TUNI Luottamuksellinen - Confidential (3Y)

EXPLORING THE MECHANICAL CHARACTERISTICS OF TUMOUR MICROENVIRONMENT IN COLORECTAL CANCER



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1 INTRODUCTION

Colorectal cancer (CRC) is among the leading causes of cancer-related deaths globally, driven by its invasive nature [1, 2]. Cancer invasion is a complex process significantly influenced by the dynamics of the tumour microenvironment (TME), a network of cells, signalling factors, and supporting structures that collectively sustain cancer growth. [3, 4]

A critical aspect of the TME is its **physical attributes**, which exert various mechanical cues on the tumour, and vice versa. Although the role of mechanical forces in cancer is well recognised, research has been limited by a lack of comprehensive *in vitro* models with many focusing solely on modulating matrix stiffness. [3] Recent evidence [5] indicates that tissue mechanics are not adequately described by a simple stiff-versus-soft paradigm. Instead, tissues exhibit **viscoelastic** behaviour, combining both elastic and viscous characteristics, which profoundly influences cellular responses and tumour progression.

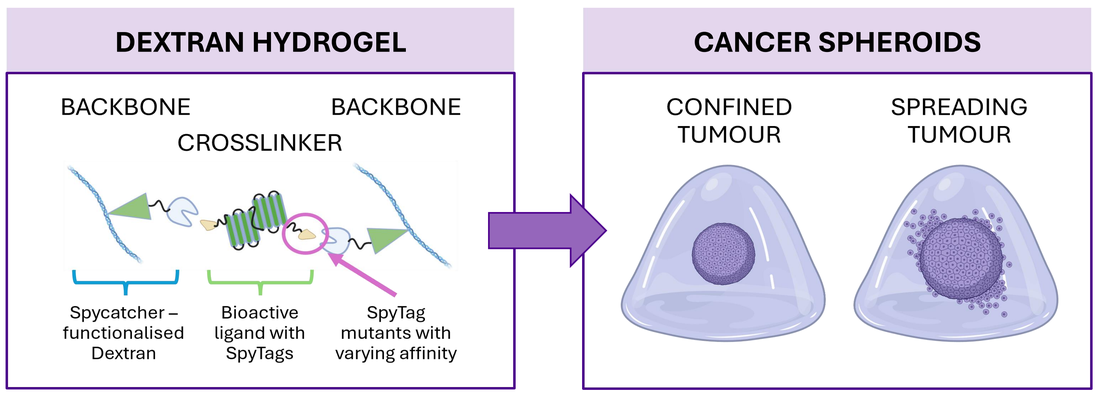
In the current study, we utilise colorectal carcinoma spheroids cultured in novel dextran –based hydrogels with tunable crosslinker-strength to investigate **how changes in the mechanical remodelling capacity and viscoelasticity of the TME affect cancer progression**.

2 MATERIALS AND METHODS

Cell line
- HCT-116 colorectal carcinoma cell line (ATCC, CCL-247)
- Embedding in gels as single cells. Natural spheroid formation over seven (7) days.

Methods
- 3D CRC cell culture
- Phase contrast microscopy
- Real Time Quantitative PCR (RT-qPCR)

