplex immunofluorescence staining of sections with Leica Bond RX autostainer and Akoya Biosciences Opal 7-color kit.
- Two panels were created to compare soft and stiff tumors
- Quantitative image analysis completed with HALO v 3.6 imaging software, tumors were partitioned into four zones, tumor-liver interface (Z4), peripheral (Z3), paracentral (Z2), and central (Z1).
- Data analyzed in GraphPad Prism where P-values of <0.05 were considered significant.

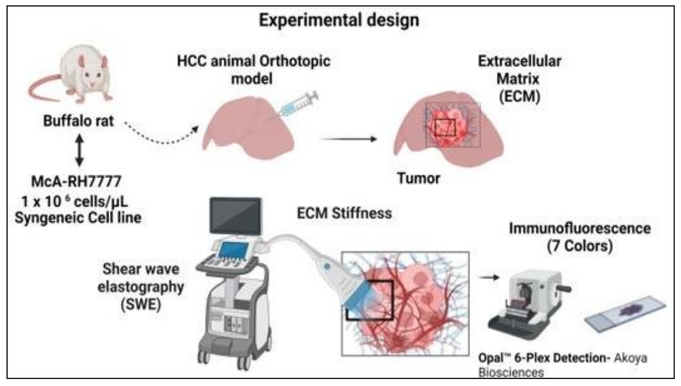

Figure 1. Experimental Design. Graphic demonstrating the workflow from tumor cell implantation to sectioning and staining by histology

Marker	Opal
IBA-1 (MACROPHAGES)	480
SMOOTH MUSCLE ACTIN	520
S100A4 (FSP1)	570
GFAP (STELLATE CELLS)	620
TGF-BETA	690
VIMENTIN	780
NUCLEAR COUNTERSTAIN	DAPI

Marker	Opal
CD34	480
GFP (TUMOR)	520
CD45	570
VIMENTIN	620
KI-67	690
ARGINASE-1 (HEPATOCYTES)	780
NUCLEAR COUNTERSTAIN	DAPI

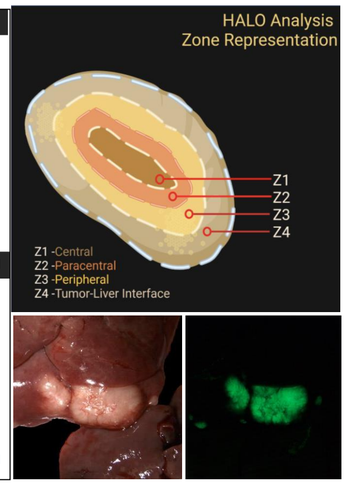

Figure 2. Markers and Zone Differentiation. Multiplex Immunofluorescence stain legend indicating the components being analyzed within the liver tumors **(Left).** HALO zone partitioning representation and gross pathology of liver tumors with GFP. **(Right).**

Results

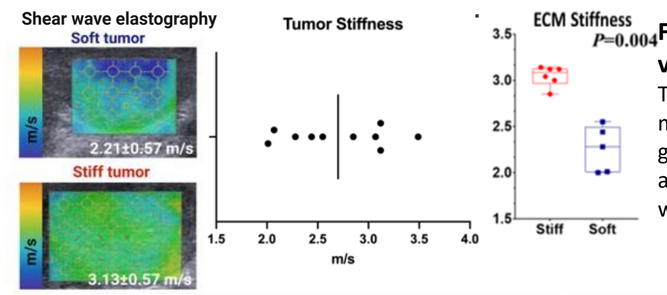

Figure 3. Tumor Stiffness Measurements via Shear Wave Elastography (SWE). Tumor stiffness ranged from 2.01 m/s to 3.49 m/s with a mean of 2.7 m/s. Tumors with greater than average stiffness were designated as stiff compared to tumors less than 2.7 m/s which were characterized as soft.

