Intestinal Crypts Assume the Fetal Position in Response to Injury

Adam R. Pont[1] and Kelley S. Yan[1,*]
[1]Columbia Center for Human Development, Columbia Stem Cell Initiative, Department of Medicine, Division of Digestive and Liver Diseases, and Department of Genetics & Development, Columbia University Medical Center, New York, NY 10032, USA
*Correspondence: ky2004@cumc.columbia.edu
https://doi.org/10.1016/j.stem.2018.07.013

Distinct stem/progenitor cells generate intestinal epithelium during fetal and postnatal life. In a recent issue of *Nature*, Nusse and Savage et al. use helminth infection to show that Lgr5$^+$ intestinal stem cells are replaced by fetal-like progenitors following injury, suggesting that some fetal developmental pathways are repurposed during injury-induced tissue regeneration.

The intestinal epithelium of adult mice is a highly self-renewing tissue that functions as a protective barrier for interfacing with the external environment, a source of secreted products, and a surface for absorption. Establishment and maintenance of the intestinal epithelium requires two temporally distinct types of stem/progenitor cells that are found during fetal development versus postnatally, distinguished by their gene expression and differential mechanisms of regulation. During embryonic development, the mouse intestine begins as a tube lined by pseudostratified epithelium harboring stem/progenitor cells dedicated to cellular expansion in a Wnt-pathway-independent manner. In preparation for postnatal life, the intestinal epithelium develops into a monolayer with villi (harboring differentiated cells) connected by intervillus regions with stem/progenitor cells during the late prenatal period (~E14.5) (Chin et al., 2017). Crypts subsequently form from the intervillus regions in the postnatal period to complete the progression into a mature adult tissue pattern. In adult tissue, Lgr5$^+$ intestinal stem cells (ISCs) reside in the niche provided by the intestinal crypts of adult mice and support steady-state physiological regeneration. Lgr5$^+$ ISCs are regulated by Wnt signaling, specifically Wnt and R-spondin ligands, that function cooperatively to maintain the stem cell state and to control stem cell number. Around E15.5, stem cell proliferation becomes dependent on Wnt signaling (Chin et al., 2017), which continues throughout adult life, in contrast to that of the Wnt-independent early fetal stage.

There is emerging evidence that aspects of this developmental trajectory may be revisited in the context of intestinal injury, highlighted by an elegant new report by Nusse and Savage et al. that used a helminth infection model to investigate the epithelial response to tissue damage in adult mice. *H. polygyrus* is a co-evolved pathosymbiont of mice that is relatively globally non-toxic and easily visualized because it forms granulomatous lesions in the small intestinal wall. Upon *H. polygyrus* infection, crypts adjacent to granulomas were enlarged and hyperproliferative but had paradoxically lost expression of Lgr5$^+$ ISC markers. Punch biopsy isolation and RNA-seq of the granuloma-associated crypts (GACs) revealed 277 differentially expressed genes enriched in interferon-signaling targets, including *Ly6a*, which encodes the protein Sca-1 (Nusse et al., 2018). Interestingly, Sca-1 marks proliferative cells including hematopoietic stem cells in mice, but is notably absent in humans. Sca-1 was increased in GACs, and then decreased as the granulomas resolved. Interestingly, Sca-1 expression was also inversely correlated with *Lgr5* expression and was not seen in a different type of helminth infection that did not actually induce crypt injury. In addition, *H. polygyrus* infection of interferon-gamma null mice did not result in Sca-1 upregulation or crypt alteration, indicating interferon dependence.

The loss of Lgr5$^+$ ISCs with *H. polygyrus* infection within the context of intact hyperproliferative crypts raised the question of how exactly the epithelium was maintained. Sca1$^+$ and Sca1$^-$ crypt cells were isolated from infected mice and cultured in clonogenic organoid assays. Sca1$^+$ crypt cells grew into cystic spheroids without budding in organoid culture, with morphology and gene expression resembling that of previously reported fetal epithelium (Fordham et al., 2013; Mustata et al., 2013) in addition to Wnt/R-spondin-independent proliferation, in contrast to that of Lgr5$^+$ ISCs. Furthermore, the cells lacked differentiation markers and exhibited high expression of multiple fetal intestinal markers including *Gja1*, identified in previous gene expression profiling of fetal intestine (Mustata et al., 2013). RNA-seq showed enrichment of fetal intestinal markers (and de-enrichment of stem cell, enterocyte, and Paneth cell markers) in the GACs, indicating that the crypts had adopted a fetal-like state in response to tissue damage.

