Original research

Iqgap3-Ras axis drives stem cell proliferation in the stomach corpus during homeostasis and repair

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ABSTRACT

Objective Tissue stem cells are central regulators of organ homeostasis. We looked for a protein that is exclusively expressed and functionally involved in stem cell activity in rapidly proliferating isthmus stem cells in the stomach corpus.

Design We uncovered the specific expression of Iqgap3 in proliferating isthmus stem cells through immunofluorescence and in situ hybridisation. We performed lineage tracing and transcriptomic analysis of Iqgap3+ isthmus stem cells with the Iqgap3-2A-tdtTomato mouse model. Depletion of Iqgap3 revealed its functional importance in maintenance and proliferation of stem cells. We further studied Iqgap3 expression and the associated gene expression changes during tissue repair after tamoxifen-induced damage. Immunohistochemistry revealed elevated expression of Iqgap3 in proliferating regions of gastric tumours from patient samples.

Results Iqgap3 is a highly specific marker of proliferating isthmus stem cells during homeostasis. Iqgap3+ isthmus stem cells give rise to major cell types of the corpus unit. Iqgap3 expression is essential for the maintenance of stem potential. The Ras pathway is a critical partner of Iqgap3 in promoting strong proliferation in isthmus stem cells. The robust induction of Iqgap3 expression following tissue damage indicates an active role for Iqgap3 in tissue regeneration.

Conclusion Iqgap3 is a major regulator of stomach epithelial tissue homeostasis and repair. The upregulation of Iqgap3 in gastric cancer suggests that Iqgap3 plays an important role in cancer cell proliferation.

INTRODUCTION

Characterisation of the mechanisms underlying the proliferation, plasticity and lineage commitment of adult stem cells is necessary for understanding the preneoplastic events leading to gastric cancer. In the stomach corpus, the gastric gland can be subdivided into four zones: the pit, the isthmus, the neck and the base. Classical radioactive tracer experiments indicated rapidly proliferating cells in the isthmus, which were revealed by electron microscopy to be granule-free stem cells. The isthmus region is therefore accepted to be the active stem cell zone of the corpus. Zymogenic chief cells located at the base of the stomach corpus are fully differentiated, postmitotic cells. Interestingly, in 2010, various studies reported that chief cells acquire proliferative capacity, suggesting that

Significance of this study

What is already known on this subject?
- Highly proliferative isthmus stem cells in the stomach corpus are multipotent.
- Iqgap3 is necessary and sufficient for cell proliferation.

What are the new findings?
- Identification of cytoskeletal scaffold protein Iqgap3 as a specific marker for actively cycling isthmus stem cells.
- Iqgap3 is required for promoting Ras-driven proliferation of isthmus stem cells.
- Iqgap3 is required for the maintenance of the stem cell transcriptional programme.
- Robust induction of Iqgap3 during tissue repair is associated with expansion of isthmus stem cells and dedifferentiation of chief cells.
- Tissue repair is accompanied by gene expression signatures associated with carcinogenesis. The commonality of increased Iqgap3 expression in tissue repair and cancer potentially indicates a driver role for Iqgap3 in ‘a wound that does not heal’.

How might it impact on clinical practice in the foreseeable future?
- Identification of Iqgap3 as a specific marker for rapidly proliferating isthmus stem cells and cancer cells may provide molecular insights to proliferation of gastric cancer stem cells.
- Robust induction of Iqgap3 during tissue repair indicates a potential mechanism for gastric cancer initiation.
chief cells may be progenitors for the preneoplastic spasmolytic polypeptide-expressing metaplasia.\textsuperscript{1,2} In 2013, Troy+chief cells were reported to serve as quiescent stem cells, which can be induced to proliferate during tissue damage to regenerate the entire gastric unit.\textsuperscript{3} A different view emerged in 2015, when Mist1+quiescent stem cells were identified in the isthmus and proposed to be the origin of gastric cancer. Notwithstanding this finding, Choi \textit{et al} used targeted expression of oncogenic Kras\textsuperscript{5,6} in Mist1+chief cells to contend that metaplasia arises from transformation or dedifferentiation of chief cells.\textsuperscript{3} Leushacke \textit{et al} identified the expression of established stem cell factor Lgr5 in a subset of chief cells and observed that Kras(G12D) induction in Lgr5+chief cells, in the presence of injury, led to metaplastic lesions. Together with the analysis of Lgr5-expressing human gastric cancer tissues, they proposed that gastric cancer originated from Lgr5-expressing chief cells.\textsuperscript{8}

