Transitional basal cells at the squamous–columnar junction generate Barrett’s oesophagus

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In several organ systems, the transitional zone between different types of epithelium is a hotspot for pre-neoplastic metaplasia and malignancy1–3, but the cells of origin for these metaplastic epithelia and subsequent malignancies remain unknown1–3. In the case of Barrett’s oesophagus, intestinal metaplasia occurs at the gastro-oesophageal junction, where stratified squamous epithelium transitions into simple columnar cells4. On the basis of a number of experimental models, several alternative cell types have been proposed as the source of this metaplasia but in all cases the evidence is inconclusive: no model completely mimics Barrett’s oesophagus in terms of the presence of intestinal goblet cells5–8. Here we describe a transitional columnar epithelium with distinct basal progenitor cells (p63+ KRT5-KRT7+) at the squamous–columnar junction of the upper gastrointestinal tract in a mouse model. We use multiple models and lineage tracing strategies to show that this squamous–columnar junction basal cell population serves as a source of progenitors for the transitional epithelium. On ectopic expression of CDX2, these transitional basal progenitors differentiate into intestinal-like epithelium (including goblet cells) and thereby reproduce Barrett’s metaplasia. A similar transitional columnar epithelium is present at the transitional zones of other mouse tissues (including the ano-rectal junction) as well as in the gastro-oesophageal junction in the human gut. Acid reflux-induced oesophagitis and the multilayered epithelium (believed to be a precursor of Barrett’s oesophagus) are both characterized by the expansion of the transitional basal progenitor cells. Our findings reveal a previously unidentified transitional zone in the epithelium of the upper gastrointestinal tract and provide evidence that the p63+ KRT5-KRT7+ basal cells in this zone are the cells of origin for multi-layered epithelium and Barrett’s oesophagus.

Barrett’s oesophagus is the precursor lesion of oesophageal adenocarcinoma, which has increased in incidence by approximately 800% over the past four decades8. Histologically, Barrett’s oesophagus is characterized by the replacement of the stratified squamous epithelium of the distal oesophagus with simple columnar epithelial cells that often express characteristics of intestinal differentiation (for example, CDX2 and ALCAN blue)3,9. During disease progression, multilayered epithelium (MLE), which is composed of cells with both squamous and columnar features, has been considered as a precursor for Barrett’s oesophagus10. However, it remains unclear which cells give rise to MLE and Barrett’s oesophagus. Five models have been proposed to explain the pathogenesis of Barrett’s oesophagus (Extended Data Fig. 1), each involving different cell types. These include the transdifferentiation of oesophageal squamous epithelium,5,6 or circulating bone marrow cells11, and the expansion of submucosal glandular epithelium12, gastric cardia mucosa7 or residual embryonic cells (RECs) located at the squamous–columnar junction (SCJ)8. Some of these studies have presented inconsistent evidence between in vitro and in vivo experiments5,6,13 and none of the experimental models recapitulates the pathological changes characteristically associated with Barrett’s oesophagus in humans, such as the presence of intestinal goblet cells5–8,11. We therefore considered the possibility that other cell types function as the cells of origin for MLE and Barrett’s oesophagus.

Overexpression of the transcription factor SOX2 leads to basal cell hyperplasia in the squamous epithelium of Krt5CreER;R26lox;AG-lapx-stop-lox/Sox2-IREs-EGFP (also known as Krt5CreER/ROSA26Sox2-lox;AG-lapx-stop-lox/Sox2-IREs-EGFP; hereafter referred to as Krt5CreER/R26lox–GFP) mice4 (Extended Data Fig. 2a). In this study, we found a hyperplastic transitional columnar epithelium at the SCJ, which in mice is located at the junction of the forestomach and hind stomach (Fig. 1a, d, Extended Data Fig. 2f) (n = 7). This epithelium consists of basal cells (p63 KRT5+]) and luminal cells (KRT8+]), in contrast to the neighbouring keratinized stratified squamous epithelium, the KRT7+ cells secrete mucin (Alcin blue periodic acid–Schiff (PAS)−) and express the intestinal marker AGR2 (Extended Data Fig. 2b, c). Furthermore, the expanded transitional epithelium expresses the Barrett’s oesophagus marker KRT715 (Fig. 1b). The pathological presentation is similar to human MLE, as both squamous (p63 KRT5+) and columnar (KRT8 KRT7+) cells are present. To determine whether basal cells serve as progenitors for the columnar epithelium, we examined the expression of GFP and SOX2 in Krt5CreER;R26lox–GFP mutant mice. Previous studies have shown that genetic lineage tracing allows the identification of stem and/or progenitor cells in a range of tissues16,17. We found that lineage-tagged GFP+ cells are present not only in the stratified squamous epithelium14 but also in the amplified transitional epithelium (SOX218) (Fig. 1b, Extended Data Fig. 2d). These findings support the notion that basal cells serve as progenitors for the SCJ transitional epithelium. Notably, the adjacent columnar cells lining the cardia mucosa are GFP−, indicating that they are not derived from basal progenitor cells (Fig. 1a–c, Extended Data Fig. 2e). Conversely, the transitional columnar epithelium does not express the cardia mucosal protein Claudin18 (Fig. 1c, Extended Data Fig. 2e). The cardia mucosa (Lgr519) consistently does not contribute to the transitional epithelium in Lgr5CreER;R26lox–GFP mice2 (Extended Data Fig. 2g). Furthermore, bile...
Acid reflux, which is a strong risk factor for Barrett's oesophagus, also leads to the expansion of the transitional columnar epithelium in mice undergoing oesophageo-gastroduodenal anastomosis surgery (Extended Data Fig. 3a, c). Lineage tracing demonstrated that the expanded columnar epithelium is generated by p63+ basal progenitor cells in p63CreER;R26GFP (also known as Tcp1CreER;R26GFP) mice (Extended Data Fig. 3c). Notably, metaplastic cells were not observed in the oesophagus or other parts of the forestomach (Extended Data Fig. 3b). These findings, derived from models driven by both genetic and environmental changes, suggest that basal cells (p63+ KRT5+) in the transitional epithelium serve as progenitors for KRT7+ Barrett's oesophagus-like epithelium in mice (Fig. 1d).

