

Supplementary Information for

A human organoid system that self-organizes to recapitulate growth and differentiation of a benign mammary tumor

Stefan Florian, Yoshiko Iwamoto, Margaret Coughlin, Ralph Weissleder,
Timothy J. Mitchison

Corresponding authors: Stefan Florian, Timothy J. Mitchison
Email: stefan.florian@charite.de, timothy_mitchison@hms.harvard.edu

This PDF file includes:

Figs. S1 to S2
Captions for movie S1

Other supplementary materials for this manuscript include the following:

Movie S1

Supplementary Figures

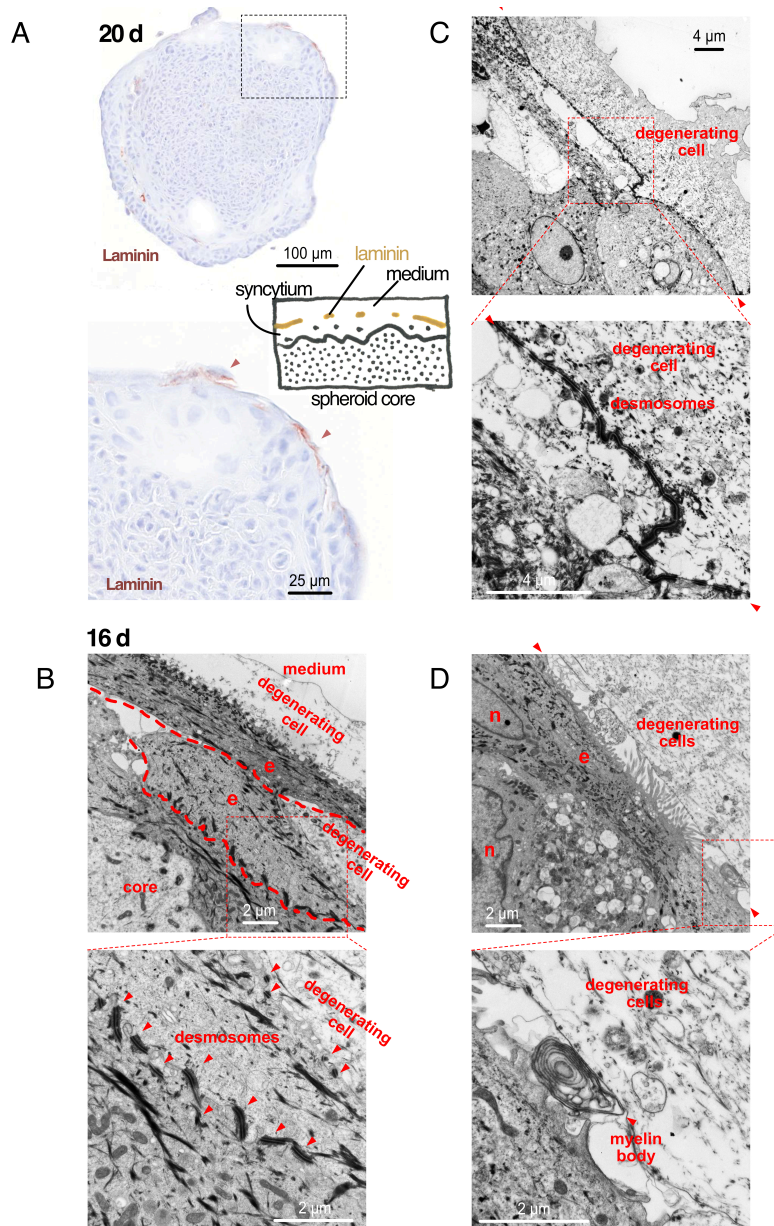


Fig. S1. Epithelial polarization starts with formation of peripheral layers of elongated cells

(A) A 20 day old spheroid formed through aggregation of 10000 cells from the immortalized HMT-3522 S1 normal mammary cell line. Antibody staining for laminin, counterstaining with hematoxylin. The rectangle represents the region shown at higher magnification in the lower panel. Arrowheads point to border segments that stain positive for laminin. Image from Fig. 6, shown here for context. The following images show the peripheral zone marked in Figure 1 B, analyzed by electron microscopy.