In 2017, we uncovered strong activity of an enhancer element of Runx1 (eR1), a haematopoietic and human hair follicle stem cell factor, in proliferating isthmus stem cells, as well as a small number of chief cells.\textsuperscript{9} Kras\textsuperscript{G12D} expression in eR1+isthmus stem cells induced foveolar hyperplasia and elimination of parietal cells.\textsuperscript{3} However, Kinoshita \textit{et al} detected induction of Lgr5 in isthmus stem cells after tissue injury and proposed that isthmus stem cells and cells from the neck lineage, instead of chief cells, contributed to metaplasia.\textsuperscript{10} Arguing that the contradictory data may have stemmed from the specificity of the chief cell markers, Hata \textit{et al} identified Gpr30 as a specific marker of chief cells, with no expression in isthmus stem cells.\textsuperscript{11} Expression of Kras\textsuperscript{G12D} in Gpr30+chief cells did not result in metaplasia but, instead, contributed to chief cell population and a compensatory expansion of neck lineage, derived from Kitl+isthmus stem cells.\textsuperscript{3} It was further argued that since most chief cells are lost during metaplasia, they are unlikely to be involved in gastric carcinogenesis.\textsuperscript{11} Certainly, after acute oxyntic injury, the increased activity of Lrig1-expressing isthmus stem cells has been shown to contribute to tissue regeneration.\textsuperscript{12} During Helicobacter pylori-induced chronic injury, it was reported that increased proliferation and accelerated differentiation of Lrig1-expressing cells gave rise to surface mucous cells and chief cells, which subsequently produced spasmolytic polypeptide-expressing metaplasia.\textsuperscript{11} The origin of metaplasia continues to be highly debated, in part due to the expression of some markers in both isthmus and chief cell populations, progenitor cell plasticity, effects from stem cell niche as well as injury.

Two models have been suggested for the homoeostasis of the corpus: (1) long-lived stem cells supported by the stem cell niche at the isthmus zone is proposed to maintain the gastric epithelium and, however, many of these markers were expressed in other cell types and, thus not specific for proliferating isthmus stem cells;\textsuperscript{13} and (2) two independent stem cell populations potentially maintain the corpus—the rapidly cycling isthmus stem cells support the pit, isthmus and neck regions, while the mostly quiescent chief cells support the base region.\textsuperscript{14-16}

The study of isthmus stem cell behaviour will provide insights to the molecular mechanism underlying cancer initiation. Sox2, Lrig1, TFF2, Mist1, eR1, Bmi1 and Kitl have all been reported to mark stem/progenitor cells in the isthmus; however, many of these markers were expressed in other cell types and, thus not specific for proliferating isthmus stem cells.\textsuperscript{16,17,11,12,16,18} Actively cycling isthmus stem cells were also studied via their expression of proliferation markers Stathmin1 (Stmn1) and Mki67.\textsuperscript{19,20} Several signalling pathways, such as Notch, Sonic hedgehog and AMP-dependent protein kinase, were reported to govern proliferation of gastric epithelial cells.\textsuperscript{19-21} In particular, isthmus stem cell proliferation was found to be driven by the Notch pathway.\textsuperscript{22} Yet, these studies did not reveal the presence of an exclusive and definitive stem cell marker in proliferative isthmus stem cells.

