ENGINEERING DORMANCY:
Insights from a 3D Model of Microscopic Colorectal Cancer Liver Metastasis

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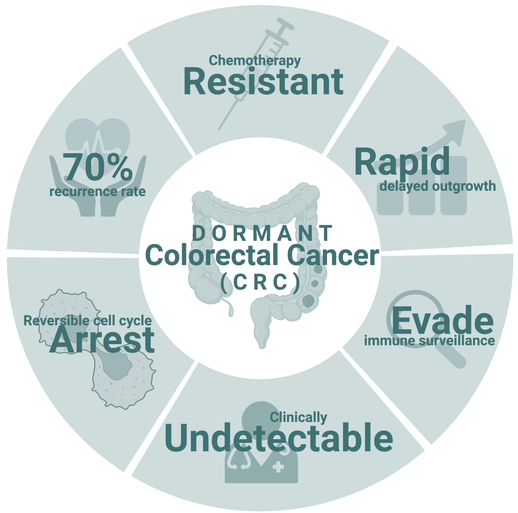
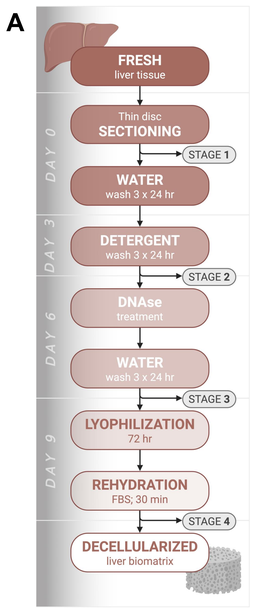
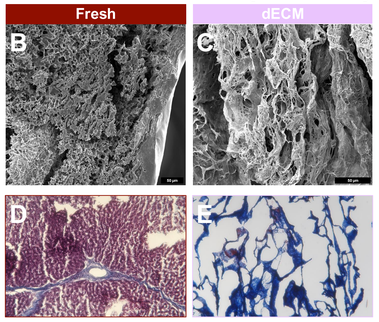
BACKGROUND AND MOVITATION



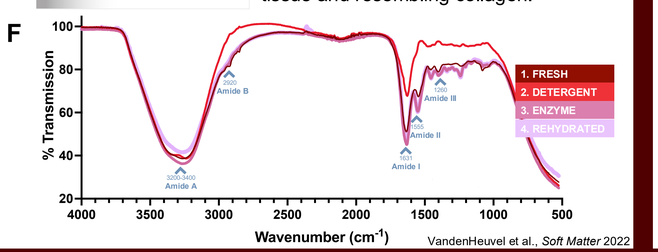
Objective: Create *in vitro* model of dormant microscopic CRC liver metastases (μCRLM) to facilitate mechanistic, diagnostic and therapeutic discovery

DECELLULARIZED EXTRACELLULAR MATRIX (dECM)



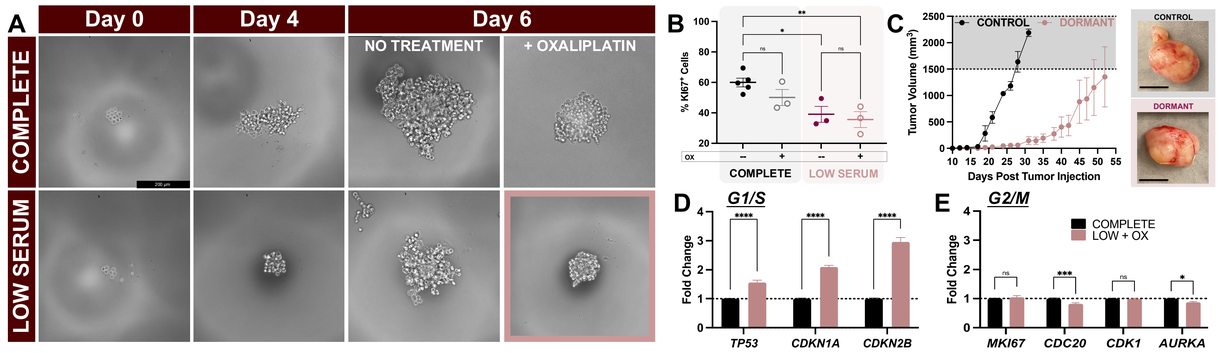


A) Tissue processing to generate dECM liver scaffolds. **B)** SEM images show efficient decellularization of fresh tissue to generate **C)** the dECM scaffold. **D)** Trichrome staining similarly demonstrates successful removal of cells (red) to produce **E)** collagen (blue) scaffolds with intact porous architecture. **F)** FTIR spectra show biochemical composition upheld through decellularization, with dECM scaffolds matching fresh liver tissue and resembling collagen.