These results prompted us to test whether a non-keratinized transitional columnar epithelium exists in the SCJ of normal mice. As previously described18,19, the keratinized stratified squamous epithelium consists of basal cells (p63+ KRT5+ KRT7+) and differentiated suprabasal cells (Loricrin+ Involucrin+) (Fig. 2a and Extended Data Fig. 4a). We also identified a narrow non-keratinized epithelial zone at the SCJ, which was composed of basal cells (p63+ KRT5+ KRT7+) and luminal cells (KRT7+ Loricrin+ Involucrin+ Claudin18) (Fig. 2a and Extended Data Fig. 4a, b). Next, we investigated whether the proliferative transitional basal cells (Ki67+) serve as progenitors for the luminal cells (Extended Data Fig. 4c). We first performed lineage tracing with the p63CreER mouse line20. As expected, p63CreER labels the squamous basal cells and their progeny (Fig. 2b); scattered individual basal cells in the transitional epithelium were also labelled 24 hours after a single low dose of tamoxifen (Fig. 2b). Two weeks after injection, a single isolated lineage-labelled cells (Tomato+/KRT7+) confined to the transitional epithelium was observed (Fig. 2b), suggesting that KRT7+ cells are generated by transitional basal cells. We then established a Krt7CreER knockin mouse line. Notably, a single low-dose tamoxifen injection induced recombination in the basal cells of the transitional epithelium, but not in any of the cells in the neighbouring squamous epithelium (Fig. 2b). The lineage-labelled cells expanded and generated the transitional epithelium (KRT7+) (Fig. 2b). Together, these lineage tracing results support the idea that the distinct basal cells (p63+ KRT7+) serve as progenitors for the SCJ transitional epithelium.

Using both 2D and 3D clonal culture, we tested whether the transitional basal cells self-renew and differentiate to reconstitute the transitional epithelium. We used the nerve growth factor receptor (p75) to purify basal progenitor cells21,22. In the SCJ, p75+ basal cells include two populations, squamous (p63+ KRT7+) and transitional (p63+ KRT7+) basal cells (Extended Data Fig. 4d, e). Consistently, fluorescence-activated cell sorting (FACS)-sorted single basal cells expanded to form two types of colony: p63+ KRT7+ and p63+ KRT7+ (Fig. 2c). These colonies can be maintained for six passages in the presence of Wnt3A, R-spondin1, Noggin and Fgf10; these growth factors were successfully used to maintain squamous basal cells and Barrett’s oesophagus organoids (n = 5)14,22,23. The two distinct basal progenitor populations did not interconvert when reseeded at single-cell densities even after five passages. Moreover, two types of epithelium (keratinized and non-keratinized) were generated by the two basal cell populations in both air–liquid interface culture and 3D organoid culture (Fig. 2d, Extended Data Fig. 4f). Notably, the p63+ KRT7+ transitional basal cells generated only a non-keratinized transitional epithelium (KRT7+ Loricrin+) and the p63+ KRT7+ squamous basal cells consistently produced only a keratinized squamous epithelium (Loricrin+) (Fig. 2d). These findings demonstrate that two distinct basal progenitor populations exist in the upper gastrointestinal tract, where they are individually responsible for maintaining the keratinized stratified squamous and non-keratinized transitional epithelia (Fig. 2e). A transitional epithelium composed of p63+ KRT7+ basal and KRT7+ luminal cells is also present in several other tissues, including the rectum–anus SCJ and the cervix-SCJ (Extended Data Fig. 4h, i) in mice.

Ad has been previously reported that RECs, a quiescent epithelial population of cells (p63+ KRT7+), persist on the upper luminal surface of the gastric epithelium and serve as the cell of origin for Barrett’s oesophagus8 (Extended Data Fig. 1e). This previous study also reported that RECs expanded following the loss of basal cells on tamoxifen injection, in adult Krt14CreER;R26GFP mice8. The authors proposed that RECs (p63+ KRT7+) and basal cells (p63+ KRT7+) are two unrelated populations in development, and that during development p63+ KRT7+ basal cells expand from the oesophagus.
to the forestomach and gradually replace the initial columnar cells (p63+ KRT7+, arrowheads) except at the SCJ where they become quiescent RECs8 (Extended Data Fig. 5a). The authors further proposed that deletion of p63 (also known as Tcp1) blocks the downward expansion of basal cells, and consequently that the forestomach is lined with p63− KRT7+ RECs. By contrast, we found that KRT7 and p63 are co-expressed in both stratified squamous and SCJ transitional epithelium at embryonic day (E)11.5, E12.5, E14.5 and E16.5 (Extended Data Fig. 5c, d). In the squamous epithelium of the oesophagus and forestomach, basal cell loss of expression of KRT7 at E18.5; basal cells in the SCJ transitional epithelium remain KRT7+ at this stage (Extended Data Fig. 5c, d). Lineage tracing demonstrated that the columnar epithelium lining the p63−/−/− oesophagus, forestomach and SCJ is derived from p63 promoter active basal progenitors in p63CreER/Cox2erتض (p63-null) mouse embryos (Extended Data Fig. 5e–h). Consistent with previous findings24, E18.5 p63-null mice retain a simple columnar oesophageal epithelium, which is similar to the normal epithelium lining the oesophagus and forestomach in E11.5 mice25 (Extended Data Fig. 5i).