In this study, we show that eR1+isthmus stem cell proliferation is driven by Iqgap3, a member of the Iqgap (IQ motif containing GTPase activating protein) cytoskeletal scaffold family that was reported to be necessary and sufficient for cell proliferation.\textsuperscript{23} Knockdown of Iqgap3 in undifferentiated cell lines, such as embryonal carcinoma NTERA-2 and gastric carcinoma HGC-27, resulted in reduction of stem cell-associated NANOg and OCT4 gene expression, as well as induction of differentiation. Therefore, Iqgap3 represents a functionally indispensable stem cell specific factor, which regulates stem cell function in homoeostasis and tissue damage repair. Our work further revealed that Iqgap3 induction during tissue repair is associated with the acquisition of oncogenic traits. Finally, we found a strong correlation between Iqgap3 expression and proliferating gastric cancer cells in tumours isolated from gastric cancer patients. Together, these data reveal a mechanism by which Ras is hyperactivated in neoplasia and identify a role for Iqgap3 in lineage plasticity and proliferation in cancer initiation.

**MATERIAL AND METHODS**

**Mice and treatment**

Wild-type (WT) C57BL/6JInv mice were obtained from InVivos Pte Ltd. The eR1-enhanced green fluorescent protein mice and the eR1-CreERT2;Rosa-Lox-Stop-Lox (LSL)-tdTomato (eR1-CreERT2;Rosa-tdTomato) mice were described previously.\textsuperscript{2,24} To induce tdTomato expression in eR1-CreERT2;Rosa-tdTomato mice for short lineage tracing experiments, 6–8 weeks old mice were given a single intraperitoneal injection of 2mg of tamoxifen (Merck) diluted in corn oil (Merck). The mice were analysed at 16 or 24 hours post-tamoxifen injection. Iqgap3-2A-tdTomato mice were generated to monitor Iqgap3 expression in the stomach (Cyagen). The 2A-tdTomato sequence was inserted before the stop codon in exon 38 (online supplemental figure S1A). Iqgap3-2A-CreERT2 mice were generated for inducing or repressing expression of the gene of interest in Iqgap3+cells (Cyagen). The 2A-CreERT2 sequence was inserted before the stop codon in exon 38 (online supplemental figure S2A). Iqgap3-2A-CreERT2 mice were bred to Rosa-tdTomato mice to generate Iqgap3-2A-CreERT2;Rosa-tdTomato mice. To induce tdTomato expression, 6–8 weeks old mice were given a single intraperitoneal injection of 2mg of tamoxifen. The mice were analysed 1-day, 3-month, 6-month and 1-year post-tamoxifen injections. Iqgap3-2A-CreERT2 mice were also bred to LSL-Kras\textsuperscript{G12D} (Kras\textsuperscript{G12D}+/−) mice to generate Iqgap3-2A-CreERT2;Kras\textsuperscript{G12D}+/− mice. To induce Kras\textsuperscript{G12D} expression, 6–8 weeks old mice were given a single intraperitoneal injection of 2mg of tamoxifen. The mice were analysed 3 months post-tamoxifen injection.

To induce tissue damage in the murine stomach, 6–8 weeks mice were given a single intraperitoneal injection of a high dose of tamoxifen (HDT) (5mg per 20g body weight). The mice were analysed at indicated time points post-tamoxifen injection.