The investigators also examined whether loss of stem cell markers with adoption of a fetal-like gene expression pattern was a general crypt response to injury (Nusse et al., 2018). Treatment with a TCRβ antibody to elicit a robust immune response resulted in a crypt response similar to that of *H. polygyrus* infection, with Lgr5$^+$ ISC loss, Sca-1 induction, and crypt hyperproliferation. Similarly, lethal irradiation revealed the same phenotype. Moreover, diphtheria-toxin-mediated ablation specifically of Lgr5$^+$ ISCs (without inducing other epithelial damage) failed to induce interferon target expression or crypt hyperplasia, but it validated the fact that mere loss of Lgr5$^+$ ISCs is sufficient to rapidly induce Sca-1 expression (Figure 1).

Previous work has shown that irradiation and other types of injury disrupt Lgr5$^+$ ISCs operative during homeostasis. Genetic lineage tracing studies have

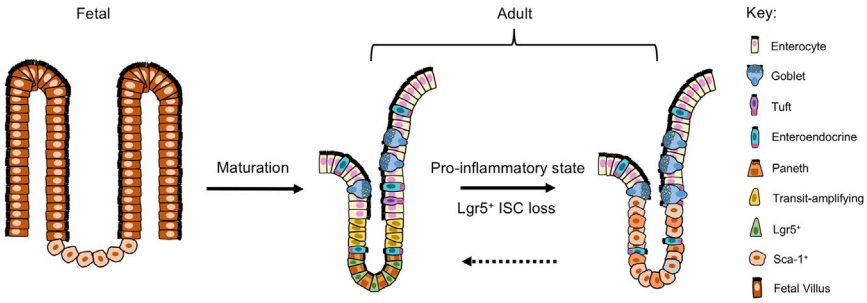

Figure 1. Model for Intestinal Epithelial Maintenance by Developmentally Distinct Pools of Stem/Progenitor Cells

Left: Fetal intestine (~E14.5) has undergone villus morphogenesis with proliferation restricted to intervillus regions expressing Sca-1$^+$ progenitor cells. Middle: Crypts form postnatally to establish adult tissue patterns to house Lgr5$^+$ ISCs and transit-amplifying cells in the Wnt-dependent ISC niche. Right: Injury to the stem cell niche, irradiation, or pro-inflammatory states result in loss of Lgr5$^+$ ISCs. The proliferative crypt is now sustained by heterogeneous Sca-1$^+$ cells with reactivation of some fetal pathways that harbor regenerative potential to repair the epithelium and restore Lgr5$^+$ ISCs.

demonstrated that non-Lgr5$^+$ ISC populations can reconstitute the epithelium in this context. Indeed, there is growing literature to support cellular plasticity of lineage-committed cells regaining developmental potential. These include cells from both secretory and absorptive lineages, which are themselves progeny derived from Lgr5$^+$ ISCs, including Dll1$^+$ secretory progenitor cells (van Es et al., 2012), Alpi$^+$ enterocyte progenitors (Tetteh et al., 2016), a population of label-retaining multipotent Paneth/enteroendocrine precursor cells (Buczacki et al., 2013), and more developmentally mature enteroendocrine cells (Yan et al., 2017). Taken together, these studies suggest that the cell type and regulatory mechanisms used during injury-induced regeneration differ from those used for steady-state epithelial renewal. In this case, Nusse and Savage et al. provide evidence that the source of Sca-1$^+$ cells upon *H. polygyrus* infection is an epithelial cell derived from the Lgr5$^+$ ISC, but the precise cell type giving rise to Sca-1$^+$ cells and its position in the lineage hierarchy remain to be determined. Furthermore, Sca-1 is broadly expressed throughout the crypts, which exhibit significant heterogeneity by single-cell RNA-seq analysis, so it is likely that additional markers will be needed to prospectively identify a fetal-like stem/progenitor sub-population mediating this regeneration.