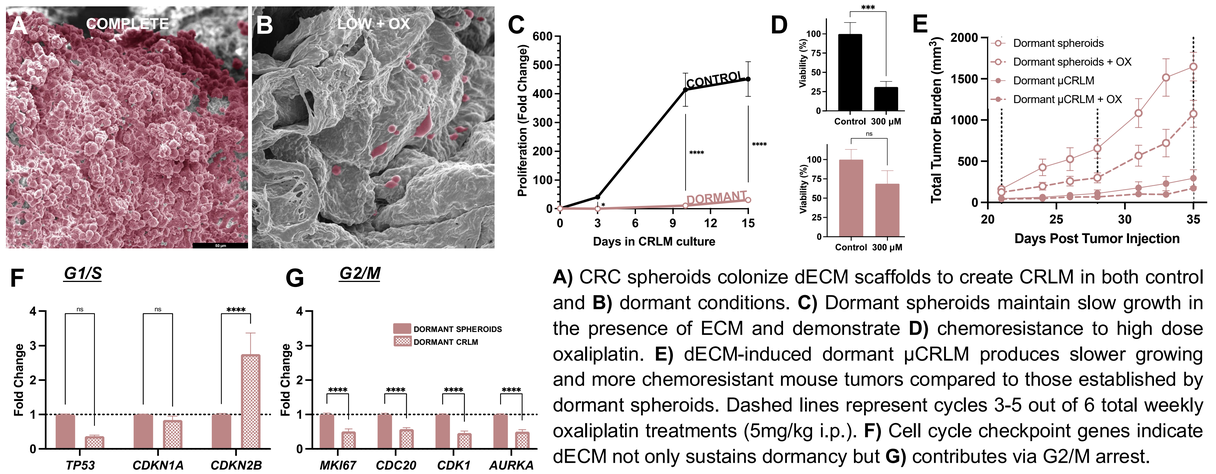
VandenHeuvel et al., *Soft Matter* 2022

NUTRIENT DEPLETION AND LOW DOSE OXALIPLATIN INDUCE DORMANCY VIA G1/S ARREST



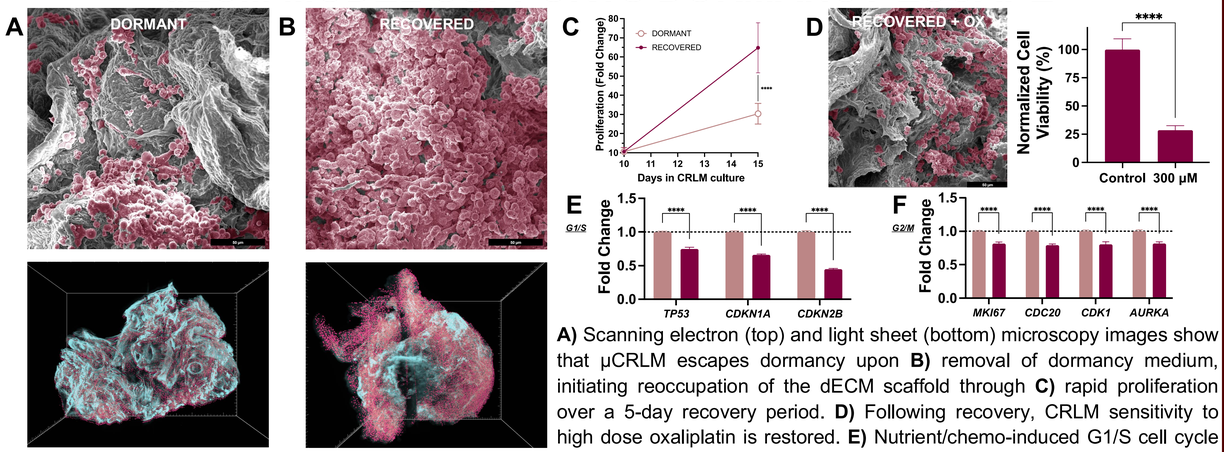
A) 3D HCT116 CRC tumor spheroids under suppressive growth conditions of serum starvation and low dose oxaliplatin chemotherapy. **B)** Reduction in Ki67 protein expression validates the assumption that reduced spheroid size is due to reduced proliferation rather than cell death, indicating a dormant phenotype. **C)** This slowed proliferation results in functional dormancy of spheroids which grow more slowly than controls as *in vivo* mouse flank tumors. **D)** Gene expression analysis indicates nutrient depletion and low dose oxaliplatin chemotherapy combine to induce CRC cell cycle arrest in the G1/S phase rather than **E)** the G2/M phase.