Barrett’s oesophagus is characterized by the increased expression of CDX2, a transcription factor that is critical for intestinal development. Ectopic CDX2 expression promotes the differentiation of gastric secretory epithelium into intestinal-like cells (such as goblet cells)27. Ectopic CDX2 expression, however, fails to promote the intestinal differentiation of the squamous epithelium of the oesophagus8. To test whether CDX2 expression drives intestinal differentiation of the transitional basal progenitor cells, we first generated Krt5CreER;R26rtTA;otet-CDX2-T2A-mCherry mice (Extended Data Fig. 6a). Ectopic CDX2 expression initially promoted the expansion of the transitional epithelium (p63+ KRT7+) one week after doxycycline exposure (Extended Data Fig. 6b). Four weeks after the onset of CDX2 expression, the metaplastic cells (KRT8+) began to express AGR2; this was accompanied by a reduction in the levels of p63 (Extended Data Fig. 6c). Eight weeks after the onset of CDX2 expression, Barrett’s oesophagus epithelium (p63− KRT7+ KRT8+) was also present in the SCJ, although p63+ cells persisted in parts of the metaplastic epithelium that were reminiscent of MLE (Extended Data Fig. 6d). Intestinal metaplasia became more prominent 13 weeks after the onset of CDX2 expression (Fig. 3a) and the Barrett-like domains contained numerous goblet cells (MUC2+ TFF3+) with characteristic mucin-secreting vesicles (Alcian blue+ PAS+) (Fig. 3b, d, Extended Data Fig. 7a, b). Notably, a mixture of MLE (p63+ KRT5+ KRT7+ KRT8+) and Barrett’s oesophagus
logical changes were observed in the SCJ of Krt7CreER,R26rtTA;otet-CDX2 of doxycycline for 11 weeks (Extended Data Fig. 8a–c). Similar patho-

Fig. 7f). Metaplasia persisted and expanded even after the withdrawal

epithelium of the oesophagus and forestomach (Extended Data

scissed (Extended Data Fig. 7d). By contrast, the levels of p63 and

increased in metaplastic epithelium isolated by laser capture micro-

multiple intestinal genes (including Vil1, Agr2, Muc2, Muc4 and Tff3)
in the presence of Wnt3A, Noggin, R-Spondin and Fgf10. More impor-
tantly, p63

KRT7 

−

and p63

KRT7 

+ 

basal cell populations sorted by FACS can be passaged up to four times

underlying basal cells is considerably expanded in patients with long-
term bile acid reflux (Fig. 4d, Extended Data Fig. 11b–d). The transitional epithelium with

underlying basal cells is considerably expanded in patients with long-
term bile acid reflux (Fig. 4e, Extended Data Fig. 11e). The expan-
ded of the transitional epithelium was more prominent in biopsies that contained a mixture of MLE and Barrett’s oesophagus (Fig. 4f,

Extended Data Fig. 11f, g). The expression of p63 and KRT5 was lost

in samples that contained only Barrett’s oesophagus (n = 12), whereas

Barrett’s epithelium gained CDX2, Villin 1 and AGR2 expression (Extended Data Fig. 11h).

We have identified a transitional columnar epithelium at the SCJ of

mice, which is the exclusive site of Barrett’s oesophagus and its pre-
cursor MLE. We have used lineage tracing, in vitro 2D culture and

organoid culture to support the idea that a distinct basal cell population

(p63

KRT5 

−

KRT7

+ 

KRT7

+ 

) was detected in the expanded SCJ (Fig. 3c, Extended Data Fig. 7c). Furthermore, the transcript levels of multiple intestinal genes (including Vil1, Agr2, Muc2, Muc4 and Tff3) increased in metaplastic epithelium isolated by laser capture micro-

dissection (Extended Data Fig. 7d). By contrast, the levels of p63 and

KRT5 were substantially downregulated (Extended Data Fig. 7d).

No metaplastic changes were observed in the SCJ of otet-CDX2 mice

(Fig. 3b, Extended Data Fig. 7e), and CDX2 did not induce intesti-
nal differentiation of squamous basal progenitor cells in the stratified

epithelium of the oesophagus and forestomach (Extended Data

Fig. 7f). Metaplasia persisted and expanded even after the withdrawal of doxycycline for 11 weeks (Extended Data Fig. 8a–c). Similar patho-

logical changes were observed in the SCJ of Krt7CreER,R26rtTA;otet-CDX2 mice after CDX2 expression (Fig. 3e, f, Extended Data Fig. 9a–c). As expected, neither MLE nor Barrett’s metaplasia developed in the neigh-
bouring squamous epithelium (Fig. 3e, f). Consistently, intestinal meta-
plasia occurred only in p63

KRT7

+ 

(and not in p63

KRT7 

− 

) basal cells, on overexpression of CDX2 in vitro (Extended Data Fig. 10). These results support the idea that the transitional basal progenitor cells that maintain the SCJ transitional epithelium generate Barrett’s oesophagus under experimental conditions.