**RESULTS**

**Iqgap3 is specifically expressed in rapidly proliferating isthmus stem cells**

Using immunofluorescence staining and RNA in situ hybridisation (ISH), we found that Iqgap3 is strongly coexpressed with the proliferation marker Ki67 (figure 1A–D), thereby identifying Iqgap3 as a marker of rapidly proliferating cells at the isthmus zone. Using an Iqgap3-2A-tdTomato mouse, we found...
Figure 1  Expression of Iqgap3 in the isthmus of corpus epithelium. (A) Schematic diagram of a gastric unit in the corpus of the mouse stomach. (B) Immunofluorescence (IF) staining for Iqgap3, Ki67 and E-cadherin (E-cad) on the corpus of wild-type (WT) mouse stomach (n=3). (C) Quantification of Iqgap3+ cells in isthmus or base (n=3). Error bar represents SD from 2025 Iqgap3+ cells of 3 mice. The Ki67+ region was defined as the isthmus zone. (D) In situ hybridisation (ISH) for Iqgap3 (green) and Mki67 (red) on the corpus of WT mice (n=2). (E) IF staining for tdTomato and Ki67 on the corpus of Iqgap3-2A-tdTomato reporter mice (n=3). (F) Flow cytometry to isolate tdTomato/Iqgap3 high expression epithelial cell fraction (Iqgap3 high) and tdTomato/Iqgap3 low or negative expression epithelial cell fraction (Iqgap3 low/neg) from stomach of Iqgap3-2A-tdTomato reporter mice (n=5). (G) qPCR for Cdh1, tdTomato, Iqgap3, Mki67, Stathmin1 (Stmn1), Lgr5, Bhlha15 (Mist1) and Tnfrsf19 (Troy) mRNA from isolated tdTomato high (Iqgap3 high) and tdTomato low/neg (Iqgap3 low/neg) gastric epithelial cells. mRNA expression was normalised by Gapdh expression (n=3). Error bars represent SD scale bar=100 µm. qPCR, quantitative PCR.
robust tdTomato expression in Ki67+ cells at the isthmus (figure 1E; online supplemental figure S1A,B). We next isolated Iqgap3+tdTomatohigh EpCAMhigh epithelial cells by flow cytometry (figure 1F). Quantitative PCR (qPCR) showed that tdTomato, Iqgap3 and Mki67 mRNA were strongly enriched in the Iqgap3+tdTomatohighEpCAMhigh epithelial cells, relative to the Iqgap3+tdTomato+low/neg population (figure 1G, upper panel). Strong enrichment of Stm1 mRNA—previously shown to mark cycling ishthmus stem cells—further confirmed the proliferative nature of Iqgap3+tdTomatohigh cells (figure 1G, lower panel). Lgr5 and Troy expression were low in Iqgap3+tdTomatohigh cells, suggesting that Iqgap3+ cells are stem cells distinct from Lgr5+cells (figure 1G, lower panel). Similarly, Mist1 expression—earlier reported to mark chief cells and quiescent isshthmus stem cells in the corpus—was relatively low in Iqgap3+ tdTomatohigh cells (figure 1G, lower panel). We had previously shown that the activity of the Ruxn1 enhancer element, eR1, could be used to identify adult stem cells in stomach tissues.9 eR1-CreERT2;Rosa-tdTomato mice were treated with tamoxifen to examine eR1+and Iqgap3 + cells in vivo. After 16 hours of short lineage tracing, eR1+cells partially coincided with Iqgap3-expressing cells at the isthmus, likely because of the variegated expression intrinsic to the mouse model (online supplemental figure S1C). This indicated that Iqgap3+cells harbour isshthmus stem cells.

The Igap family comprises three genes, namely Igap1, 2 and 3.25 Unlike Igap3, the expression of Igap1 and 2 were not confined to the isthmus, but observed throughout the gastric unit (online supplemental figure S1D–G). Therefore, Igap3—alone in the Igap family—plays a unique role in driving proliferation of isshthmus stem cells.

Iqgap3+ isthmus stem cells are multipotent and responsible for core homeostasis of corpus glands

We next used Iqgap3-2A-CreERT2;Rosa-tdTomato mice to perform lineage tracing of Iqgap3+ cells (figure 2A,B; online supplemental figure S2A,B). One-day post-tamoxifen injection revealed strong coexpression of Ki67 and tdTomato at the isthmus (figure 2C; online supplemental figure S2B); at 3-month, 6-month and 1-year post-tamoxifen injection, we observed progressive expansion of tdTomato+cells that span almost the entire gastric gland (figure 2C). tdTomato expression overlapped with markers of various corpus lineages, including mucous pit, neck, parietal and chief cells (figures 1A and 2D,E). Analysis of the lineage tracing events at 6-month post-tamoxifen injection revealed that ~50% of the glands showed tdTomato positivity spanning from the pit to neck region, while ~40% showed tdTomato positivity from the pit to the transition regions (see #1 and #2 in figure 2F). About 10% of the glands showed tdTomato+cells spanning from the pit to base region (see #3 in figure 2F). At 1-year post-tamoxifen injection, tdTomato positivity spanning the pit to base region had increased to ~55% of the glands (figure 2F). XZ-plane imaging further confirmed tdTomato+ cells in the neck (GS-II+/Gif–), transition (GS-II+/Gif+) and chief cell populations (GS-II–/Gif+) (online supplemental figure S2C). The self-replicating nature of chief cells may be the reason why not all chief cells were labelled with tdTomato, even after 1 year of tracing. Nevertheless, our time course data indicated that lineage tracing initiated at the isthmus and that tdTomato+ cells are multipotent stem cells.