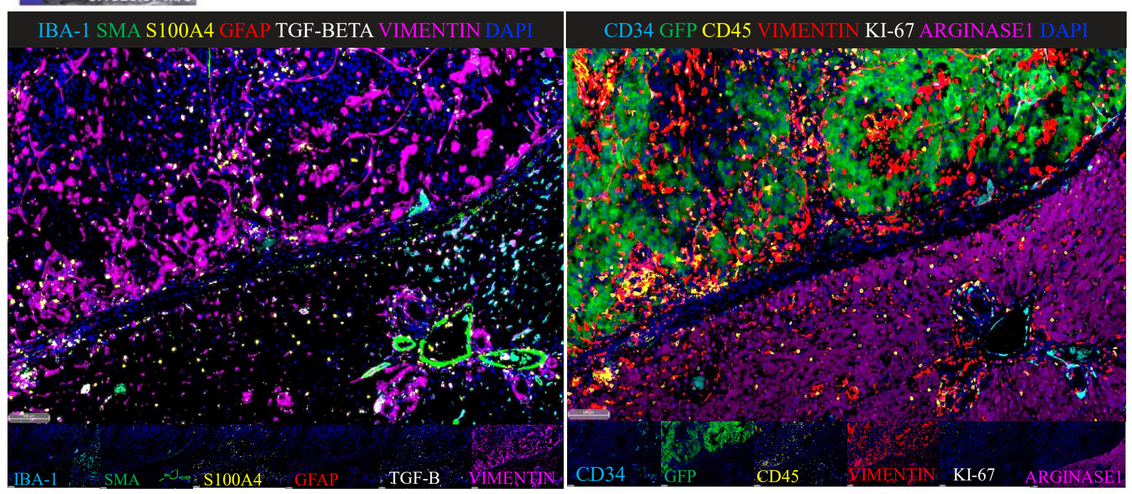

Figure 4. Multiplex Immunofluorescence Panels. Two panels created to analyze tumor content in stiff and soft tumors, each with six markers of different colors for distinction.

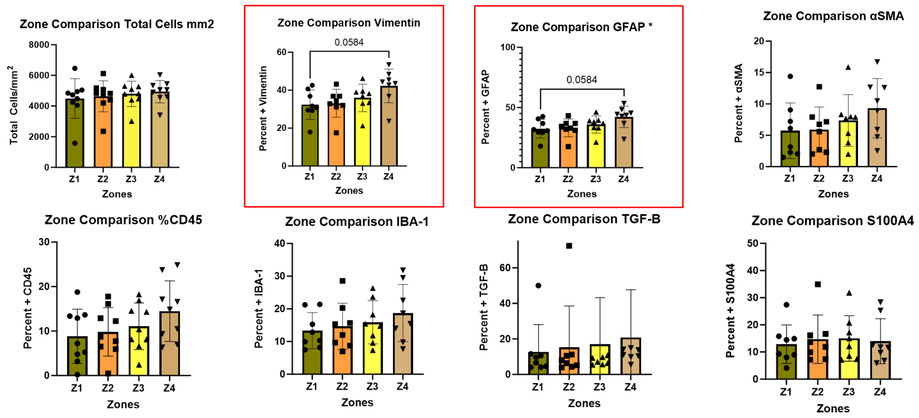

Figure 5. Zone Comparisons. Vimentin and GFAP content were significantly higher at the tumor liver interface (TLI). A trend was also present for increased SMA+, CD45+, IBA-1+, TGF-beta+ cells at the TLI.

