News & Views

THE EMBO JOURNAL

Everything has its time: Id2 clocks embryonic specification of Lgr5$^+$ gut stem cells

Kai Kretzschmar[1,2] & Hans Clevers[1,2,3]

While adult intestinal stem cells and their niche are well characterised, their developmental specification is still poorly understood. In this issue of *The EMBO Journal*, Nigmatullina *et al* (2017) show that maturation of Lgr5$^+$ progenitors in the small intestinal epithelium during murine embryonic development is controlled by the transcription factor Id2.

See also: **L Nigmatullina *et al*** (April 2017)

Maintenance of epithelial tissues is fuelled by adult stem cells, which reside in specialised niches. The stem cells of the small intestinal epithelium are located in the crypt bottom (Clevers, 2013). The maintenance of intestinal stem cells is controlled by the Wnt/β-catenin signalling cascade. Consequently, Lgr5, both target gene and component of the Wnt/β-catenin pathway, was discovered to be a marker of adult intestinal stem cells (Barker *et al*, 2007). However, the developmental origin of Lgr5$^+$ intestinal stem cells and their specification during embryonic development remained unknown. It was suggested earlier that intestinal stem cells appear at embryonic day 14.5 (E14.5) during a developmental stage, when the epithelium reorganises by forming villi and inter-villus regions (Guiu & Jensen, 2015). In contrast, it was subsequently reported that *Lgr5* expression in the developing intestinal epithelium is initiated much earlier, that is at E12.5 (Shyer *et al*, 2015) during a phase of growth and elongation (Guiu & Jensen, 2015). It was also proposed that the pseudostratified intestinal epithelium is uniformly composed of equipotent Wnt-active progenitors at this stage (Shyer *et al*, 2015). Now Nigmatullina *et al* (2017) resolve the apparent discrepancies between these earlier reports utilising genetic lineage tracing, messenger RNA sequencing, *in situ* hybridisation and organoid technology.

Nigmatullina *et al* (2017) first compared the transcriptional profile of EpCAM$^+$ embryonic intestinal epithelium with that of Lgr5-EGFP$^+$ adult stem cells. More than 4,500 genes—about half of the transcriptome—were differentially expressed between embryonic and adult epithelial cells. The adult epithelium mainly expressed genes associated with antimicrobial defence as well as ribosomes and mitochondria. In contrast, the embryonic transcriptional profile was enriched in genes encoding members of the transcriptional machinery and transcription factors. Notably, inhibitors of canonical Wnt signalling such as *Sfrp1* and *Sfrp2* were expressed at higher levels in embryonic tissue, while markers of adult intestinal stem cells such as *Lgr5*, *Ascl2*, *Cd44* and *Olfm4* were barely detectable or completely absent in the embryonic transcriptome. The authors then went on to analyse Lgr5-EGFP expression at later stages. In contrast to observations by Shyer *et al* (2015), < 1% of epithelial cells were found to be Lgr5-EGFP$^+$ at this stage with the number of positive epithelial cells increasing to more than 15% at E17.5. Using genetic lineage tracing (Kretzschmar & Watt, 2012), the authors further found that significant tracing of Lgr5 cell progeny was not found until E14.5 when assessed 1 day after tamoxifen injection. Transcriptional profiling of the Lgr5$^+$ cells at this stage showed that Lgr5$^+$ progenitors expressed genes involved in cell cycle progression and proliferation, as well as markers of intestinal stem cells such as *Ascl2, Cd44* and *Olfm4*. Yet, these were expressed at much lower levels compared to mature adult stem cells, while markers of differentiation were remarkably downregulated. These data suggested that functional Lgr5$^+$ embryonic progenitors born at E13.5 have the capacity to contribute to the adult stem cell pool, as previously shown by Shyer *et al* (2015).