dTomato+ isthmus cells from corpus units isolated from Iqgap3-2A-CreERT2;Rosa-tdTomato mice readily formed organoids, further confirming the stem potential of Iqgap3+ isthmus cells (figure 2G). Induction of differentiation via removal of Wnt3a and R-spondin1 from the organoid culture media (termed ENFG as opposed to WENFRG)26 led to decreased Iqgap3 mRNA, accompanied by a sharp increase in differentiation marker Muc5ac (online supplemental figure S2D,E). Iqgap3 expression was therefore specific to stem cells and shows rapid reduction on differentiation in ENFG media. Iqgap3+tdTomato+ organoids showed upregulation of Muc5ac protein on differentiation (online supplemental figure S2F). The organoids derived from Iqgap3+cells possessed PgC+/GS–II+, PgC+/ GS–II– and PgC–/GS–II+ cells, as well as Gif+/GS–II+ and Gif+/ GS–II– cells, demonstrating that Iqgap3+ isthmus cells differentiated to the chief cell, mucus-neck cell and transition cell lineages in our organoid culture system (online supplemental figure S2G,H). In addition, the Iqgap3+ cell-derived organoids also showed expression of Ki67, but not H, K-ATPase (online supplemental figure S2J). Iqgap3+tdTomato+ cells therefore generate pit, neck and chief cells in vivo.

To understand the function of Iqgap3 in maintaining stemness, we performed siRNA-mediated knockdown of Iqgap3 in a well-established model for pluripotency and differentiation, the human embryonal carcinoma NTERA-2 cell line. Depletion of Iqgap3 led to significant reductions in mRNA and protein expression levels of stem cell factors such as NANOG, OCT4 and KLF4 (figure 2H,I). We also observed an increase of the gli fibrillar acidic protein, which is expressed on differentiation to astrocytes (figure 2H,I). This proof-of-concept experiment indicates that Iqgap3 is necessary for the maintenance of stem cell gene expression signature and not just a marker for proliferation.

Iqgap3 drives stem cell proliferation by promoting the Ras-extracellular signal-regulated kinase signalling pathway

To identify the transcriptional programmes integral to the isthmus stem cell state, we next isolated Iqgap3+tdTomatohigh and Iqgap3+tdTomato+low/neg expressing cells by flow cytometry (figure 3A). RNA-sequencing revealed that Iqgap3+tdTomatohigh cells were highly enriched in Iqgap3, Mki67, Mist1, Kitl mRNA, while showing low expression levels of chief cell markers PgC and Gpr30 (online supplemental figure S3A). Gene set enrichment analysis (GSEA) confirmed that Iqgap3+tdTomatohigh stem cells are observed from Lgr5 + cells (figure 3B; online supplemental figure S3B). The transcriptional profile of Iqgap3+tdTomatohigh cells mapped closely with the short-term haematopoietic stem cell gene signature ST-HSIC (online supplemental figure S3C,D). The enriched expression of HSC self-renewal—and asymmetric cell division-associated genes in the Iqgap3+tdTomato+high fraction further reinforced the notion that Iqgap3+ cells possess stem cell properties (online supplemental figure S3E). Notch, Hedgehog and Wnt pathways were not significantly upregulated (online supplemental figure S3F). Conversely, the strong upregulation of Myc-target, E2F-target and Ras signalling genes in Iqgap3+tdTomato+high cells identified these pathways as core programmes driving isthmus stem cell proliferation (figure 3C). Notably, key components of the Ras signalling pathway, namely Erbb2, Erbb3, Fgfr2, Fgfr3, Met and Ras, were enriched in Iqgap3+tdTomato+high stem cells (figure 3D).

