Exploring how ECM stiffness influences breast cancer cell sensitivity to cell cycle inhibitors

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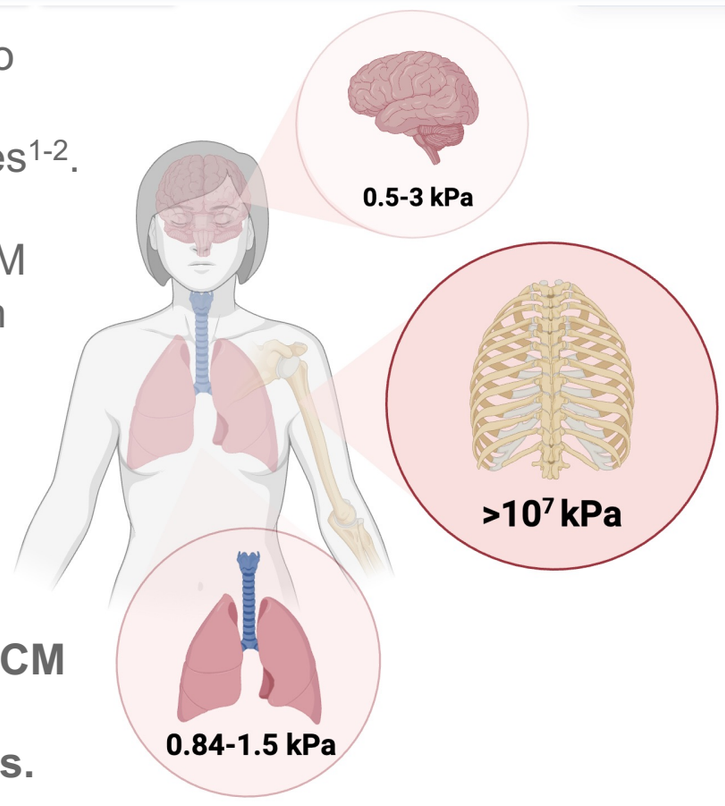
Introduction

- Metastatic breast cancers disseminate into extracellular matrices (ECM) with varying stiffness, from soft brain to stiff bone niches[1-2].
- Mechanotransduction and changes to ECM stiffness can influence tumour progression and invasiveness[3-5].
- Limited studies have explored how these metastatic niches could impact cell responses to cell cycle inhibitors.

My PhD project aims to investigate how ECM stiffness can regulate HR+/HER2- breast cancer cell responses to CDK4/6 inhibitors.



Methods

- HR+/HER2- breast cancer models were used to conduct CDK4/6 inhibitor (CDK4/6i; Palbociclib) response assays in varying hydrogel stiffness.
- Cell morphology and epithelial-mesenchymal transition will be monitored in increasing stiffness.
- 2D/3D MCF7 reporter cell lines were used to examine cell cycle protein expression during CDK4/6i treatment