The authors did not stop here, as it remained unresolved how Lgr5$^+$ progenitor specification is controlled. Expression data obtained for the embryonic epithelium at E11.5 revealed genes that were expressed significantly higher than in Lgr5$^+$ adult stem cells and were previously associated with regulating adult stem cell fate decisions. Amongst these genes was *Id2*, most highly expressed at E11.5 and known to encode a transcriptional regulator promoting differentiation and suppressing tumour formation in the embryonic intestinal epithelium (Russell *et al*, 2004). Nigmatullina and co-workers therefore analysed mutant mice with homozygous deletion of *Id2* (*Id2* KO). In *Id2* mutant mice, Lgr5-EGFP$^+$ cells were detected significantly earlier at E9.5. More than one-third of the epithelial cells was Lgr5-EGFP$^+$ 2 days later with positivity

1 Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW) and University Medical Centre (UMC) Utrecht, Utrecht, The Netherlands. E-mail: h.clevers@hubrecht.eu
2 Cancer Genomics Netherlands, UMC Utrecht, Utrecht, The Netherlands
3 Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands
DOI 10.15252/embj.201796482 | Published online 15 March 2017

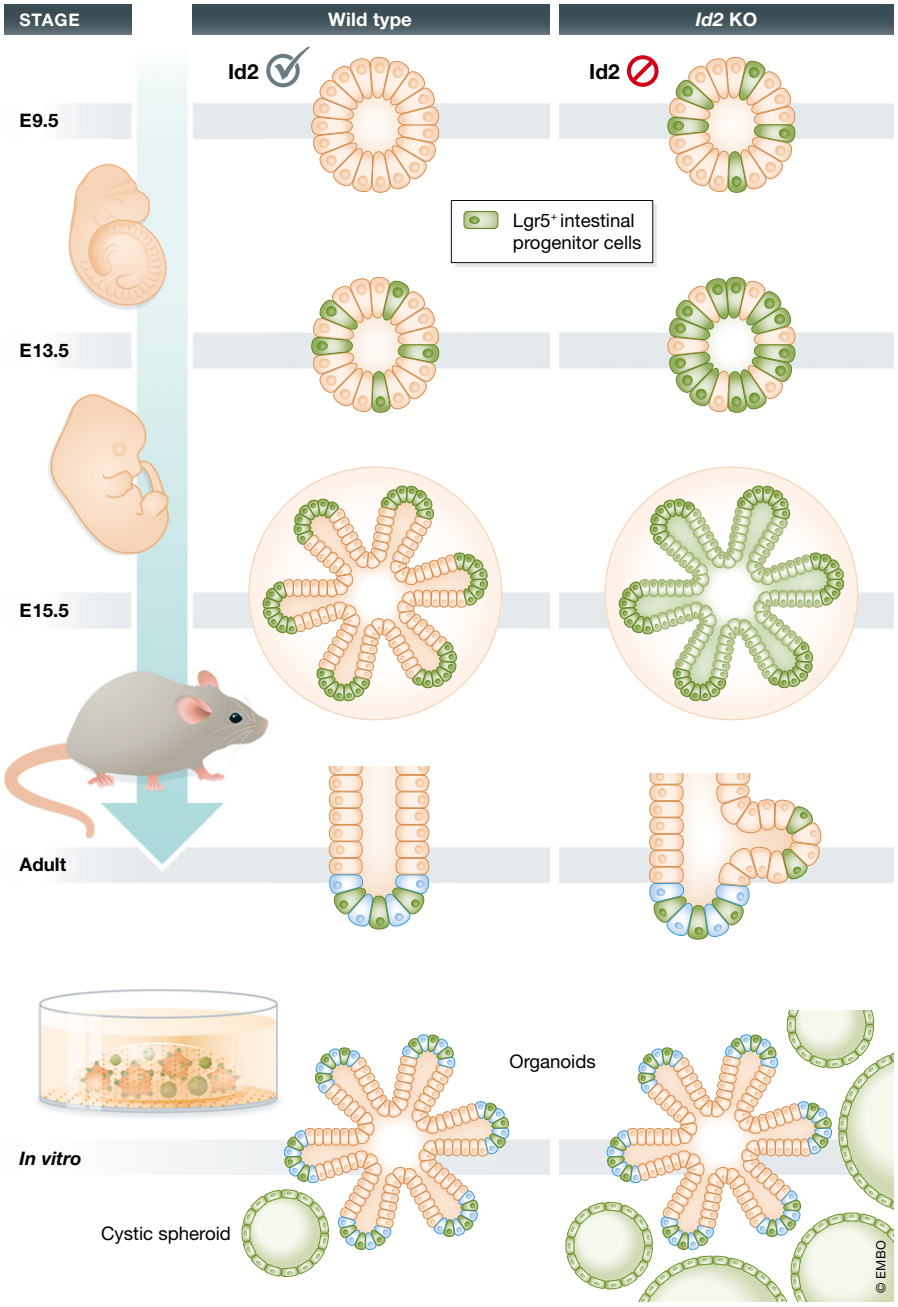

Figure 1. Loss of Id2 causes precocious appearance of Lgr5$^+$ intestinal progenitor cells during embryonic development.
In wild-type mice with functional Id2, Lgr5$^+$ intestinal progenitor cells (green) appear in the pseudostratified epithelium at E13.5 and become restricted to the inter-villi region at E15.5 residing in the crypt bottom in adult animals. In *Id2*-deficient mice, Lgr5$^+$ intestinal progenitor cells are born prematurely at E9.5, increase in number at E13.5 and remain highly enriched at later embryonic stages beyond E15.5. In adult *Id2* knock-out mice, ectopic Lgr5$^+$ cells are a major cause of intestinal neoplasms. *In vitro* cultures of *Id2*-deficient Lgr5$^+$ intestinal progenitors have a much higher organoid-forming capacity and also produce more cystic spheroids than their wild-type counterparts.

doubling again at E15.5. Gene expression analysis revealed that Id2 antagonises expression of several other intestinal stem cell markers such as *Lrig1*, *Prominin-1/Cd133* and *Tnfrsf19/Troy* as well as activation of Wnt/β-catenin signalling in the embryonic epithelium. The authors, however, found no evidence of the epithelium being affected by the mesenchymal niche in *Id2*-deficient mice. This is in contrast to the study by Shyer *et al* (2015) demonstrating that spatial restriction of the progenitor pool is controlled by mesenchymal BMP signalling following morphological changes and induction of endodermal Shh expression.

