Mapping tumor cell drug response as a function of matrix context using combinatorial cell microarrays

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Introduction: Carcinoma progression and metastasis are directed by interactions between epithelial tumor cells and components of their microenvironment. In particular, cell–extracellular matrix (ECM) interactions are known to influence tumor growth, metastatic potential, and sensitivity or resistance to therapy. However, the complexity of ECM composition within the *in vivo* tumor microenvironment has limited understanding of the underlying mechanisms. Here, we have utilized a high-throughput cell microarray-based approach to investigate the impact of defined combinations of ECM proteins on tumor cell function and drug response.

Materials and Methods: Our overall approach is summarized in Fig. 1A. Lung adenocarcinoma (A549, HCC1833) cells were seeded onto a polyacrylamide hydrogel substrate spotted with ECM proteins and subsequently treated with drugs for 48 h, after which we evaluated cell adhesion, apoptosis, and proliferation using immunofluorescence. Using this approach, we quantitatively evaluated the effects of 55 different ECM environments comprising all 2-factor combinations of 10 ECM proteins in response to a panel of drugs including an alkylating agent (cisplatin) and six receptor tyrosine kinase inhibitors (cabozantinib, gefitinib, nilotinib, vandetanib, and sunitinib). We further examined whether expression of the neuroendocrine transcription factor *ASCL1*, which has been previously associated with tumor progression and poor patient outcome, altered cell response to ECM context.

Results and Discussion: We observed differential cell adhesion to ECM in the array format (Fig. 1B). Tumor cell death was dependent on ECM context, effects that were further specific to each drug treatment and expression of *ASCL1* in tumor cells (Fig. 1C). For instance, we observed that *ASCL1*$^{+/+}$ cells on type I collagen (C1) alone were resistant to sunitinib treatment. The addition of tenascin C (TC) to the ECM was sufficient to induce caspase-3-mediated apoptosis (Fig. 1D). Overall, 11.7% of ECM combinations for WT and 15.6% for *ASCL1*$^{+/+}$ cells induced sensitivity to treatment with our panel of drugs (Fig. 1E). We additionally observed changes in cell proliferation independent of the modulatory effects of ECM on tumor cell response to drug (Fig. 1F).

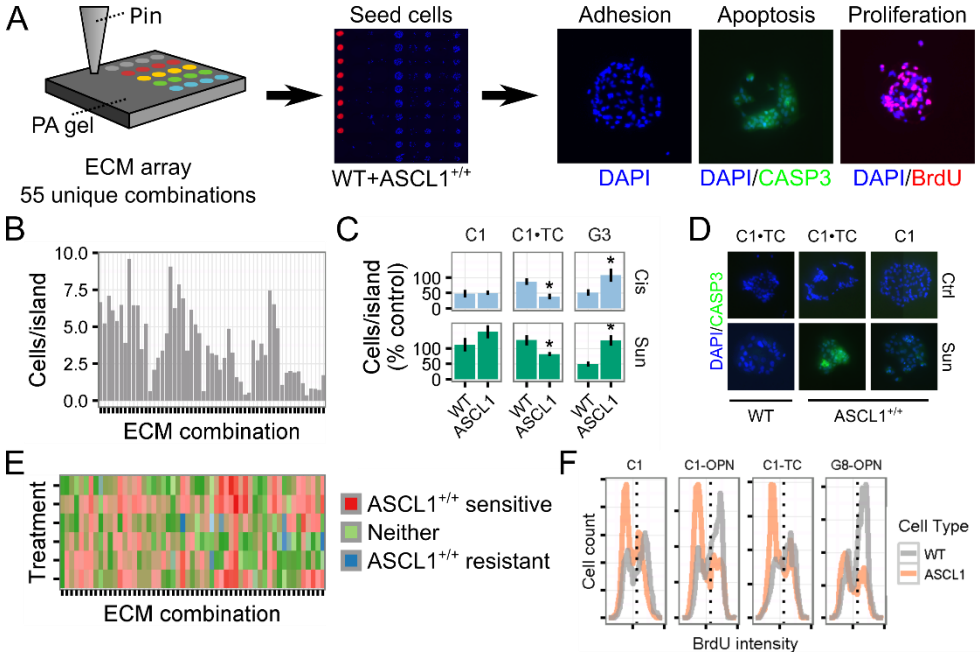

Figure 1: (A) Schematic of overall approach. (B) Cells/island for 55 combinations, highlighting dependence of adhesion on ECM composition. (C) Selected combinations demonstrating ECM-, drug-, and *ASCL1*-specific effects on cell survival. (D) Induction of caspase-3-mediated apoptosis in sunitinib-treated *ASCL1*$^{+/+}$ but not WT cells on C1•TC. (E) Heat map showing sensitivity and resistance for all ECM combinations as a function of drug treatment. (F) Selected combinations demonstrating ECM- and *ASCL1*-specific effects on proliferation. Dotted line is cutoff for BrdU-positive cells

Conclusions: We have demonstrated an engineered array platform for mapping the combinatorial impact of ECM context on tumor cell function and drug response. Our results suggest that expression of specific ECM in lung adenocarcinomas may impact therapeutic efficacy and tumor growth. Continuing work utilizing this approach aims to: (1) further define the molecular mechanisms by which interactions with ECM drive lung carcinogenesis and tumor cell drug resistance; and (2) integrate our findings with clinical observations and patient outcomes.