Extracellular Matrix in Hydrogel-based Organoids Regulate Epigenetics and Signaling in Prostate Cancers

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Statement of Purpose: Following treatment with androgen receptor (AR) pathway inhibitors, ≈20% of prostate cancer patients progress by shedding their AR dependence. These tumors undergo epigenetic reprogramming turning castration-resistant prostate cancer adenocarcinoma (CRPC-Adeno) into neuroendocrine prostate cancer (CRPC-NEPC). No targeted therapies are available for CRPC-NEPCs, and there are minimal organoid models to study these aggressive tumors. Here, using a combination of patient tumor proteomics, RNA sequencing, spatial-omics, and a synthetic hydrogel-based organoid, putative extracellular matrix (ECM) cues that regulate the phenotypic, transcriptomic, and epigenetic underpinnings of CRPC-NEPCs are defined. The synthetic organoids suggest the regulatory role of ECM in modulating signaling and epigenetics of prostate cancer cells, regulating, therapeutic response to targeted therapies in CRPC-NEPCs, and they enable the discovery of therapies to overcome resistance (see [1]Mosquera et al, Advanced Materials, 2021).

Methods: Maleimide-functionalized polyethylene glycol (PEG-4MAL) were functionalized with ECM-mimicking short peptides[1]. Cells were grown for 1 week and RNA seq, DNA methylation, imaging, drug treatment, and flow cytometry were performed[1]. Mice bearing tumors obtained at week 5 were treated with ONC201 administered at 125 mg/kg once a week by oral gavage for a total of 5 weeks. All methods are described in detail in [1]Mosquera et al, Advanced Materials, 2021.

Results: We performed transcriptomic analysis on 111 patients and a combination of specialized techniques such as proteomics, spatial omics, and microscopy analysis on patient biopsies to define ECM and related microenvironment in CRPC-Adeno and CRPC-NEPC **(Fig. 1A-C)**. Informed by these findings, we developed a synthetic polymer-based, hydrogel platform of PEG-4MAL to grow CRPC-Adeno and CRPC-NEPC patient tumor cells under the defined ECM microenvironment, which resulted in distinct morphology of cancer organoids (**Fig 1D**). RNA sequencing further revealed distinct gene mobilization in CRPC-NEPC OWCM 155 and CRPC-Adeno OCMW-1358 cells grown in various ECM conditions (**Fig 1E**). ECM in PEG-4MAL organoids modulated the expression of the epigenetic regulator EZH2, histone 3 trimethylation (**Fig 1F**), and response to EZH2 inhibitor[1]. The CRPC-NEPC patient tumor cells grown in PEG-4MAL hydrogels functionalized with fibronectin-like REDV peptide and RGD peptide ECMs shared higher similarities of promoter methylation compared to Collagen-1-mimicking GFOGER ECM and the patient sample (**Fig. 1G**). In comparison to untreated organoids, when treated with EZH2i, we observed a loss of actin cytoskeleton structure in tumors grown in Matrigel[1]. In contrast, PEG-4MAL hydrogel-based organoids did not show the same actin loss. Although RGD conditions did not reduce cell growth under EZH2i conditions, they seem to disrupt the actin symmetry. We next determined whether the integrins were regulating EZH2 activity, that is, trimethylation of histone 3, through actin cytoskeleton. We added Rho-associated protein kinase (ROCK) inhibitor Y-27632 to the REDV organoid culture and observed that ROCK inhibition reduced the percentage of H3k27Me3+ cells and expression level of H3k27Me3. Our findings provide evidence that ECM may regulate the activity of epigenetic markers, such as EZH2. The ECM type distinctly regulated the response to small molecule inhibitors of epigenetic targets and Dopamine Receptor D2 (DRD2), the latter being an understudied target in neuroendocrine tumors. In vivo patient-derived xenograft in immunocompromised mice showed an anti-tumor response when treated with a DRD2 inhibitor (**Fig 1H**). Finally, we demonstrate that therapeutic response in CRPC-NEPCs under drug-resistant ECM conditions can be overcome by first cellular reprogramming with epigenetic inhibitors, followed by DRD2 treatment[1].

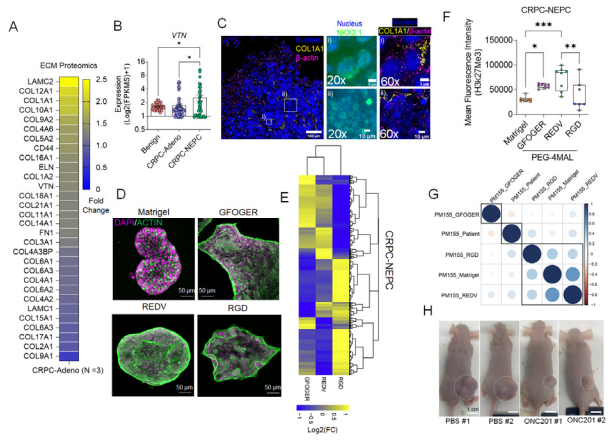

Fig. 1 Extracellular Matrix in Synthetic Hydrogel-Based Prostate Cancer Organoids Regulate Therapeutic Response to EZH2 and DRD2 Inhibitors. A) Mass spectrometry analysis of ECM components (left) and integrin signaling components (right) in primary CRPC-Adeno tumors relative to adjacent normal tissue (average of n=3). **B)** RNA-seq transcriptomic analysis of ECM components and integrins in CRPC-Adeno and CRPC-NEPC patient tumor biopsies, as compared to benign tissues (n=74 CRPC-Adeno, n=37 CRPC-NEPC, n=34 benign samples). Data presented as Mean ± SEM. **C)** Multiplexed single-cell spatial omics RNA analysis of prostate tumors. **D)** Representative confocal imaging of organoid morphology across ECM conditions (DAPI: purple, Actin: green). **E)** Heatmap of differentially expressed genes in CRPC-NEPC OWCM-155 and CRPC-Adeno OWCM-1358 cultured across GFOGER, RGD, and REDV-functionalized PEG-4MAL hydrogel and normalized to Matrigel. **F)** Flow cytometry analysis of H3k27Me3 expression across organoid conditions (n=5 per condition). **G)** Pearson correlation of methylation profiles between patient samples and organoids. **H)** DRD2 inhibitor ONC201 reduces tumor volume in the PDX-engrafted nude mouse model of CRPC-NEPC.

Conclusion: Here, informed by an extensive tumor microenvironment analysis, synthetic hydrogel-based prostate cancer organoids are developed to grow patient tumor cells under the defined microenvironment conditions, leading to the discovery of novel therapeutics.

References: [1]Mosquera et al, Advanced Materials, 2021.