Regulation of cancer cell metabolism and proteostasis by mechanical tissue cues

Category: Experimental Hematology / Oncology

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Background and Objectives

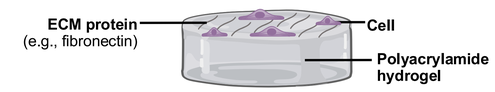
- The **integrated stress response** (ISR) is a signalling pathway that regulates **protein synthesis** in response to diverse stressors (e.g., amino acid scarcity) and is implicated in **cancer progression** and **treatment resistance**.
- Phosphorylation of the α subunit of eukaryotic translation initiation factor 2 (**eIF2α**) attenuates global **mRNA translation**, while enhancing selective translation of mRNAs, including activating transcription factor 4 (**ATF4**). This promotes an adaptive cellular response to aid recovery and survival to re-establish homeostasis or, alternatively, induce cell death.

- Tissue **stiffness**, a mechanical property of the tumour microenvironment determined by the extracellular matrix (ECM), influences cell behaviour through **mechanotransduction** (e.g., via **YAP/TAZ**).
- Stiffer tumour tissue promotes the proliferative, invasive, and metastatic capacity of cancer cells, and promotes drug resistance by impeding drug delivery.

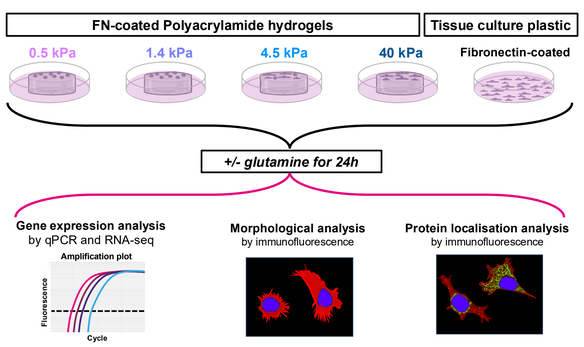
- Given the importance of both mechanotransduction and the ISR in cancer biology, we hypothesise that **stiffness may modulate ISR activation**, thereby linking mechanical cues to stress adaptation in tumor cells.

Methods

- **Solid cancer cell lines** (A375, A375-MA2, MiaPaCa-2, Saos-2, and T98G) were cultured on fibronectin-coated **polyacrylamide hydrogels** with stiffness ranging from **0.5 to 40 kPa.**
- Morphological analysis was performed to assess stiffness sensitivity of cell lines, whereby cell area (um^2) and circularity was measured via immunofluorescence and quantified using Fiji software.
- Gene expression of key YAP/TAZ targets, *CYR61* or *CTGF*, was assessed by qRT-PCR.



- **ISR** pathway was activated in A375 melanoma cells by **glutamine starvation** and assessed by qRT-PCR, RNA sequencing, and immunofluorescence to evaluate global gene expression and regulation of selected ISR and **proteostasis networks.**



Results

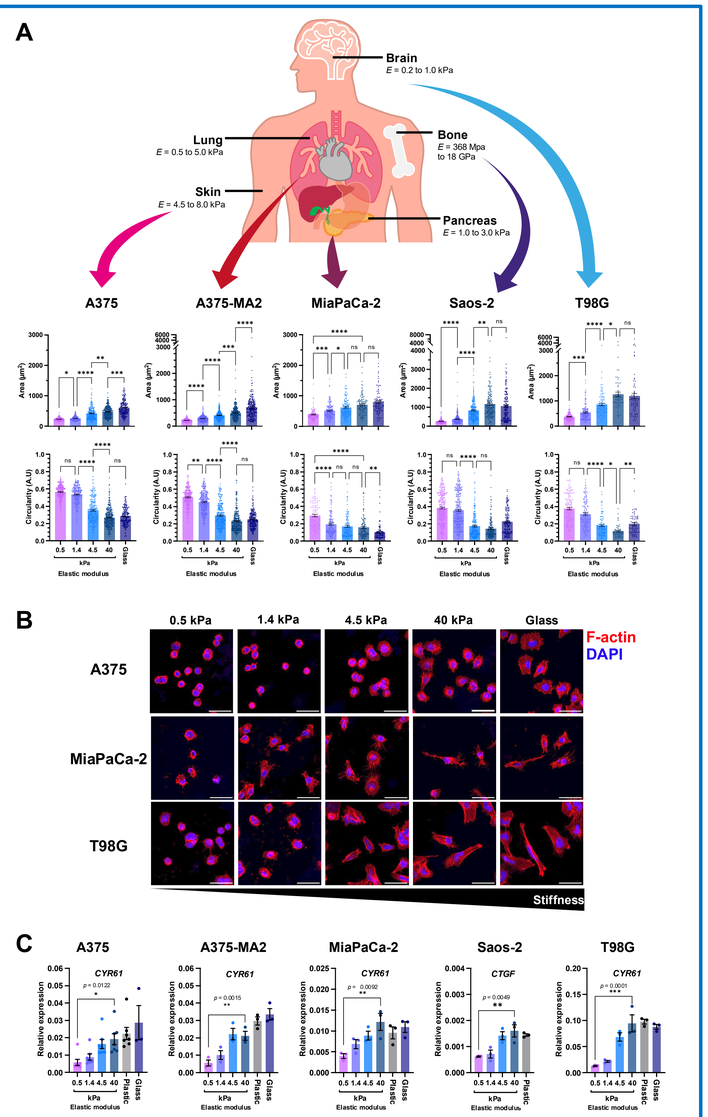


Figure 1. Solid cancer cell lines exhibit stiffness-dependent changes in morphology and YAP/TAZ activity.