Next, we investigated whether the transitional epithelium is also present in the human SCJ; unlike in the mouse model, the human SCJ is located at the gastro-oesophageal junction (Fig. 4a). Notably, a columnar epithelium composed of basal (p63

KRT5 

−

KRT7

+ 

) and luminal (p63

KRT5 

+ 

KRT7

+ 

) cells (similar to that present in mice) was observed in the human SCJ (Fig. 4a–c). Flow cytometric analysis

indicated the presence of two distinct basal progenitor populations

(p63

KRT7

− 

and p63

KRT7

+ 

) (Extended Data Fig. 11a). The two basal cell populations sorted by FACS can be passaged up to four times

in the presence of Wnt3A, Noggin, R-Spondin and Fgf10. More impor-
tantly, p63

KRT7

+ 

(but not p63

KRT7 

− 

) basal cells generate intesti-
nal-like epithelial cells in organoid culture upon CDX2 overexpression

(Fig. 4d, Extended Data Fig. 11b–d). The transitional epithelium with

underlying basal cells is considerably expanded in patients with long-
term bile acid reflux (Fig. 4e, Extended Data Fig. 11e). The expan-
ded of the transitional epithelium was more prominent in biopsies that contained a mixture of MLE and Barrett’s oesophagus (Fig. 4f,

Extended Data Fig. 11f, g). The expression of p63 and KRT5 was lost

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Barrett’s epithelium gained CDX2, Villin 1 and AGR2 expression (Extended Data Fig. 11h).

We have identified a transitional columnar epithelium at the SCJ of

mice, which is the exclusive site of Barrett’s oesophagus and its pre-
cursor MLE. We have used lineage tracing, in vitro 2D culture and

organoid culture to support the idea that a distinct basal cell population

(p63

KRT5 

−

KRT7

+ 

) maintains this transitional columnar epithelium at homeostasis. We further show that these transitional basal progenitor cells differentiate into intestinal cells, including goblet cells, following

CDX2 expression. Notably, a transitional epithelium with underlying

p63

KRT7

− 

basal cells is also present in the human gastro-oesophageal junction and expansion of these transitional basal progenitors is associated with the development of Barrett’s oesophagus; moreover, similar transitional epithelia exist in at least two other tissue junctions.
Characterization of the progenitor cells for these additional transitional epithelia and investigations into how they respond to pathological conditions, such as acid reflux, hormonal modulation, virus infection and chemical agents are likely to yield critical information related to the aetiology of tissue-specific metaplasia and tumorigenesis.

Online Content  Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions M.J. and J.Q. designed experiments, analysed data and wrote the manuscript. M.J. and J.Q. generated the oet-Cdx2 transgenic and Krt7CreER knockin mouse lines. M.J. performed immunostaining, imaging, laser microdissection, flow cytometry, organoid culture and mouse genetics. H.L. performed qPCR and immunostaining; Y.-C.L. and J.Y. generated the CDX2 knockin mouse lines. M.J. performed immunostaining, imaging, laser microdissection, flow cytometry, organoid culture and mouse genetics. H.L. performed the acid reflux surgery. X.L., H. Wu, J.Z. and J.S. assisted with imaging. M.J. and J.S. performed the acid reflux surgery. X.L., H. Wu, J.Z. and J.S. assisted with imaging. J.X. and D.-K.L. provided the p63CreER knockin mouse lines. M.J. performed immunostaining, imaging, laser microdissection, flow cytometry, organoid culture and mouse genetics. H.L. and J.Q. performed the acid reflux surgery. X.L., H. Wu, J.Z. and J.S. assisted with imaging. M.J. and J.S. performed the acid reflux surgery. X.L., H. Wu, J.Z. and J.S. assisted with imaging. J.X. and D.-K.L. performed the p63CreER mouse line. Z.Z., L.Z., J.A.A., T.C.W., A.R.S., Q.W., H.C. and X.S. provided human MLE and Barrett’s oesophagus samples.

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Mice. Krt5CreER; Krt5TA; R26lox-stop-loxp-Sox2-IRES-GF and Lgr5CreER and Lgr5CreERT2 mice have been previously described2,7,13,28,29,30. R26lox-stop-cre and R26lox-stop-flb were purchased from the Jackson Laboratory. p63CreER mice were provided by J.X.30. The otet-CDX2-T2A-mCherry transgenic line was generated by pronuclear injection of linearized DNA. The resulting transgenic mice are maintained on a C57BL/6 and 129SvEv mixed background. Two individual lines were used as a consistent expression pattern. The Krt5CreERT2 knockin line was generated by replacing the CreERT2 cassette at the ATG of the Krt7 gene and maintained on a C57BL/6 and 129SvEv mixed background. To lineage trace Lgr5 cells, 2 mg tamoxifen per 20 g bodyweight was injected into Lgr5CreERT2, R26lox-stop-flb or mice or controls. To line trace p63+ and KRT7+ cells, p63CreER; R26lox-stop-cre and Krt5CreERT2, R26lox-stop-flb mice were intraperitoneally injected with low-dose tamoxifen (0.2 mg per 20 g body weight). To overexpress CDX2, Krt5CreERT2; otet-CDX2-T2A-mCherry mice (6–8 weeks old) were given doxycycline (Dox) drinking water (2 mg ml−1) every other week and Krt7CreERT2; R26lox-stop-cre; otet-CDX2-T2A-mCherry mice were administrated with four doses tamoxifen, followed by Dox drinking water. Samples were collected for analysis at a range of time points (indicated in the text). otet-CDX2-T2A-mCherry mice provided with Dox water were included as controls. All mice were maintained in Columbia University's animal facility according to institutional guidelines. All mouse experiments were conducted in accordance with procedures approved by the Institutional Animal Care and Use Committee at Columbia University. All relevant ethical regulations were followed.