0.1 kPa — 10^7 kPa

1. Decreased MCF7 sensitivity to Palbociclib in low hydrogel stiffness

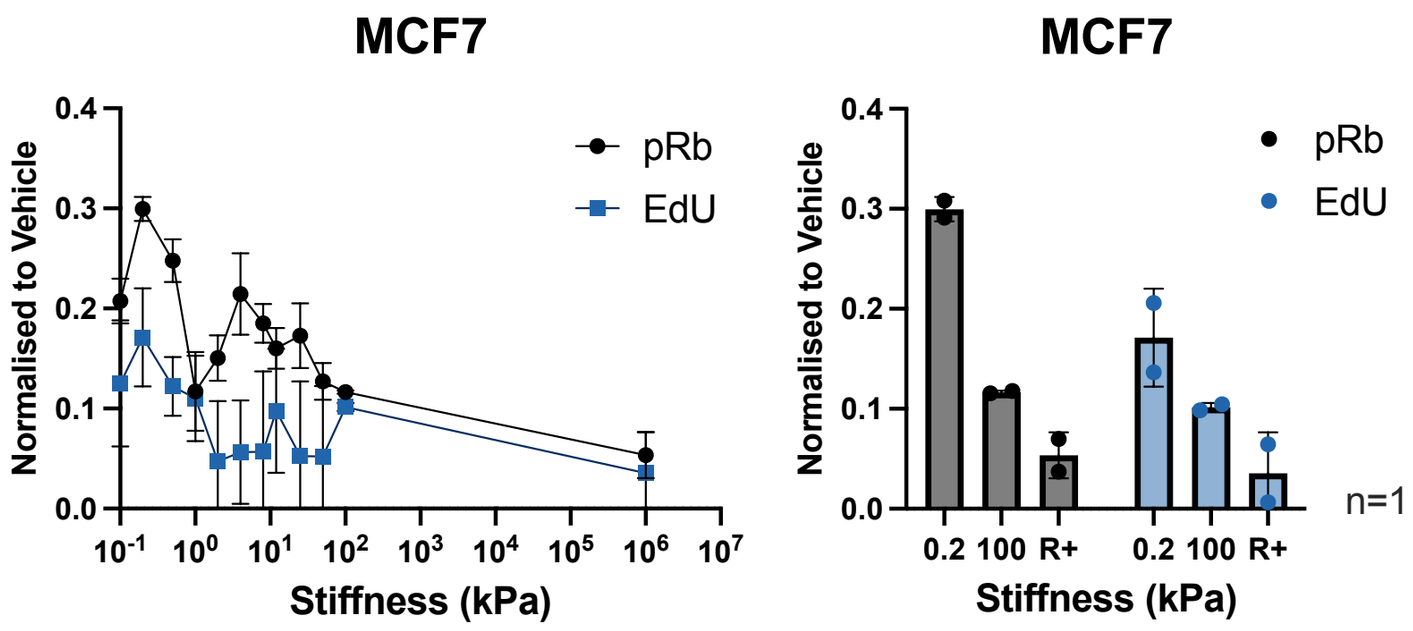


Figure 1: Mean fold change of pRb+ and EdU+ expressing MCF7 cells in varying hydrogel stiffness (0.1kPa-100kPa, R+; rigid matrix at ~10^7kPa) with Palbociclib treatment (1μM) normalised to vehicle-treated cells (DMSO).

2. Increased p21+ cells in Palbociclib 2D MCF7s

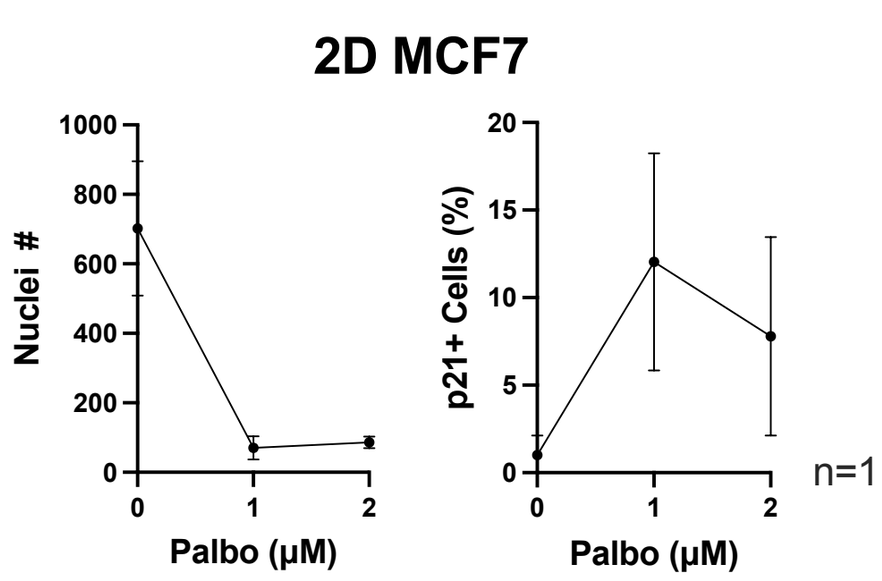
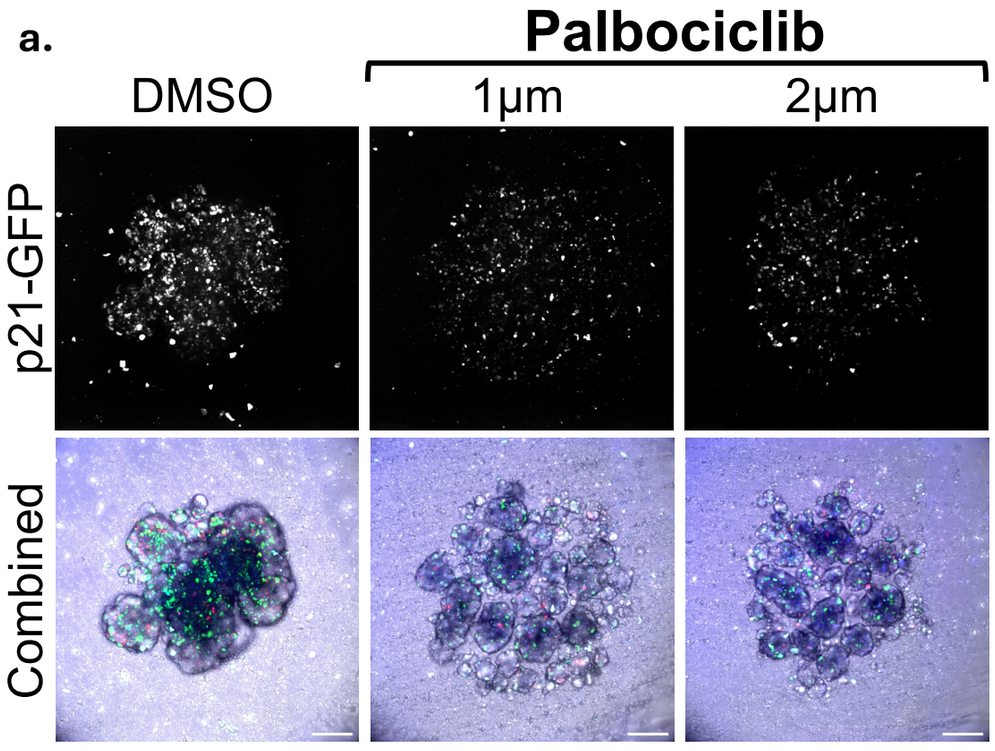


