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Linking stem cell function and growth pattern of intestinal organoids

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ABSTRACT

Intestinal stem cells (ISCs) require well-defined signals from their environment in order to carry out their specific functions. Most of these signals are provided by neighboring cells that form a stem cell niche, whose shape and cellular composition self-organize. Major features of this self-organization can be studied in ISC-derived organoid culture. In this system, manipulation of essential pathways of stem cell maintenance and differentiation results in well-described growth phenotypes.

We here provide an individual cell-based model of intestinal organoids that enables a mechanistic explanation of the observed growth phenotypes. In simulation studies of the 3D structure of expanding organoids, we investigate interdependences between Wnt- and Notch-signaling which control the shape of the stem cell niche and, thus, the growth pattern of the organoids. Similar to *in vitro* experiments, changes of pathway activities alter the cellular composition of the organoids and, thereby, affect their shape. Exogenous Wnt enforces transitions from branched into a cyst-like growth pattern; known to occur spontaneously during long term organoid expansion. Based on our simulation results, we predict that the cyst-like pattern is associated with biomechanical changes of the cells which assign them a growth advantage. The results suggest ongoing stem cell adaptation to *in vitro* conditions during long term expansion by stabilizing Wnt-activity.

Our study exemplifies the potential of individual cell-based modeling in unraveling links between molecular stem cell regulation and 3D growth of tissues. This kind of modeling combines experimental results in the fields of stem cell biology and cell biomechanics constituting a prerequisite for a better understanding of tissue regeneration as well as developmental processes.

1. Introduction

Self-renewal of the intestinal tissue is substantially impaired in several developmental diseases (Markel et al., 2008) and its efficiency decreases during ageing (Martin et al., 1998). Moreover, the intestinal tissue is not capable of macroscopic re-growing after resection leading to short bowel syndrome (Donohoe and Reynolds, 2010). Tissue engineering applying *in vitro* expanded intestinal stem cells (ISCs) has been envisioned as a possible therapeutic approach.

In order to enable such approaches long-term *in vitro* culture of ISCs has been tried for decades. Sato et al. (2009) successfully established an intestinal organoid culture that enables long term tissue expansion. Mouse organoids require specific culture supplements (EGF, Noggin, R-spondin: ENR) for expansion. Under standard ENR conditions organoids grow in a branched pattern, resampling crypt- and villi morphology of the intestine. Moreover, they contain all types of cells present in the mouse intestine, including ISCs, Paneth cells (PCs), goblet cells (GCs) and enterocytes (ECs). Organoids obtained from mouse intestinal tumors grow in a cystic pattern without Noggin and R-spondin and show a quantitatively different cellular composition (Sato et al., 2009).

The establishment of intestinal organoid culture together with the development of sophisticated mouse reporter systems provided new insights into ISCs organization in the last years (Merker et al., 2016). For instance, Farin et al. (2012) demonstrated a link between the molecular regulation of Wnt, the cellular composition of the organoids and their growth pattern. In particular, they showed that increased Wnt-activity changes the growth pattern of the organoids from branched to cyst-like, while in parallel the number of PCs per organoid bud increases. Moreover,

Abbreviations: ISC, intestinal stem cell; PC, Paneth cell; GC, goblet cell; EC, enterocyte; ENR, EGF- Noggin- R-spondin; NET, model polymer network; PSET, parameter set

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they found that PC-derived Wnt-3 is required for intestinal organoid culture, and that deletion of Wnt-3 can be rescued by adding exogenous Wnt-3a. Notably, Wnt-activity in ISCs can be bistable. This bistability originates from a positive feedback loop of the TCF/β-catenin complex (Schuijers et al., 2015). Switches between the states are associated with lineage specification, where high Wnt-activity is specific for ISCs and PCs and low Wnt-activity for ECs and GCs. The absolute level of Wnt-activation can be modulated e.g. by changing R-spondin 1 (in the following R-spondin) concentrations in the culture medium. R-spondin suppresses Rnf43 and Znrf3, which are repressors of Frizzled (Wnt-) receptors (Farin et al., 2016). Experiments by Yin et al. (2014) manipulating the activity of the Wnt- and the Notch- pathway demonstrated that the cellular composition of organoids depends specifically on culture supplements and that nearly pure ISC organoids can be grown.

Despite the experimental progress, a comprehensive mechanistic model explaining ISC self-organization in organoids is currently missing. There are only a few computational approaches to describe organoid growth (Pin et al., 2015; Langlands et al., 2016). In 2012, we have introduced a first individual cell-based model approach (Buske et al., 2012) which builds on our intestinal crypt model (Buske et al., 2011). This computational model allowed for simulations of the self-organization of the ISC niche within an organoid. Here, we provide an extension of this model that enables a mechanistic explanation of how alterations of the Wnt- and Notch- pathways impact on the organization of the ISC-niche and organoid growth pattern. For this purpose, we transferred regulatory principles of Wnt- and Notch- signaling, that we have successfully applied to describe intestinal crypts (Thalheim et al., 2016), to our organoid model. Thereby, we link for the first time evolving 3D morphology of intestinal organoids to molecular regulation of these pathways in individual cells.