Bile acid reflux model. To generate bile acid reflux mouse models, oesophago-gastroduodenal anastomosis was performed by X.C., as previously described11. In brief, eight-week-old p63CreER; R26lox-stop-cre mice injected with four doses tamoxifen, followed by Dox drinking water. Samples were collected for analysis after 1 week. All studies were conducted in accordance with the relevant ethical regulations and were approved by the institutional animal research committee.

Isolation of SCJ basal progenitor cells and FACS sorting. Mouse or human SCJ regions were cut and incubated in dispase for 30 min at room temperature. Digestion was stopped by adding DMEM medium with 10% FBS. The epithelium was detached from the mesenchyme with forceps and digested for 20 min in 0.1% trypsin. Digestion was stopped by adding DMEM medium with 10% FBS. The epithelium was then cultured in vitro for four days. For immunostaining of p63 and KRT7 and the other for reseeding onto 24-well plates for flow cytometric analysis, isolated single cells were fixed by 4% paraformaldehyde in PBS for 30 min. After fixation, the samples were permeabilized with 0.5% Triton X-100 for 1 hour at room temperature. After permeabilization, the samples were incubated with primary antibodies in 1% bovine serum albumin (BSA) for 1 hour at room temperature. After washing with PBS, the samples were incubated with secondary antibodies in 1% BSA for 1 hour at room temperature. After washing with PBS, the samples were incubated with a mounting medium containing 1 mg/ml DAPI for 1 hour at room temperature. The samples were then coverslipped and observed using a Zeiss LSM 710 confocal microscope.

Statistical analysis. Statistical analysis was performed using GraphPad Prism software. Data are expressed as mean ± standard error of the mean (SEM), and statistical significance was assessed using unpaired two-tailed Student's t-test. Two-tailed P < 0.05 was considered statistically significant.

Supplementary Information. Further data that support the findings of this study are available within the paper and its Supplementary Information. All relevant ethical regulations were followed. Basic information about patients is listed in Supplementary Table 2.
Multiple models have been proposed to explain the cell of origin for Barrett’s oesophagus. 

**a** Transdifferentiation of the stratified squamous oesophageal epithelium into Barrett’s epithelium. 

**b** Transdifferentiation of circulating bone marrow cells into Barrett’s epithelium. 

**c** Expansion of the oesophageal submucosal gland leads to Barrett’s oesophagus. 

**d** Stem and progenitor cells (Lgr5⁺) in the cardia mucosa differentiate into Barrett’s oesophagus. 

**e** Expansion of the quiescent RECs at the SCJ leads to Barrett’s oesophagus formation.

None of the studies that form the basis of these models recapitulates the pathological changes characteristically associated with Barrett’s oesophagus in humans, such as the presence of intestinal goblet cells.
Extended Data Figure 2 | Expansion of the columnar epithelium at the SCJ in Krt5\textsubscript{CreER},R26\textsubscript{Sox2-GFP} mutants. a, Generation of Krt5\textsubscript{CreER},R26\textsubscript{Sox2-GFP} (SOX2 overexpression) mutants. b, The columnar epithelium secretes mucin, as indicated by Alcian blue and PAS staining. \(n = 7\) per group. c, The mucin-secreting cells (AGR2\textsuperscript{+}) are derived from KRT5\textsuperscript{+} basal progenitor cells, as verified by the lineage tracing tag GFP. \(n = 7\). d, High magnification image of Fig. 1b to show that expanded GFP\textsuperscript{+} KRT7\textsuperscript{+} basal progenitor cells invade the underneath of the cardia mucosa on SOX2 overexpression. \(n = 7\). e, High magnification image of Fig. 1c, showing that expanded basal cells (KRT5\textsuperscript{+}) invade and intercalate with the cardia mucosal epithelium (CLDN18\textsuperscript{+}) on SOX2 overexpression. Note that KRT5\textsuperscript{+} cells (arrow and arrowheads) do not express CLDN18. Conversely, CLDN18\textsuperscript{+} cells do not express KRT5 (asterisk). \(n = 7\). f, The columnar epithelium does not express the squamous cell marker loricrin. \(n = 7\). g, Co-staining of X-gal with KRT5 and p63 indicates Lgr5\textsuperscript{+} cardia progenitor cells do not contribute to KRT5\textsuperscript{+} or p63\textsuperscript{+} basal cells in Lgr5\textsubscript{CreER},R26\textsubscript{LacZ} mice with both short-term tracing and long-term tracing. \(n = 3\) per group. Scale bars, 20\(\mu\text{m}\).
Extended Data Figure 3 | Basal progenitor cells contribute to columnar metaplasia following bile acid reflux induced by anastomosis surgery. a, Oesophageal–duodenal anastomosis and lineage tracing in p63CreER;R26tdTomato mice. b, Metaplasia does not occur at the distal oesophagus, where the anastomosis surgery site is located (arrow). Note that Alcian blue, PAS and CDX2 label the intestinal (but not the squamous) oesophageal epithelium. n = 5. c, Lineage-labelled transitional epithelial cells (tdT+) expand and resemble the MLE, expressing the columnar markers (KRT7, KRT8) and basal cell markers (KRT5 and p63). The expanded epithelium secretes mucin, as indicated by Alcian blue and PAS staining. n = 5. Scale bars, 20 μm.
Extended Data Figure 4 | The transitional columnar epithelium is present at the SCJ of normal mice. a, The transitional epithelium (asterisk) does not express involucrin and loricrin, markers labelling the stratified squamous epithelium. n = 11. b, Basal cells of the transitional epithelium express KRT5 but not the cardia epithelial marker CLDN18. n = 11. c, The KRT7⁺ transitional basal cells are highly proliferative (Ki67⁺, arrowheads). n = 3. d, The transitional basal cells of the SCJ express p75, KRT7 and KRT5, but not KRT8. Note that p75 and KRT5 are also expressed in the neighbouring squamous basal cells. n = 5. e, FACS analysis reveals p75⁺ basal cells include two subpopulations, squamous basal cells (p63⁺ KRT7⁻) and transitional basal cells (p63⁺ KRT7⁺). n = 3 independent experiments. f, A representative culture of p63⁺ KRT7⁻ and p63⁺ KRT7⁺ basal progenitor cells. Note that p63⁺ KRT7⁺ transitional basal cells in the colony are loosely arranged, unlike the cobblestone characteristic of the squamous basal cell colony (p63⁺ KRT7⁻). n = 5 per group. g, p63⁺ KRT7⁻ and p63⁺ KRT7⁺ basal progenitor cells generate keratinized (Loricrin⁺ KRT7⁻) and non-keratinized (Loricrin⁻ KRT7⁺) epithelium, respectively, in organoid culture. n = 5 per group. h, i, The transitional epithelium is also present at the SCJ of the anus (h) and cervix (i). Lineage tracing with the Krt7CreER mouse line confirmed that KRT7⁺ cells serve as progenitors for the transitional epithelium. n = 3 per group. Scale bars, 20 μm.
Extended Data Figure 5 | Loss of p63 prevents the stratification of KRT7⁺ columnar epithelium during embryonic development.