We, therefore, investigated the activity of the Ras pathway in isthmus stem cells in vivo. Immunostaining showed that Hras, Nras and Kras were all expressed in the corpus, partially overlapping with Ki67 expression (figure 3E; online supplemental figure S4A,B). Her2 is known to promote Ras pathway activation.27 28 Immunostaining indicated the expression of Her2 and Ras downstream effector phosphorylated Erk (p-Erk) in the
The Iqgap3-expressing cells in the isthmus are multipotent stem cells. (A) Iqgap3-2A-CreERT2;Rosa-tdTmato mouse model. (B) Experimental strategy for lineage tracing time course. (C) IF staining for Ki67 and tdTomato on the corpus of Iqgap3-2A-CreERT2;Rosa-tdTmato mice at 1 day (1 d), 3 months (3 m), 6 months (6 m) and 1 year (1 y) post-tamoxifen induction (p.i.) (n=3). Lineage tracing, LT. (D, E) IF staining for tdTomato and markers of major stomach differentiated cells (Muc5ac, H,K-ATPase, GS-II and Gif) on the corpus of Iqgap3-2A-CreERT2;Rosa-tdTmato mice at 1 year post-tamoxifen induction (n=3). (F) Quantification of lineage tracing on the corpus of Iqgap3-2A-CreERT2;Rosa-tdTmato mice at 6 months and 1 year post-tamoxifen induction. (n=3). The tdTomato+ lineage tracing glands were categorised into pit to neck (LT #1: Pit - Neck), pit to mucus-neck/chief cell transition (LT #2: Pit-Transition) and pit to base (LT #3: Pit – Base). A total of 167 tdTomato+ glands from three mice (6 months) or 172 tdTomato+ glands from three mice (1 year) were counted. Error bars represent SD data sets were analysed by one-way ANOVA. ***P<0.001. (G) tdTomato expression in the isolated corpus gastric units from Iqgap3-2A-CreERT2;Rosa-tdTmato mice at 20–24 hours post-tamoxifen administration (top). Corpus organoids were generated from tdTomato + cells (n=2). (H) qPCR for IQGAP3, NANOG, OCT4, KLF4, cMYC, SOX2, CD44v9 and GFAP mRNA from IQGAP3 knockdown embryonic stem cell line NTERA-2. mRNA expression was normalised by GAPDH expression (n=3). Error bars represent SD data sets were analysed by Student’s t-test. *P<0.05, **P<0.01. (I) Immunoblot for IQGAP3, Nanog, Oct4, KLF4, CD44v9, GFAP and GAPDH from knockdown embryonic stem cell line NTERA-2 (n=3). Scale bar=100 µm. ANOVA, analysis of variance; GFAP, glial fibrillar acidic protein; IF, immunofluorescence; qPCR, quantitative PCR.
Figure 3  Iqgap3 regulates stem cell proliferation via Ras-ERK pathway. (A) Average of RNA expressions in sorted Iqgap3^{high} and Iqgap3^{low/NEG} cell fractions are shown. (B) Gene set enrichment analysis (GSEA) showing enrichment of Lgr5-negative (LGR5neg) and—high (LGR5pos) corpus epithelial cell gene signature from public datasets (GSE86603) in Iqgap3^{high} and Iqgap3^{low/NEG} cell fractions. P values determined by a weighted Kolmogorov–Smirnov-like statistic and adjusted for multiple hypothesis testing. (C) GSEA showing enrichment of Myc target gene signature, E2F targets gene signature, Ras pathway gene signature in Iqgap3^{high} and Iqgap3^{low/NEG} cell fractions. (D) Heat MAP showing expression of representative Ras-ERK pathway genes in Iqgap3^{high} and Iqgap3^{low/NEG} cell fractions. Gene expression levels are shown in Z-score of CPM of RNA-sequencing. (E–G) IF staining for HRAS, Ki67, E-cad, HER2 and phosphorylated ERK (p-Erk) on the corpus of wild-type (WT) mice (n=3). (H) Immunoprecipitation for the interaction of Venus-tagged Iqgap3 (Venus-Iqgap3) with Myc-tagged HRAS (Myc-Hras) or Myc-tagged HrasG12V (Myc-HrasG12V) and immunoblot for cell lysate from Venus-Iqgap3/Myc-Hras/Myc-HrasG12V expressed 293T cells. (I) Experimental strategy to suppress Iqgap3 expression by shRNA in organoids from WT mice. (J) Quantification of size of Iqgap3 knockdown organoids from 0 passage (6 days post doxycycline (Dox) treatment, before passage), first passage (8 days postpassage) and second passage (7 days postpassage) (n=2). Error bars represent SD from each population. Data sets were analysed by one-way ANOVA. Scale bar=100 µm. ANOVA, analysis of variance; CPM, counts per million; ERK, extracellular signal-regulated kinase; NES, normalised enrichment score.
Ki67 + proliferating cells at the isthmus (figure 3FG). Her2\textsubscript{high} and Her2\textsubscript{low}-expressing cells isolated by flow cytometry revealed enrichment of ErbB2, Iqgap3 and Mki67 mRNA in Her2\textsubscript{high} cells, and elevated Lgr5 and Mist1 mRNA in Her2\textsubscript{low} cells (online supplemental figure S4C,D). Kras, Hras and Nras mRNA were detected in both Her2\textsubscript{high} and Her2\textsubscript{low} cells, with Kras and Hras showing significantly higher expression in Her2\textsubscript{high} cells (online supplemental figure S4E). Iqgap3 expression, therefore, positively correlates with the activity of Erk signalling cascade. Indeed, immunoprecipitation revealed that the interaction of Iqgap3 with the constitutively active Hras\textsubscript{G12V} mutant was associated with increased p-Erk in the total cell lysate (figure 3H).