Figure 2: Nuclei count (left) and percentage of p21+ expressing cells (right) in 7-day Palbociclib (0, 1, and 2μM) treatment with 2D wild-type MCF7s.

3. Decreased p21 protein expression in 3D MCF7 spheroids with 7-day Palbociclib treatment



MCF7 (TagBFP2-H2B, mCherry-Geminin, p21-GFP)
Scale bar: 200μm

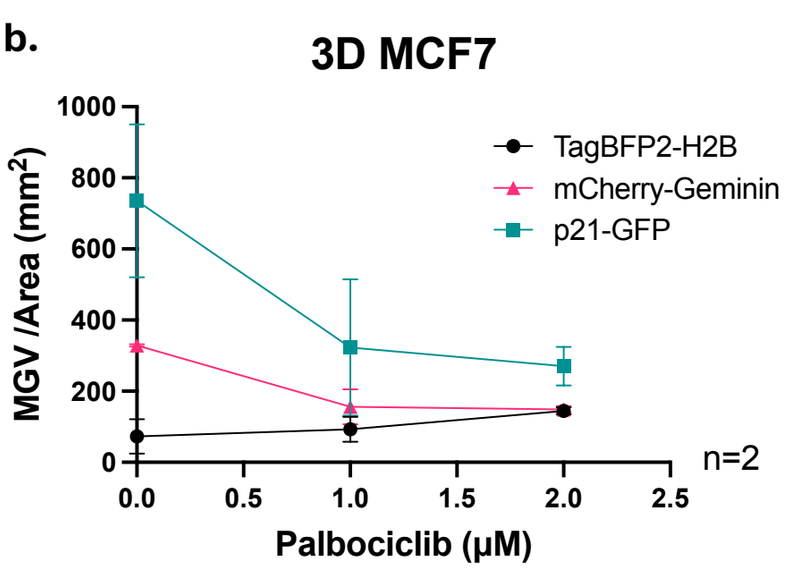
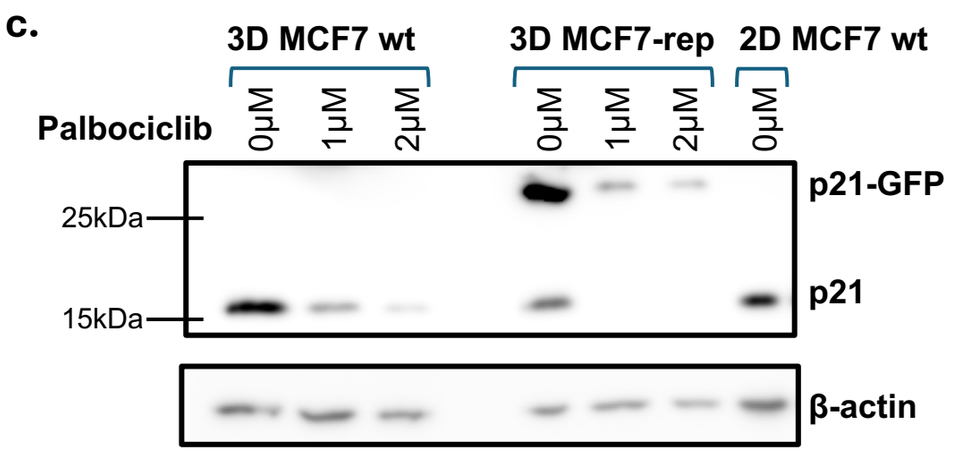
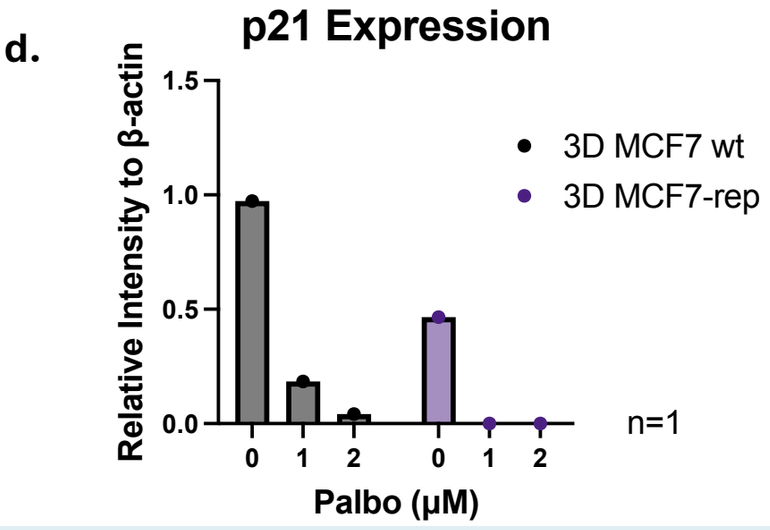


Figure 3: a). p21-GFP expression in 3D MCF7 fluorescent reporter cells and b). quantification of mean gray values (MGV) normalized to total spheroid area in 7-day Palbociclib treatment. c-d). MCF7 p21 protein expression from a western blot.



MCF7-rep: MCF7 (TagBFP2-H2B, mCherry-Geminin, p21-GFP)



Future Work

Evaluate cell morphology and mesenchymal transition in HR+/HER2- breast cancer cells with varying matrix stiffness.

RNAseq and proteomics of cells in varying ECM stiffness with CDK4/6i treatment to identify mechanotransduction pathways regulating cell sensitivity to CDK4/6i.

Assess the population of quiescent and senescent cells in 2D/3D arrested cells, post-CDK4/6i treatment, to identify for CDK4/6i persistence.

References

1. van Uden DJP, van Maaren MC, Strobbe LJA, Bult P, van der Hoeven JJ, Siesling S, et al. Metastatic behavior and overall survival according to breast cancer subtypes in stage IV inflammatory breast cancer. Breast Cancer Res. 2019;21(1):113
2. Guimaraes CF, Gasperini L, Marques AP, Reis RL. The stiffness of living tissues and its implications for tissue engineering. Nat Rev Mater. 2020;5(5):351–70.
3. Gehler S, Ponik SM, Riching KM, Keely PJ. Bi-directional signaling: extracellular matrix and integrin regulation of breast tumor progression. Crit Rev Eukaryot Gene Expr. 2013;23(2):139–57.
4. Horta CA, Doan K, Yang J. Mechanotransduction pathways in regulating epithelial-mesenchymal plasticity. Curr Opin Cell Biol. 2023;85:102245.
5. Xu Z, Yu J, Chen D, Liu Y, Zhang G, Guo Q, et al. Extracellular matrix stiffness regulates the proliferation and migration capacities of lymphatic endothelial cells via FAT1. Front Cell Dev Biol. 2025;13:1667154.