a, Proposed REC model of downward expansion of p63⁺ KRT7⁻ basal cells and retreatment of p63⁻ KRT7⁺ embryonic cells. b, The columnar epithelium lining the mouse forestomach and the SCJ expresses p63, KRT7 and KRT8 from E11.5 to E16.5. Note that Claudin18 expression is limited to the hindstomach epithelium. n = 5. c, Expression of KRT7 is restricted to the SCJ transitional epithelium at E18.5, and basal cells (arrowheads) express p63, KRT5, KRT7 and low levels of KRT8, but not CLDN18. n = 5. d, Gradual restriction of KRT7 expression to the SCJ transitional epithelium during development. Initially, the simple columnar epithelium lining the forestomach and SCJ expresses both p63 and KRT7.

On stratification, the forestomach epithelium loses KRT7 expression but basal cells at the SCJ maintain expression of both p63 and KRT7.

e, Lineage tracing of epithelial progenitor cells (p63 promoter active) in p63CreER/CreER; R26LacZ (p63-null) mutants. f, Whole-mount X-gal staining of the oesophagus and stomach isolated from p63CreER/CreER; R26LacZ mutants and p63CreER/CreER; R26LacZ controls. n = 3. g, h, The simple columnar epithelium that lines the forestomach (g) and oesophagus (h) of p63-null mutants is derived from basal progenitor cells (p63 promoter active), as indicated by X-gal staining. n = 3 per group. i, Normal oesophagus and forestomach is lined by simple columnar epithelium at E11.5. n = 3. Es, oesophagus; FS, forestomach; HS, hindstomach; Tr, trachea. Scale bars, 20 μm.
Extended Data Figure 6 | Ectopic CDX2 expression promotes intestinal metaplasia of the transitional epithelium at the SCJ. 

a, Targeted expression of CDX2 in basal progenitor cells of Krt5<sup>CreER<sup>, otet-CDX2-T2A-mCherry mice. Dox water was given every other week for three months to induce CDX2 and mCherry expression. 
b, CDX2 overexpression leads to the expansion of the transitional epithelium at the SCJ at the first week of Dox treatment. 

n = 3 per group. 
c, Dox treatment for four weeks promotes further expansion of the transitional epithelium at the SCJ and the epithelium presents MLE characteristics, with the co-expression of the columnar markers KRT8 and KRT17 and the squamous marker p63. Note that some basal cells start to lose p63 expression as they gain the expression of the AGR2 intestinal marker. 

n = 3 per group. 
d, Intestinal metaplasia is apparent after eight weeks of Dox treatment; some metaplastic cells lose p63 expression. 