(B-D) EM images of 16 day old HMT-3522 S1 spheroids grown as in (A). (B) Red dashed lines follow cell borders. (C) Arrowheads mark desmosome-lined border between two syncytial layers. (D) Arrowheads mark border between a detaching degenerating cell layer and the adjacent viable layer. So called myelin bodies as a typical sign of cell degeneration with loss of membrane integrity.

n: nucleus, e: elongated cell

embryonic development mouse/human

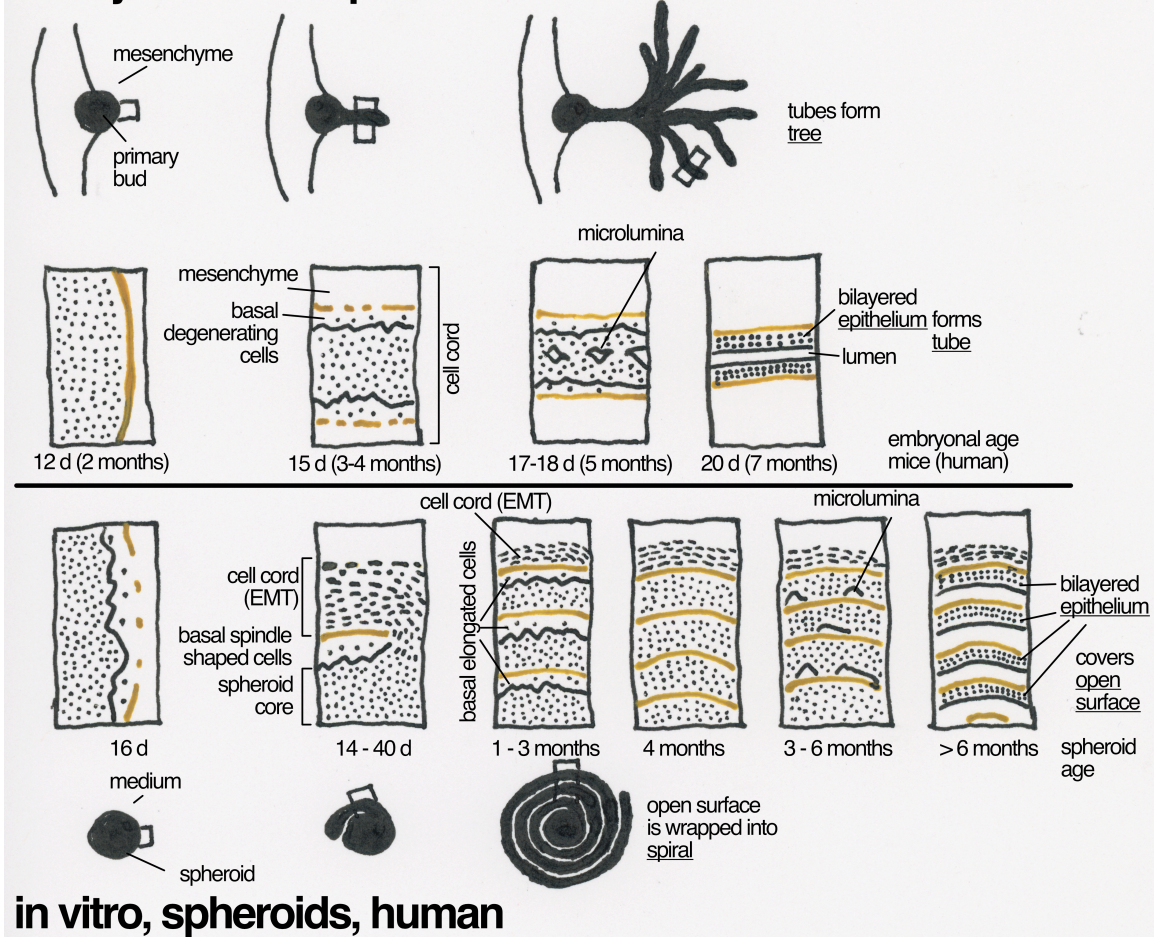


Fig. S2. Epithelial polarization starts with formation of peripheral layers of elongated cells

Overview of mouse embryonic development as described (1). Embryonic age in the mouse from (1), in humans (2). Note that basal cell decay has only been described in mice. Lower panel: Development of HMT-3522 S1 spheroids. Some of the events, especially disappearance of basal elongated cells and emergence of microlumina, can overlap and are separated here and in the examples chosen for Fig. 4 and 5, for clarity. See Fig. 6A for a summary of the whole dataset.

Movie S1.

A 4 day old spheroid imaged for 55 days using a transmitted light microscope at 37°C with 5% CO₂. Note the initial growth of a cell cord which then grows on the spheroid surface to form a cell layer concentric with the core spheroid. The movie was acquired on an Incucyte live cell imaging system (Essen BioScience) in the transparent U-bottom shaped spheroid culture plate that was also used to culture spheroids.

SI References

1. Hogg NA, Harrison CJ, Tickle C (1983) Lumen formation in the developing mouse mammary gland. *J Embryol Exp Morphol* 73:39–57.
2. Standring S (2008) in *Gray's anatomy : the anatomical basis of clinical practice*, eds Standring S, Gray H (Churchill Livingstone/Elsevier, Edinburgh), pp 915–938.