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Quantitative analysis of epithelial cystogenesis revealed serum factors and mechanical restrictions as triggers for lumen initiation.

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INTRODUCTION: Epithelial organs (e.g. lung, kidney) contain a monolayer of polarized cells facing a continuous lumen. The mechanism(s) regulating formation and maintenance of the lumen are currently unclear. Madine-Darby canine kidney (MDCK) cells embedded in collagen-based matrix form self-enclosed monolayers of polarized cells with a central lumen (cysts). Such three-dimensional (3D) cultures are widely used as a model system to study epithelial morphogenesis [1,2].

METHODS: Two stably transfected MDCK strain II cells clones expressing fluorescent protein tags (YMD and GMD cells in the following) were used to prepare 3D cultures as described elsewhere [2]. We characterized individual MDCK cysts' development in 4D by reconstructing the 3D geometry of each cell aggregate from the deconvolved XY-stacks collected at day 1, 4, 7, 10, and 13. From the reconstructed 3D object we obtained a measure of the total aggregate volume and surface. To correlate the mechanical response of collagen gel with lumen formation, we measured the viscoelastic properties of collagen gels using the rheometer Physica MCR 300 (Anton Paar GmbH. Graz, Austria) as described elsewhere [3].

RESULTS: We observed four different stages during cystogenesis (Figure 1): single cell (SC), cell aggregate (CA), cell aggregate with lumen (where more than one layer of cells encloses a single lumen or multiple lumens; CAL), and cyst (a lumen enclosing monolayer). For both cell clones a wide time-range for lumen initiation and cyst formation was observed. Interestingly, all CA to CAL transitions were achieved in a narrow range of total aggregate volume suggesting that either the aggregate volume or a specific cell number in the aggregate trigger lumen initiation. To test the effect of growth factors and mechanical properties of collagen on lumen initiation, we analysed cystogenesis in 3D culture with increased FCS concentration or more rigid collagen preparation.

An increase in matrix rigidity correlates with a slight delay in lumen initiation while a higher percentage of FCS in the medium strongly enhances lumen initiation.

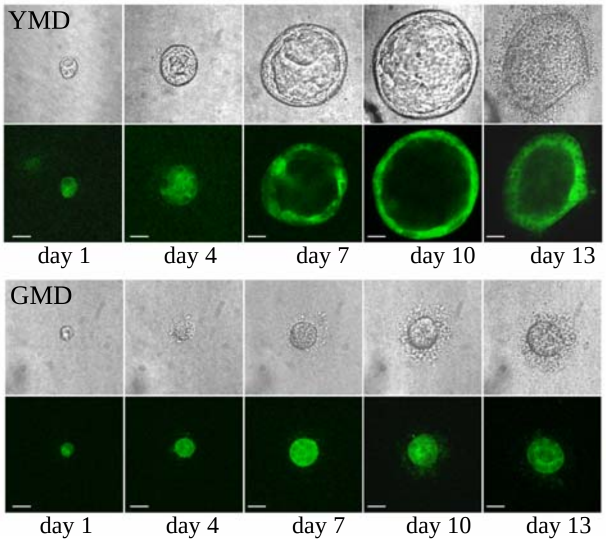

Fig. 1: Middle section of DIC (upper row) and fluorescence (lower row) confocal XY-stacks collected at day 1, 4, 7, 10, and 13 of cystogenesis in representative YMD and GMD evolutions. Bars: 10 µm (YMD at day 1, 4, 7 and GMD), and 20 µm (YMD at day 10, and 13).

DISCUSSION & CONCLUSIONS: Using quantitative analysis of time-lapse confocal microscopy images we derived information about physical conditions under which lumens are initiated. Our data suggest that small cell aggregates initiate lumen in a narrow volume and cell number range that depends on local properties of cell-ECM interactions, nutrients and growth factors [4].

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