- Solid cancer cell lines of varied tissue origin responded to substrate stiffness of polyacrylamide hydrogels in a similar manner; with **reduced cell area and increased cell circularity on softer substrates**, as opposed to increased cell area and reduced cell circularity on stiffer substrates (**Fig. 1A-B**).
- Expression of key YAP/TAZ target genes, *CYR61* or *CTGF*, increased in cells on stiffer substrates, indicating **upregulated YAP/TAZ activity** (**Fig. 1C**).

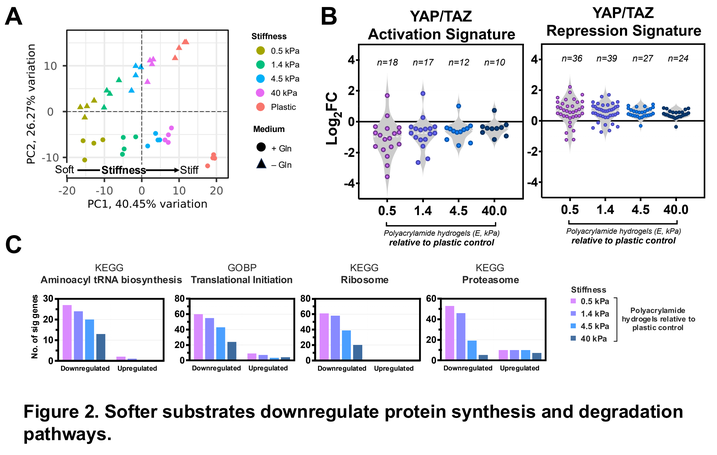


Figure 2. Softer substrates downregulate protein synthesis and degradation pathways.

- **Whole transcriptome analysis** of A375 cells on substrates of varied stiffness by principal component analysis revealed a progressive stiffness-dependent variation in gene expression profile both in the presence or absence of glutamine (**Fig. 2A**).
- Expression of a broader range of YAP/TAZ targets by RNA-seq, using 'Activation' or 'Repression' gene signatures, revealed progressively downregulated YAP/TAZ activity with reducing stiffness (**Fig. 2B**).
- Gene set enrichment analysis revealed that stiffness, alone, regulates a variety of protein turnover-related gene sets (**Fig. 2C**).

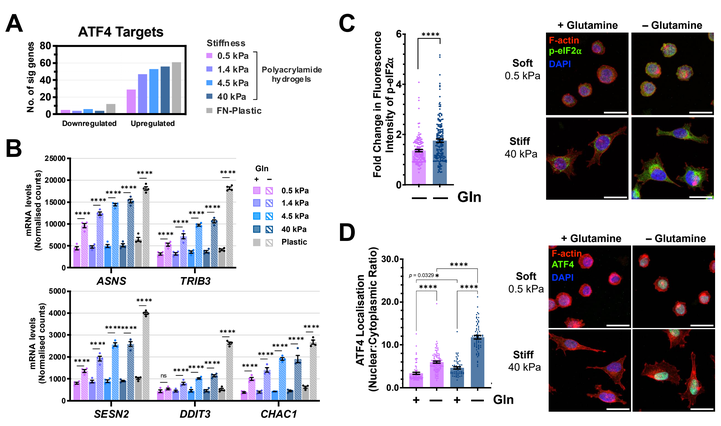


Figure 3. Extent of ISR activation induced by glutamine starvation is stiffness-dependent.

- Glutamine-starved A375 cells exhibited a stiffness-dependent difference in the expression of ATF4 targets (**Fig. 3A**), including expression of key ISR-activated genes (**Fig. 3B**).
- We examined whether the sensing or execution of the ISR was altered in A375 cells on soft (0.5 kPa) or stiff (40 kPa) polyacrylamide hydrogel substrates. This revealed increased phosphorylation of eIF2α in cells on stiff versus soft substrates (**Fig. 3C**) and a higher nuclear/cytoplasmic (N/C) ratio of ATF4 in cells on stiffer substrates (**Fig. 3D**).

Conclusion

- Cancer cells of varied tissue origin respond to substrate stiffness in a similar manner, with similar morphological and transcriptional outcomes.

- Cancer cells progressively downregulate protein turnover mechanisms as substrate stiffness decreases, including those involved in translation.

- Stress sensing was increased in cells on stiffer substrates than those on softer substrates at the transcriptional level.

- Cells on softer substrates exhibited weaker ISR induction when induced by glutamine starvation.

- ISR sensing (p-eIF2α) and execution (ATF4 nuclear localisation) were increased in cells on stiffer substrates.

- Cellular stress responses highly depend on the stiffness of the microenvironment with potential implications for cancer therapy.

- Our study highlights that caution should be implemented when interpreting cellular stress response studies in cells on tissue culture plastic due to the supraphysiological stiffness of tissue culture plastic.

Acknowledgements

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