To address whether Id2 negatively regulates protein stability of β-catenin and consequentially β-catenin/TCF-dependent transcription, Nigmatullina *et al* (2017) performed cell culture experiments using organoid cultures that have emerged as an essential method to study stem cells and developmental biology *in vitro*. This technology was utilised by the authors to demonstrate that *Id2*-deficient Lgr5$^+$ progenitors have a higher organoid-forming capacity compared to their *Id2*-sufficient counterparts. *Id2* KO organoids grew faster, bigger and started to express markers of neoplastic transformation, as previously reported for the intestines of neonatal *Id2* mutant mice (Russell *et al*, 2004).

Taken together, this study sheds new light on the mechanism of developmental specification of the intestinal epithelial progenitors. Nigmatullina and colleagues demonstrate, in contrast to an earlier study (Shyer *et al*, 2015), that these Lgr5$^+$ embryonic progenitors represent a subpopulation of the intestinal epithelial cells. This observation is similar to earlier descriptions of the adult stem cell niche at the crypt bottom, as only about 15 Lgr5$^+$ stem cells are present per crypt (Clevers, 2013). Id2 was shown to be a critical regulator of this spatial restriction by inhibiting Wnt/β-catenin signalling. Upon Id2 loss, Lgr5$^+$ progenitors were precociously born and were increased in numbers *in vivo*. Established intestinal organoids generated from *Id2*-deficient mice were also hyperproliferative in culture. Collectively, the current study strongly suggests that epithelial Id2 controls specification of intestinal progenitors in a cell-intrinsic manner. Now, it will be important to address a possible role for epithelial–mesenchymal bidirectional signalling in the regulation of the progenitor emergence in the embryonic intestine in future.

References

Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H (2007) Identification of stem cells in small intestine

and colon by marker gene Lgr5. *Nature* 449: 1003 – 1007

Clevers H (2013) The intestinal crypt, a prototype stem cell compartment. *Cell* 154: 274 – 284

Guiu J, Jensen KB (2015) From definitive endoderm to gut-a process of growth and maturation. *Stem Cells Dev* 24: 1972 – 1983

Kretzschmar K, Watt FM (2012) Lineage tracing. *Cell* 148: 33 – 45

Nigmatullina L, Norkin M, Dzama MM, Messner B, Sayols S, Soshnikova N (2017) Id2 controls specification of Lgr5^{+} intestinal stem cell progenitors during gut development. *EMBO J* 36: 869 – 885

Russell RG, Lasorella A, Dettin LE, Iavarone A (2004) Id2 drives differentiation and suppresses tumor formation in the intestinal epithelium. *Cancer Res* 64: 7220 – 7225

Shyer AE, Huycke TR, Lee C, Mahadevan L, Tabin CJ (2015) Bending gradients: how the intestinal stem cell gets its home. *Cell* 161: 569 – 580