dECM LIVER INDUCES DORMANCY VIA G2/M BLOCKADE



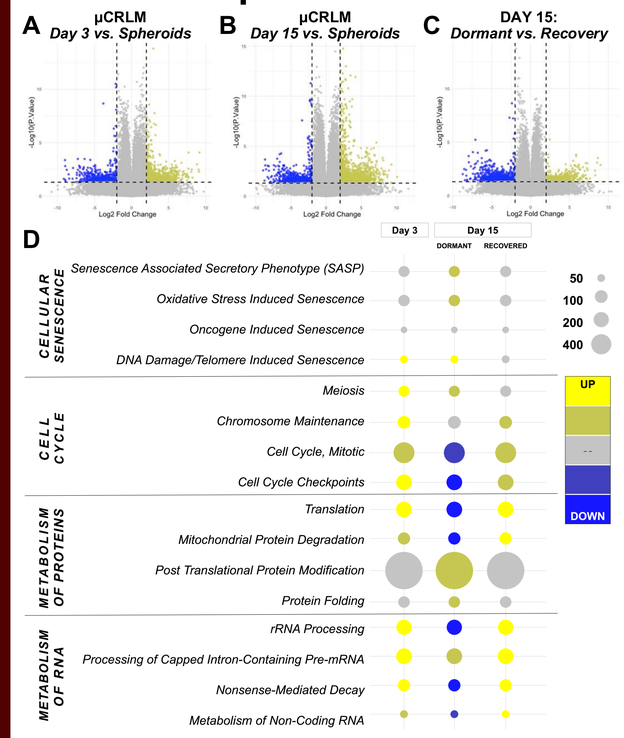
A) CRC spheroids colonize dECM scaffolds to create CRLM in both control and **B)** dormant conditions. **C)** Dormant spheroids maintain slow growth in the presence of ECM and demonstrate **D)** chemoresistance to high dose oxaliplatin. **E)** dECM-induced dormant μCRLM produces slower growing and more chemoresistant mouse tumors compared to those established by dormant spheroids. Dashed lines represent cycles 3-5 out of 6 total weekly oxaliplatin treatments (5mg/kg i.p.). **F)** Cell cycle checkpoint genes indicate dECM not only sustains dormancy but **G)** contributes via G2/M arrest.

dECM MODEL SUPPORTS DORMANCY ESCAPE



A) Scanning electron (top) and light sheet (bottom) microscopy images show that μCRLM escapes dormancy upon **B)** removal of dormancy medium, initiating reoccupation of the dECM scaffold through **C)** rapid proliferation over a 5-day recovery period. **D)** Following recovery, CRLM sensitivity to high dose oxaliplatin is restored. **E)** Nutrient/chemo-induced G1/S cell cycle arrest is reversed with recovery, but **F)** ECM-induced G2/M arrest persists.

RNA SEQUENCING REVEALS DORMANT μCRLM SIGNATURE



A) Significantly differentially expressed genes between dormant spheroids and μCRLM at day 3 and **B)** day 15, as well as **C)** recovered vs. dormant day 15. **D)** Pathway enrichment analysis (Reactome) reveals an initial active stress response during scaffold colonization (day 3), which transitions into a secretory deep senescence state (day 15). Recovery reinstates cell cycle and metabolic processes to support restored proliferation and recurrence.

CONCLUSIONS

- Nutrient depletion, in combination with oxaliplatin, produces a phenotype indicative of dormancy via G1/S cell cycle arrest in microscopic CRC spheroids
- Porcine liver decellularization produces a collagen scaffold with intact architecture and biochemical composition, generating a biomimetic nest for μCRLM
- dECM scaffolds contribute to dormancy, driving G2/M arrest and supporting slow-growing, chemotherapy resistant CRC dormancy in which cells enter a deep secretory senescence state
- dECM micrometastasis model supports CRC dormancy escape to mimic disease recurrence, enabling mechanistic discovery, biomarker identification and therapeutic screening

ACKNOWLEDGEMENTS

This work is supported by NIH NCI grant R37CA269224 and has been conducted in collaboration between Texas A&M University and MD Anderson Cancer Center