To ascertain if the Iqgap3-Ras-Erk axis contributes to organoid growth, we subjected organoids to shRNA-mediated depletion of Iqgap3 (figure 3I). Depletion of Iqgap3 using three different shRNAs (online supplemental figure S5A) resulted in drastically smaller organoids with reduced ability to be serially passaged (figure 3J; online supplemental figure S5B-D). Corpus glands were treated with two different inhibitors of the Erk pathway, U0126 and MK-88635. Immunostaining of the organoids revealed that both inhibitors resulted in severe depletion of p-Erk (online supplemental figure S5E). After 96 and 72 hours, organoid growth was strongly inhibited in a concentration-dependent manner by U0126 and MK-88335, respectively (online supplemental figure S5F,G).

**HDT treatment promotes stem cell activity and neoplastic features**

Immunostaining of the injured tissue showed reduction of neck and chief cell markers, such as GS-II and PgC (figure 5A); mRNA of differentiated cell markers Atp4b, PgC, Gif, and Muc6 were reduced as well (figure 5B). HDT-associated depletion of chief cells has been reported previously.\

Taken together with the observations by Radyk et al., the decreased expression of chief cell markers in our work suggests that the injury-induced Iqgap3+K6i67+cells at the base were stem-like cells possibly derived from reprogramming of chief cells. Conversely, Hata et al. had reported that chief cells do not dedifferentiate after HDT and that the compensatory response from neck progenitors contributed to the replacement of chief cells. Our work indicated that two complementary events, namely the expansion of Iqgap3-expressing cells from the isthmus zone and the Iqgap3-associated dedifferentiation of chief cells to stem-like cells, accelerated tissue regeneration.

RNA-sequencing and GSEA of epithelial and non-epithelial cells from tissues isolated from HDT-treated mice revealed that mitogenic genes, E2F- and Myc-target genes were upregulated, reflecting increased proliferation state of the HDT-treated cells (online supplemental figure S7A,B). Myc expression itself was strongly upregulated (2.5-fold increase) after tissue damage (online supplemental figure S7B). Myc induction was reported to activate an embryonic stem cell (ESC)-like transcriptional signature associated with diverse epithelial cancers. We observed that the ESC-like transcriptional programme, together with transcriptional programmes associated with Kras transformation, early gastric cancer and inflammation were significantly upregulated after HDT-induced injury (figure 5C,D; online supplemental figure S7C,E). An earlier study by Leushacke et al. reported upregulation of matrix metalloproteinase-7 and downregulation of sclerostin domain containing 1 after injury, which they suggested might amplify Wnt signalling to drive tissue regeneration. While we detected upregulation of various Wnt-related genes (eg, Axin2, Notch1 and Myc), there was no statistically significant trend of Wnt activation (online supplemental figure S7D,E). Instead, our data suggest that a combination of dysregulated Myc and Kras signalling—driven by injury-induced Iqgap3 expression—contributed to a less differentiated state, and a more proliferative, stem-like and neoplastic phenotype. Importantly, HDT-treated tissues showed elevated p-Erk staining labelled by tdTomato, indicating expression of Iqgap3 in chief cells (figure 4H). qPCR showed that the induction of Mki67 mRNA reflected that of Iqgap3 during tissue injury, whereas Lgr5 showed moderate increase in expression (figure 4I).