Expression of fetal markers in response to tissue injury has been recently reported in multiple contexts including NSAID drug-mediated injury to gastric epithelium (Fernandez Vallone et al., 2016) and in chemically induced colitis (Yui et al., 2018). In the latter study, the population of cells with fetal signature were Sca1$^+$, as in the current study. Taken together, these suggest that injury-induced regeneration occurs from conversion of existing cell types to adopt fetal-like pathways to rapidly reestablish the steady state, possibly by re-deriving the adult stem/progenitor cells similarly to the manner in which the tissue was originally formed.

The connections drawn in these studies between developmental programs and injury-repair, including those drawn by Nusse and Savage et al. (Nusse et al., 2018), have broad potential applications in regenerative medicine. Damage to intestinal epithelium occurs in many disease states including inflammatory bowel disease, ischemic injury, infection, and the influence of drugs. Further distillation of the precise mechanisms by which crypts can hijack fetal pathways upregulated during the injury response may enable us to specifically target these pathways to generate replacement tissues, enhance mucosal healing *in vivo*, or even prevent epithelial damage. Understanding the toolbox used by the epithelium for regeneration during this fetal-like reversion would enable development of novel therapeutics to add to the armamentarium for diseases affecting the gut epithelium and perhaps other tissues more broadly.

REFERENCES

Buczacki, S.J., Zecchini, H.I., Nicholson, A.M., Russell, R., Vermeulen, L., Kemp, R., and Winton, D.J. (2013). Intestinal label-retaining cells are secretory precursors expressing Lgr5. Nature *495*, 65–69.

Chin, A.M., Hill, D.R., Aurora, M., and Spence, J.R. (2017). Morphogenesis and maturation of the embryonic and postnatal intestine. Semin. Cell Dev. Biol. *66*, 81–93.

Fernandez Vallone, V., Leprovots, M., Strollo, S., Vasile, G., Lefort, A., Libert, F., Vassart, G., and Garcia, M.I. (2016). Trop2 marks transient gastric fetal epithelium and adult regenerating cells after epithelial damage. Development *143*, 1452–1463.

Fordham, R.P., Yui, S., Hannan, N.R., Soendergaard, C., Madgwick, A., Schweiger, P.J., Nielsen, O.H., Vallier, L., Pedersen, R.A., Nakamura, T., et al. (2013). Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury. Cell Stem Cell *13*, 734–744.

Mustata, R.C., Vasile, G., Fernandez-Vallone, V., Strollo, S., Lefort, A., Libert, F., Monteyne, D., Pérez-Morga, D., Vassart, G., and Garcia, M.I. (2013). Identification of Lgr5-independent spheroid-generating progenitors of the mouse fetal intestinal epithelium. Cell Rep. *5*, 421–432.

Nusse, Y.M., Savage, A.K., Marangoni, P., Rosendahl-Huber, A.K.M., Landman, T.A., de Sauvage, F.J., Locksley, R.M., and Klein, O.D. (2018). Parasitic helminths induce fetal-like reversion in the intestinal stem cell niche. Nature *559*, 109–113.

Tetteh, P.W., Basak, O., Farin, H.F., Wiebrands, K., Kretzschmar, K., Begthel, H., van den Born, M., Korving, J., de Sauvage, F., van Es, J.H., et al. (2016). Replacement of Lost Lgr5-Positive Stem Cells through Plasticity of Their Enterocyte-Lineage Daughters. Cell Stem Cell *18*, 203–213.

van Es, J.H., Sato, T., van de Wetering, M., Lyubimova, A., Yee Nee, A.N., Gregorieff, A., Sasaki, N., Zeinstra, L., van den Born, M., Korving, J., et al. (2012). Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. Nat. Cell Biol. *14*, 1099–1104.

Yan, K.S., Gevaert, O., Zheng, G.X.Y., Anchang, B., Probert, C.S., Larkin, K.A., Davies, P.S., Cheng, Z.F., Kaddis, J.S., Han, A., et al. (2017). Intestinal Enteroendocrine Lineage Cells Possess Homeostatic and Injury-Inducible Stem Cell Activity. Cell Stem Cell *21*, 78–90 e76.

Yui, S., Azzolin, L., Maimets, M., Pedersen, M.T., Fordham, R.P., Hansen, S.L., Larsen, H.L., Guiu, J., Alves, M.R.P., Rundsten, C.F., et al. (2018). YAP/TAZ-Dependent Reprogramming of Colonic Epithelium Links ECM Remodeling to Tissue Regeneration. Cell Stem Cell *22*, 35–49 e37.