2. Methods

2.1. The model of lineage specification

Our model is based on a set of simple principles of self-organization of the ISC-niche under homeostasis which are described in detail in (Buske et al., 2011, 2012). A sketch of these principals is shown in Fig. 1A. We assume that ISCs require Wnt- and Notch-activity for self-renewal in agreement with experimental findings (van de Wetering et al., 2002; Fre et al., 2005). If Wnt-activity decreases, modeled ISCs specify into absorptive ECs, while decreasing Notch-activity forces them to specify into secretory PCs. Notch-ligands are provided by both secretory lineages, PCs (Sato et al., 2011) and GCs (Stamataki et al., 2011), while Wnt is provided by PCs only (Sato et al., 2011; van Es et al., 2012). Thus, in the absence of exogenous Wnt, ISC-maintenance requires PCs in the direct neighborhood. Therefore, two essential parameters of the model are the minimal numbers of Wnt-secreting neighbors N_W and of Notch-ligand-presenting neighbors N_N that are required for ISC maintenance. Outside the niche, ECs require $N_{N,EC}$ Notch-ligand-presenting neighbors in order to prevent specification into GCs.

Allowing ISCs to specify into PCs independent of their environment would result in a permanently expanding ISC-PC system, i.e. an expanding ISC-niche, capable of self-maintaining. Because such expansion is not seen *in vitro* under ENR conditions, we restricted ISC-specification into PCs to those ISCs that are located in highly convex areas of the tissue (Buske et al., 2012), where the local mean curvature C is larger than $1/R_0$ (Fig. 1B). Here, R_0 is the radius of a spherical organoid in which ISCs can no longer specify into PCs. Enrichment of ISCs and PCs in highly convex areas is indeed observed for branched organoid growth (Sato et al., 2009). Moreover, the assumed principle provides an explanation, why prolonged culture of flat intestinal tissue was not successful (Booth and Potten, 2000). Key extensions of the lineage specification model compared to our original approach are explained in the Results section.

2.2. Biomechanical properties of the organoids

We described the biomechanical properties of the organoids by the properties of a closed but permanently reorganizing layer of semi-flexible polymers (Buske et al., 2012). This model allowed for simple control of the expansion and shape of the layer by adjusting two parameters: the elastic modulus of the polymers K_L and the bending

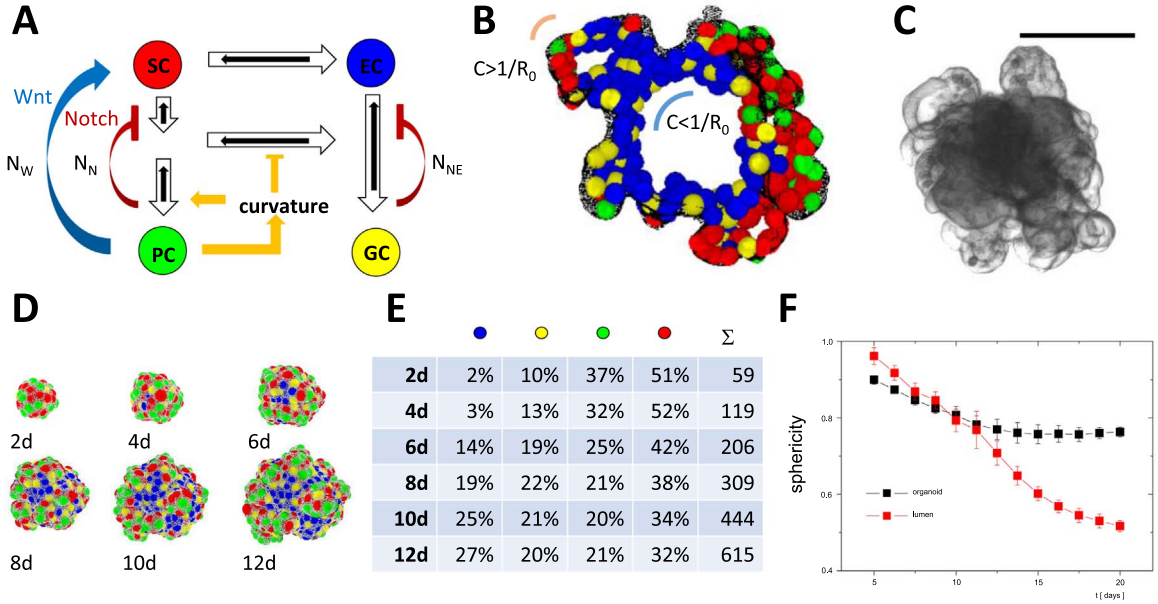

Fig. 1. The branched growth pattern. A) Regulatory circuit assumed in the model. Lineage specification (black arrows) is controlled by external signals and thus is reversible. For self-maintenance, ISCs require at least N_W and N_N neighbors that secret Wnt-3 (blue arrow) and provide Notch-ligands (brown T bar), respectively. ECs require at least $N_{N,EC}$ neighbors that provide Notch-ligands (brown T bar) to suppress GC-specification. PC-NET interactions induce local curvature that is required for PC-specification (orange arrows). B) Slice of a simulated organoid (viable cells): PCs are specified in regions with high mean curvature ($C > 1/R_0$). C) Phase contrast image of an organoid grown under ENR conditions (scale bar: 200 μm). D) Snapshots of an *in silico* growing organoid (SC: red, PC: green, GC: yellow, EC: blue; N_N=3, PSET I). Numbers indicate days (d) in culture. E) Cell type composition of the organoid shown in (D) at selected time points. F) The sphericity of organoids (PSET I) and of their lumen decrease over time indicating the formation of branches. Organoid sphericity reaches a plateau after about 10 days as the cell composition stabilizes (n = 6 organoids, mean ± SD).