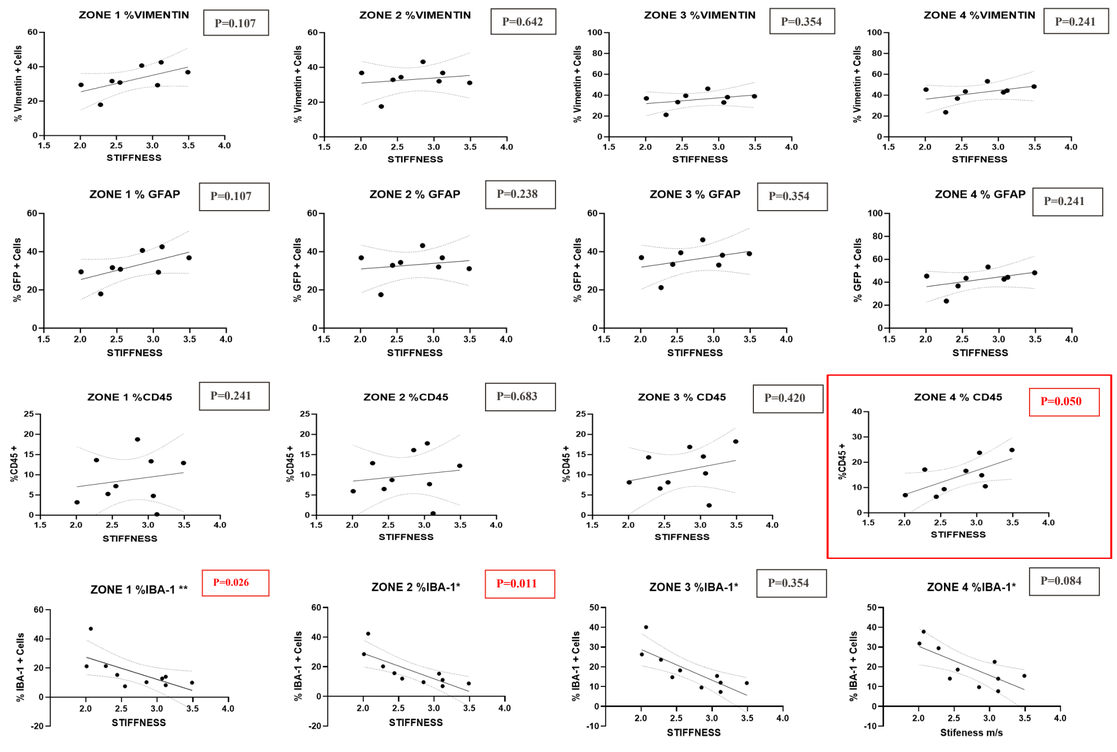

Figure 6. Stiffness and Marker Trends. Quantitative analysis of Vimentin, GFAP, CD45, and IBA-1 presence as tumor stiffness increases. Significance values found in the (TLI) in stiffer tumors for CD45 presence. IBA-1 negatively correlates with tumor stiffness in central and paracentral regions.

Discussion –

Shear wave elastography demonstrated variable tumor stiffness in a rat HCC model. We developed two 7-color mIF panels to evaluate molecular features of the tumors and determine the molecular underpinnings of tumor stiffness.

Vimentin and GFAP were significantly increased in the tumor-liver interface (TLI) (zone 4) when compared to the tumor center (zone 1). Analysis of the tumor zones also showed a trend towards increased CD45+ leukocytes and IBA-1+ macrophages at the TLI. Increased fibroblasts (vimentin+) and hepatic stellate cells (GFAP+) at the TLI create a barrier for immune infiltration resulting in poor CD45+ immune infiltration in central and paracentral areas.

Increased CD45+ immune cells at the TLI positively correlated with tumor stiffness. There was a negative correlation with IBA-1+ macrophages at the central and paracentral areas. These results suggest that increased tumor stiffness could be an indicator of poor immune infiltration into more central areas of the tumor.

A limitation in this study was the number of samples available for analysis. Eight tumors were used but could benefit from a larger sample size due to the presence of positive trends in the quantitative analysis. Additionally, HALO protocols needed extensive adjustment in order to accurately detect positive signals.

Future directions for this study could include continuing analysis for the other markers which may hold explanations as to why Immune cell and macrophage infiltration may be stopped by the abundance of fibrous cell content within stiff tumors. Additional panels to further characterize immune cell subsets may also provide additional immunopathogenic insights.

Conclusions –

- Shear wave elastography (SWE) can be utilized to detect differences in tumor stiffness in a rat HCC model.
- Increased fibroblasts and hepatic stellate cells are present at the tumor-liver interface (TLI) and create a barrier for CD45+ immune cell penetration to central areas of the tumor.
- SWE may be a non-invasive technique for predicting tumor fibrosis and immune exclusion in liver tumors.

References –

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