The molecular landscape of transformed and non- transformed cells in an engineered fibrous microenvironment

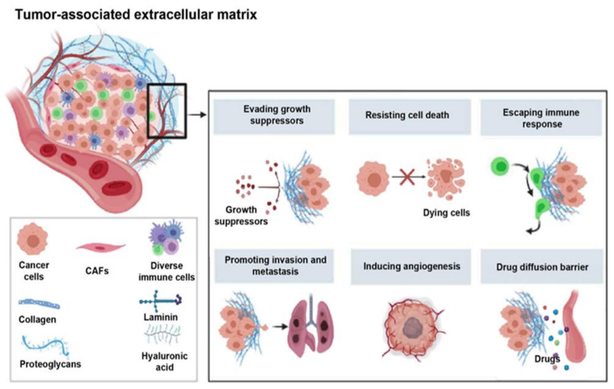
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Introduction

- Cancer is a complex disease with a plethora of hallmark characteristics and resulting in millions of fatalities every year around the world. In reality cancer is effectively a group of diseases composed of many genetically different conditions having various commonalities in the molecular mechanisms and metabolic changes (Brown et al, 2023).

- All cells reside in and respond to fibrous extracellular environments, but how these responses differ between non-transformed and transformed (cancer) cells is not well understood. This gap is important because altered mechanosensing may contribute to malignant progression.**We hypothesise that mechanosensitive responses to fibrous environments are defective in transformed cells compared to normal cells, and that targeting this defect may suppress the transformed phenotype.** To test this, we will use engineered fibrous scaffolds, including polycaprolactone (PCL) electrospinning, to create tissue-like microenvironments with tunable chemical and mechanical properties. We will compare non-transformed and transformed cell lines to assess differences in their behaviour within these matrices.

Tumor-associated extracellular matrix in solid tumors



Methods and Materials

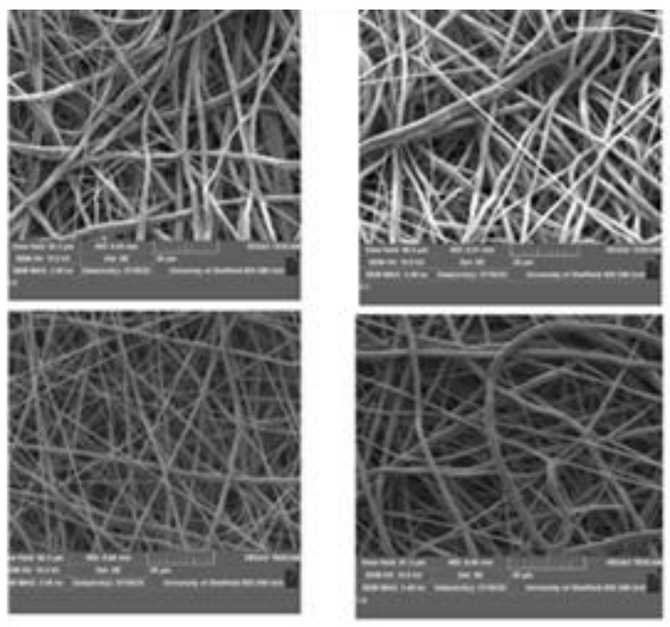


Figure 2. Scanning electron microscopy (SEM) images of electrospun polycaprolactone (PCL) scaffolds with distinct architectures. The top row shows 3D PCL scaffolds characterized by a highly porous, multi-layered fiber arrangement, enabling increased surface area and spatial complexity..

Quantitative PCR (qPCR)

(qPCR), a PCR-based technique that couples amplification of a target DNA sequence with quantification of the concentration of that DNA species in the reaction (Dymond, 2013). It Was performed on BJhtert and BJ-Ras cells to assess gene expression profiles.

RNA Sequencing

RNA sequencing (RNA-seq) is a technique used in Molecular Biology that was used to analyze the quantity and sequences of RNA in a sample using high throughput sequencing. It allows researchers to identify which genes are actively expressed and at what levels in different cells or conditions (Wang et al, 2009).