Cancer model

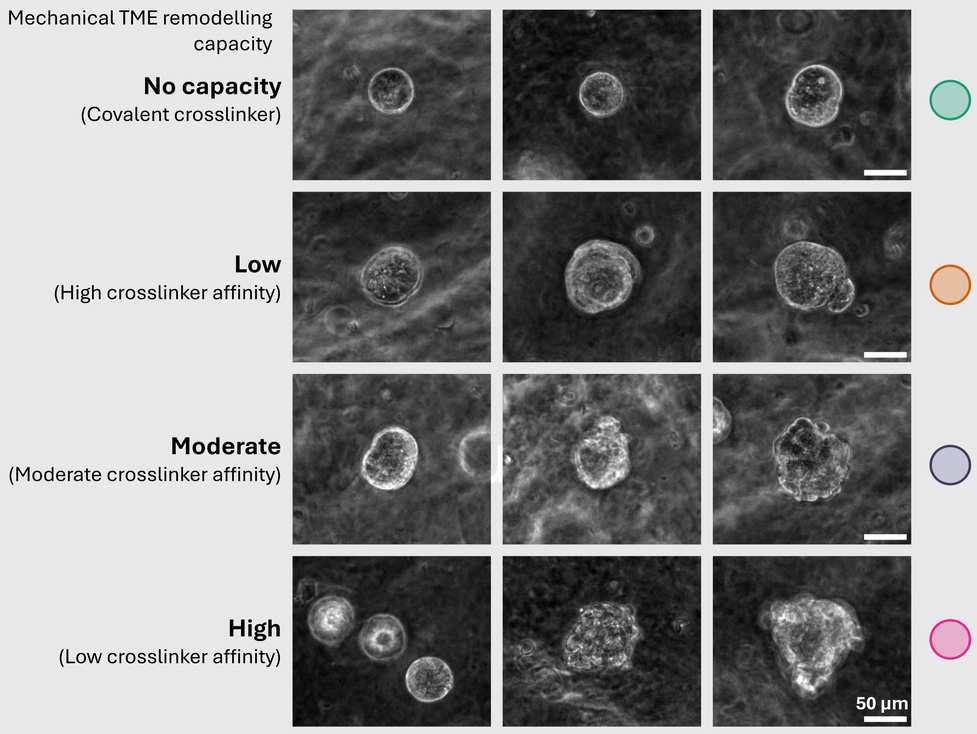


- SpyTag mutants enable crosslinker affinity and thus hydrogel viscoelasticity tuning.
- Dextran supplemented with type 1 collagen.

3 RESULTS

1) CRC spheroids proliferate and attain an invasive phenotype in environments with mechanical remodelling capacity

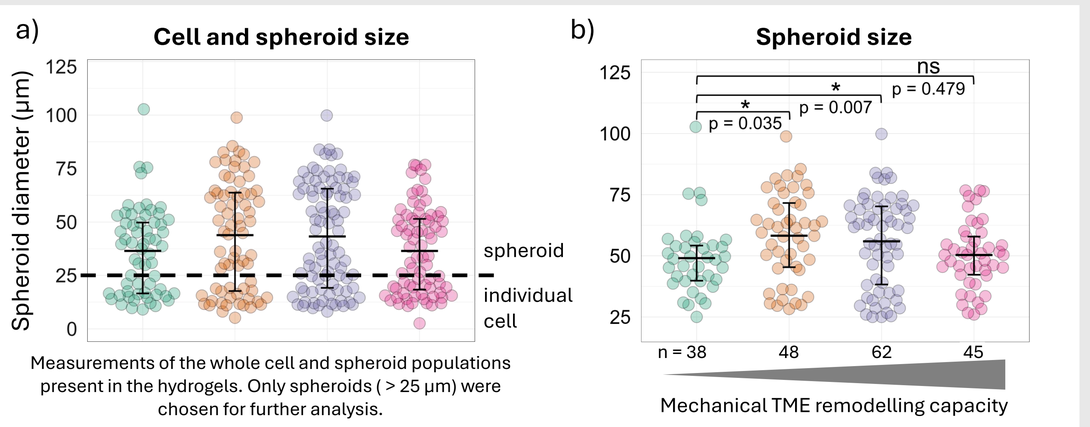
Phase contrast imaging at day 7 reveals the effect of mechanical TME remodelling capacity on HCT-116 spheroid growth-rate and phenotype. TME with High remodelling capacity results in larger, more irregularly -shaped spheroids after seven days in culture.