n = 4 per group. Scale bars, 20 μm.
Extended Data Figure 7 | See next page for caption.
Extended Data Figure 7 | Ectopic CDX2 expression promotes intestinal metaplasia of the transitional epithelium. a, Intestinal metaplasia occurs in Krt5rtTA;otet-CDX2 mutants given Dox water for three months, as shown by PAS staining. n = 5 per group. b, Goblet cells enriched with vesicles (asterisks) are present in the SCJ of Krt5rtTA;otet-CDX2 mutants after three months of Dox treatment, as shown by electron microscope. n = 3 per group. c, KRT7 is expressed by both Barrett’s oesophagus and MLE but not the neighbouring squamous epithelium. Residual CDX2 is expressed in a subpopulation of the metaplastic columnar cells, although KRT5 expression is not detectable. n = 5 per group. d, Reduced expression of basal cell genes (p63 and Krt5) and increased expression of the intestinal genes (Vil1, Agr2, Muc2, Muc4 and Tff3) during intestinalization of the SCJ after CDX2 overexpression. Not significant (ns), P > 0.05; *P < 0.05; **P < 0.001; two-tailed Student’s t-test and n = 3 independent experiments. e, Normal SCJ structure is maintained in control mice (otet-CDX2-T2A-mCherry) following three months of Dox treatment. n = 5. f, CDX2 overexpression does not promote columnar metaplasia in the oesophagus and forestomach. Squamous hyperplasia is present in the forestomach (dotted black line). Note that CDX2 expression does not induce ectopic expression of KRT7 in the stratified squamous epithelium. n = 5. G, goblet; N, nucleus. Scale bars, 20 μm.
Extended Data Figure 8 | Intestinal metaplasia is maintained even after withdrawal of Dox in Krt5\textsuperscript{TA};\texttt{tet-CDX2} mutants. 

a, Severe metaplasia develops at the SCJ of mutants that have been treated with Dox for the first 13 weeks and chased for another 11 weeks. \( n = 3 \) per group. 
b, The metaplastic cells remain at the SCJ even after withdrawal of Dox water. The metaplastic cells express KRT7 and KRT8. Note that mCherry, which indicates CDX2 expression, is not detected. \( n = 3 \) per group. 
c, Metaplastic cells maintain AGR2 and Villin 1 expression. \( n = 3 \) per group. Scale bars, 20\( \mu \text{m} \).
Extended Data Figure 9 | Ectopic CDX2 expression promotes intestinal metaplasia of the transitional epithelium in Krt7CreER;R26rtTA;otet-CDX2-T2A-mCherry mutants. a, Induction of CDX2 overexpression by combined treatment of tamoxifen and doxycycline. b, Thirty days of CDX2 expression drives the differentiation of the transitional basal cells into intestinal-like epithelium, including goblet cells, in Krt7CreER;R26rtTA;otet-CDX2-T2A-mCherry mice. n = 3 per group. c, Ninety days of CDX2 overexpression leads to prominent intestinal metaplasia of the transitional basal progenitor cells, as indicated by intestinal markers. n = 4 per group. Scale bars, 20 μm.
Extended Data Figure 10 | Intestinal metaplasia develops in air–liquid interface culture of the transitional (p63+KRT7+) but not the squamous (p63+KRT7−) basal progenitor cells on CDX2 expression. a, Dox-induced CDX2 expression. b, Intestinal metaplasia occurs in Dox-treated transitional (p63+KRT7+), but not squamous basal (p63+KRT7−), progenitor cells. Note that p63+KRT7− basal cells generate a thick keratin layer in the air–liquid interface culture and the differentiated cells express loricrin. n = 5 per group. Scale bars, 20 μm.
Extended Data Figure 11 | See next page for caption.
Extended Data Figure 11 | Different responses to CDX2 overexpression in transitional (p63+ KRT7+) and squamous (p63+ KRT7−) basal progenitor cells in vitro. Human and mouse MLE presents similar gene expression. a, Two distinct basal progenitor populations (p63+ KRT7 and p63+ KRT7−) are present at the human SCJ, as indicated by flow cytometric analysis. n = 3 independent experiments. b, Induction of CDX2 overexpression with Dox treatment of CDX2 virus-infected human SCJ basal progenitor cells. c, CDX2 overexpression promotes intestinal metaplasia of p63+ KRT7− cells. The metaplastic columnar cells are PAS+ and express Villin 1, Muc2 and TFF3. n = 6 per group. d, Ectopic CDX2 expression does not promote intestinal metaplasia of the stratified squamous epithelium in organoids formed by p63− KRT7− squamous basal cells. n = 4 per group. e, The transitional epithelium with underlying basal cells is considerably expanded in patients with long-term gastro-oesophageal acid reflux. Dotted lines indicate the basement membrane. n = 3. f, The transitional epithelium with basal cells is amplified in Barrett’s oesophagus mixed with MLE. n = 5. g, Similar phenotypic presentations of human MLE and mouse MLE developed at the SCJ following CDX2 overexpression and oesophageal–duodenal anastomosis surgery. Human MLE, n = 10; Krt5rtTA; tet-CDX2 mutant mice, n = 5; surgical mice, n = 5. h, Goblet cells in human Barrett’s oesophagus are positive for Alcian blue and PAS staining. Barrett’s epithelium loses the expression of KRT5 and p63 but maintains the expression of KRT7. Note that Barrett’s oesophagus gains CDX2, MUC2, AGR2 and Villin 1 expression. n = 12. Scale bars, 20 μm.
**Experimental design**

1. **Sample size**
   - Describe how sample size was determined.
   - The sample size estimate is based on our previous studies. We analyzed 7-10 animals for each group.

2. **Data exclusions**
   - Describe any data exclusions.
   - We did not exclude data.

3. **Replication**
   - Describe whether the experimental findings were reliably reproduced.
   - Each experiment were replicated at least 3 times and the results were reliably reproduced.

4. **Randomization**
   - Describe how samples/organisms/participants were allocated into experimental groups.
   - We did not use randomization.