Results

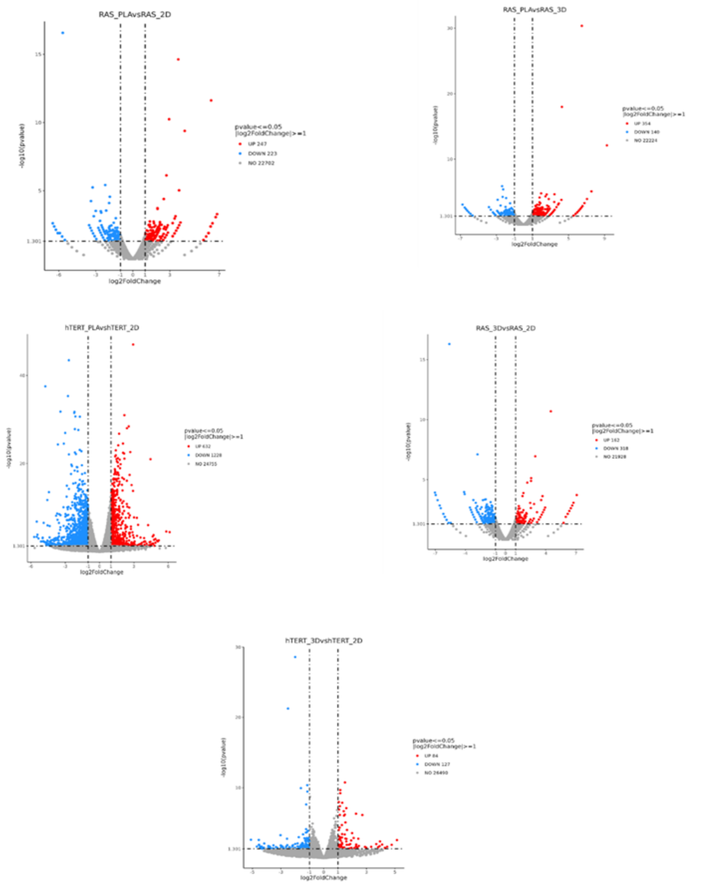


Figure 4. Differential gene Volcano Map. Volcano plots can be used to infer the overall distribution of differentially expressed genes. In this figure, The x-axis shows the fold change in gene expression between different samples, and the y-axis shows the statistical significance of the differences. Red dots represent up-regulation genes and blue dots represent down-regulation genes.

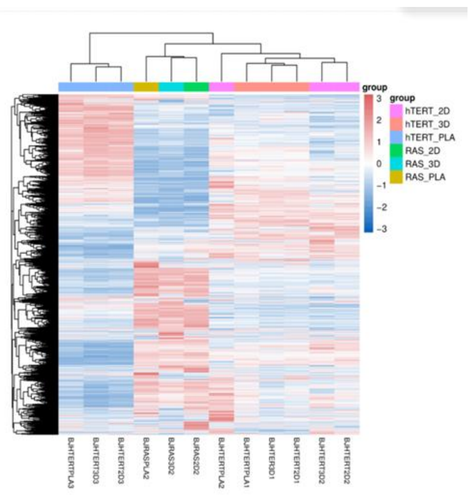


*Figure 5.*Differentially expressed genes were pooled and analysed by hierarchical clustering of FPKM values following row-wise Z-score normalisation. The heatmap displays relative expression patterns, where colours represent normalised values (−2 to 2) and are comparable across samples for each gene. Both gene- and sample-level clustering were performed, with the final output showing relationships between samples.

Methods and Materials

We fabricated these fibrous matrices using electrospinning, allowing us to *independently tune* both the mechanical stiffness and chemical composition. We have optimised fibre stiffness, seeded both non-transformed (BJhTtransformed (BJhTERT-SV40T-H-RasV12) fibroblasts ERT) and onto these scaffolds.

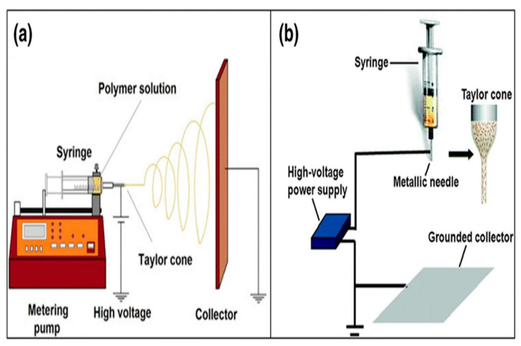


Figure 1 showing the steps of electrospinning of PCL

Results

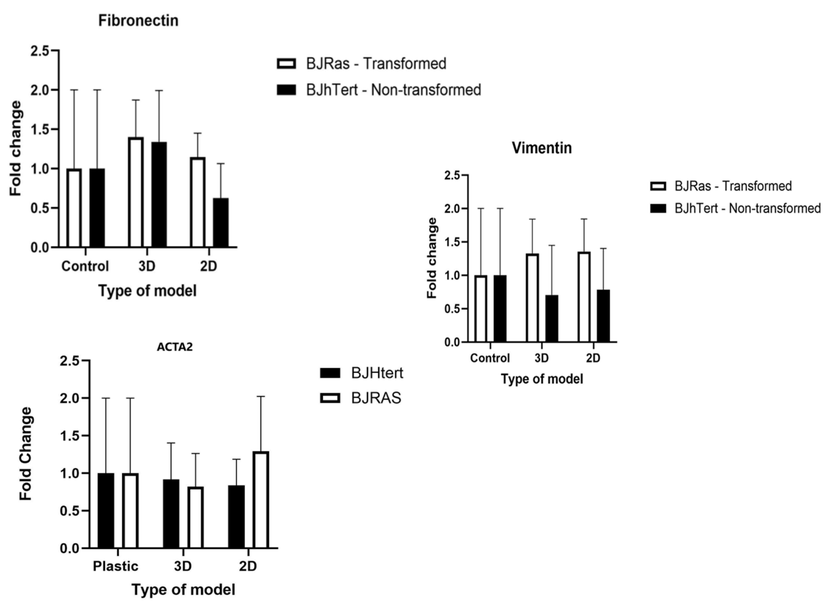


*Figure 3.Shwoing the results of q*uantitative PCR (qPCR) results illustrating the differences between the immortalized BJhtert fibroblasts and their Ras-transformed Counterparts as well as the control cells.

Conclusions

Despite extensive research on environmental stiffness in cancer progression, there is limited understanding of how dynamic mechanical cues influence cells throughout metastasis. Crucially, differences in how normal and cancer cells respond to three dimensional fibrous environments, such as the model system used here, remain poorly understood. (Kozaburo and Mayumi, 2015).

Here, we aim to address this gap by first comparing cell responses in 2D and 3D environments and then examining how normal and oncogenically transformed cells respond to different fibre conditions. Key findings from RNA-seq will be independently validated using quantitative RT-PCR.

Acknowledgments

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