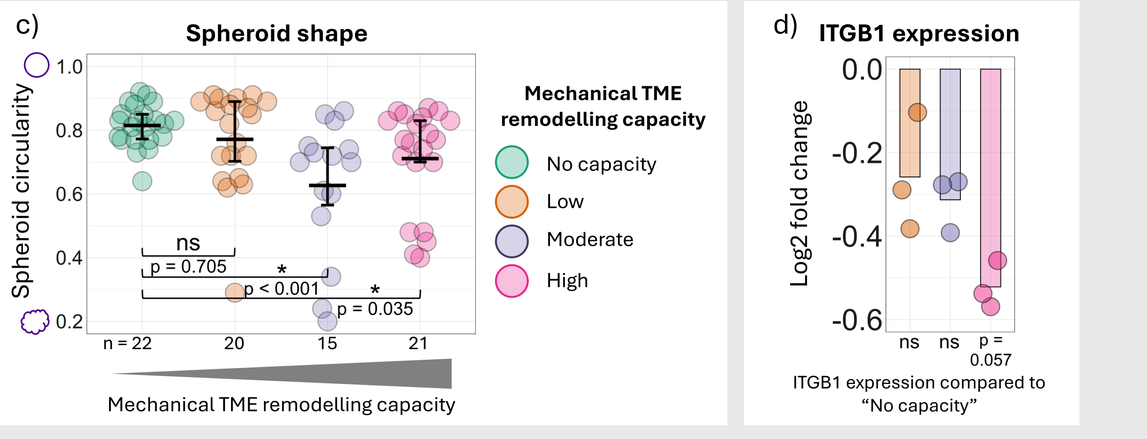
Three representative ROIs from each hydrogel. All scale bars 50 µm.

2) CRC spheroid morphology is dependent on mechanical remodelling capacity

Cell and spheroid size and shape measurements at day 7 demonstrate the effects of mechanical TME remodelling capacity on the tumour morphology. ITGB1 gene expression analysis of the spheroids suggests a loss of cell adhesion dependent on TME remodelling capacity.



Measurements of the whole cell and spheroid populations present in the hydrogels. Only spheroids (> 25 µm) were chosen for further analysis.



ITGB1 expression compared to "No capacity"

4 CONCLUSIONS

Mechanical TME remodelling capacity influences HCT-116 colorectal cancer spheroid behaviour. HCT-116 cells cultured in TME with High mechanical remodelling capacity have an increased growth-rate and form more dynamic anisotropic spheroids when compared to those cultured in TME with mechanically Low remodelling capacity. Analysis of ITGB1 expression suggests remodelling capacity –dependant loss of integrin B1 –mediated cell signalling, which is often associated with increased invasiveness. Our results indicate that the ability of CRC tumours to mechanically modify their environment plays a vital role in their ability to proliferate and attain invasive morphologies.

FIGURE LEGENDS

Mat&Met: (Left) Principle of the SpyCatcher-functionalised Dextran-based hydrogel with Fibronectin –containing SpyTag –crosslinker. (Right) Graphical abstract of the effects of viscoelasticity/mechanical TME remodelling capacity on CRC spheroid proliferation. In TME with high mechanical remodelling capacity and viscoelasticity, the spheroids can proliferate and attain invasive phenotypes.
Figure 1: Phase contrast images of HCT-116 CRC spheroids in hydrogels with Low to High mechanical remodelling capacity supplemented with with 0.5 mg/ml type 1 collagen at day 7 reveal how the ability of the tumour spheroid to modify the environment (TME) affects its' proliferation and morphology. Spheroids cultured in High remodelling capacity -environments form larger structures and grow into irregular morphologies, while those in Low or No capacity -environments remain smaller and denser in structure.
Figure 2: Measurements and analysis of cell/spheroid size (diameter) and shape (circularity). Each data point is one measured cell/spheroid. a) All hydrogel cultures at day 7 contained both spheroids (diameter > 25 µm) and individual cells (diameter < 25 µm). Only spheroids were chosen for further analysis in b), which shows how TME remodelling capacity correlates with spheroid size until the environment is highly remodellable, after which c) the spheroids attaining increasingly irregular morphologies often associated with cell shedding and invasiveness. d) Gene expression analysis with qPCR suggests that integrin B1 expression decreases in spheroids cultured in higher remodelling capacity environments indicating their increased potential for invasion.
Group differences (b, c, d) were analysed using Mann-Whitney U test versus the "No capacity" group. Crossbars (b, c) indicate mean values and whiskers denote the interquartile range (25-75 %). Two-sided $p < 0.05$ was considered significant.

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Patent pending

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