5. **Blinding**
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - No blinding was done

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. **Statistical parameters**
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   | n/a | Confirmed |
   |-----|-----------|
   | ☒   | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
   | ☒   | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
   | ☒   | A statement indicating how many times each experiment was replicated |
   | ☒   | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
   | ☒   | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
   | ☒   | The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted |
   | ☒   | A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
   | ☒   | Clearly defined error bars |

See the web collection on statistics for biologists for further resources and guidance.
Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

We use Carl Zeiss Zen Software to analyze images; Graphpad Prism 6 to analyze statistical data; DNASTAR Lasergene Software to design primers; BD FACSDiva to collect data and Flowjo to analyze flow cytometry data.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No material is restricted.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The primary antibodies used for immunostaining analysis include: rabbit anti-p63 (1:200, sc-8343, Santa Cruz Biotechnology); mouse anti-p63 (1:500, MAB4135, Millipore); chicken anti-KRT8 (1:1000, ab107115, Abcam); chicken anti-GFP (1:1000, GFP-1020, Aves Labs); rabbit anti-SOX2 (1:500, WRAB-1236, Seven Hills); mouse anti-KRT7 (1:200, Ab9021, Abcam); rabbit anti-CLDN18 (1:1000, ab203563, Abcam); chicken anti-KRT5 (1:1000, 905901, Biogenlend); mouse anti-Ki67 (1:200, 550609, BD Biosciences); mouse anti-Involucrin (1:1000, MS-126-P0; Thermo Fisher Scientific); rabbit anti-Loricrin (1:2000, 905101, Biogenlend); rabbit anti-RFP (1:100, ab62341, Abcam); rabbit anti-CDX2 (1:500, ab76541, Abcam); mouse anti-CDX2 (1:500, ab157524, Abcam); rabbit anti-Villin1 (1:200, 16488-1-AP, Protein Group); rabbit anti-AGR2 (1:200, PAS-34517, Thermo Fisher Scientific); rabbit anti-Muc2 (1:200, sc15334, Santa Cruz Biotechnology); rabbit anti-TFF3 (1:200, PAB656, Cloud-Clone Corporation).

The secondary antibodies used for immunostaining analysis include: donkey 488 anti-rabbit (1:500, 111-545-047, Jackson laboratory); donkey cy3 anti-mouse (1:500, 115-165-66, Jackson laboratory); donkey cy5 anti-chick (1:500, A-21499, Invitrogen); donkey cy5 anti-rat (1:500, 712-175-150, Jackson laboratory); donkey biotin anti-rabbit (1:500, 711-066-152, Jackson laboratory); donkey biotin anti-mouse (1:500, 715-065-150, Jackson laboratory).

The antibodies used for flow cytometry analysis include: FITC conjugated mouse anti-p75 (1:10, ab62122, Abcam), Alexa Fluor 647 conjugated mouse anti-KRT7 (1:50, sc-23876, Santa Cruz Biotechnology), rabbit anti-p63 (1:200, sc-8343, Santa Cruz Biotechnology).

The staining process was followed by manufacture’s instruction and the antibodies are validated using positive controls provided by the manufactures.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Eukaryotic cell lines were not used in this study.

b. Describe the method of cell line authentication used.

Eukaryotic cell lines were not used in this study.

c. Report whether the cell lines were tested for mycoplasma contamination.

Eukaryotic cell lines were not used in this study.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

Eukaryotic cell lines were not used in this study.
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

We use Krt5-CreER, Krt5-rtTA, R26lox-p-stop-loxp-Sox2-IRES-GFP, Lgr5-CreER, R26tdTomato, R26-LacZ, R26-rtTA, p63-CreER, otet-CDX2-T2A-mCherry and Krt7-CreER mouse strains and maintained on a C57BL/6 and 129SvEv mixed background. Sex includes male and female. We used adults (8 weeks old) and embryos (E11.5, E12.5, E14.5, E16.5 and E18.5).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Human SCJ biopsies were provided by the Columbia University department of Medicine under IRB approval at Columbia University. Slides containing human samples were provided by the Department of Pathology at the University of Rochester. Human sample include both male and female. The age of human patients are from 30 to 80 years old. Human patients include normal, MLE and Barrett’s oesophagus. The age of patients is from 30 to 80 years old. The sex of the patients are both male and female. The patients with normal, MLE and Barrett’s oesophagus diseases are included.
Flow Cytometry Reporting Summary

For all flow cytometry data, confirm that:

1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
3. All plots are contour plots with outliers or pseudocolor plots.
4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation. Mouse or human SCJ tissues are incubated with Dispase to remove mesenchyme and digested to single cells using Trypsin.
6. Identify the instrument used for data collection. We use BD Influx, BD LSRII and BD FACS Canto to collect data.
7. Describe the software used to collect and analyze the flow cytometry data. We use BD FACSDiva to collect data and flowJo to analyze the data.
8. Describe the abundance of the relevant cell populations within post-sort fractions. For mouse SCJ flow cytometric analysis, 16.3% of the total cell population are p75+ basal progenitor cells which include 84.7% p63+KRT7- and 13.3% p63+KRT7+ population. For human SCJ preparation, 10.9% of total cell preparation are p75+ basal progenitor cells which include 94.6% p63+KRT7- and 5.02% p63+KRT7+ population.
9. Describe the gating strategy used. In sample gating, cells were first gated for debris (SSC-A Vs SSC-A) and single cells (FCS-W Vs FSC-A). The single cell gate is further analyzed by live/dead staining to determine live cells vs dead cells, and the cell surface marker p75 only picks p75+ cells. The expression of p63 and KRT7 was then determined from the gated population.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☐