A population of Ki67 + cells near the gland bottom, in the area outside of the epithelium (figure 4G). These CD45 + K6i67+E-cad-cells (online supplemental figure S6E,F) are CD45 + leukocytes, most likely macrophages as described previously. To investigate whether cells with robust expression of Iqgap3 are involved in the repair of HDT-damaged tissue, Iqgap3-2A-CeER\textsubscript{2};Rosa-tdTomato mice were treated with HDT. At 14 days post HDT, the tissue morphology, confined K6i67 staining at the isthmus, and regeneration of H, K-ATPase-producing parietal cells indicated near complete repair (figure 4J,K; online supplemental figure S6G). The regenerated gastric glands showed patches of tdTomato-labelling from the bottom to the top, suggesting that Iqgap3+cells, including Lgr5+chief cells, gave rise to the regenerated parietal cells as well as multiple lineages during repair (online supplemental figure S6G).

**Iqgap3 is strongly induced following tissue injury**

We next examined the expression of Iqgap3 during tissue injury. Parietal cell protonophores such as tamoxifen, DMP-777 and L635 have been used to study the development of metaplasia. Tamoxifen was reported to cause the back wash of acid into parietal cells, resulting in parietal cell death. In addition to induction of reversible atrophy, tamoxifen treatment has been associated with increased p-Erk in the total cell lysate (figure 3H).

To ascertain if the Iqgap3-Ras-Erk axis contributes to organoid growth, we subjected organoids to shRNA-mediated depletion of Iqgap3 (figure 3I). Depletion of Iqgap3 using three different shRNAs (online supplemental figure S5A) resulted in drastically smaller organoids with reduced ability to be serially passaged (figure 3J; online supplemental figure S5B-D). Corpus glands were treated with two different inhibitors of the Erk pathway, U0126 and MK-88635. Immunostaining of the organoids revealed that both inhibitors resulted in severe depletion of p-Erk (online supplemental figure S5E). After 96 and 72 hours, organoid growth was strongly inhibited in a concentration-dependent manner by U0126 and MK-88335, respectively (online supplemental figure S5F,G).

We investigated whether cells with robust expression of Iqgap3 are involved in the repair of HDT-damaged tissue, Iqgap3-2A-CeER\textsubscript{2};Rosa-tdTomato mice were treated with HDT. At 14 days post HDT, the tissue morphology, confined K6i67 staining at the isthmus, and regeneration of H, K-ATPase-producing parietal cells indicated near complete repair (figure 4J,K; online supplemental figure S6G). The regenerated gastric glands showed patches of tdTomato-labelling from the bottom to the top, suggesting that Iqgap3+cells, including Lgr5+chief cells, gave rise to the regenerated parietal cells as well as multiple lineages during repair (online supplemental figure S6G).

RNA-sequencing and GSEA of epithelial and non-epithelial cells from tissues isolated from HDT-treated mice revealed that mitogenic genes, E2F- and Myc-target genes were upregulated, reflecting increased proliferation state of the HDT-treated cells (online supplemental figure S7A,B). Myc expression itself was strongly upregulated (2.5-fold increase) after tissue damage (online supplemental figure S7B). Myc induction was reported to activate an embryonic stem cell (ESC)-like transcriptional signature associated with diverse epithelial cancers. We observed that the ESC-like transcriptional programme, together with transcriptional programmes associated with Kras transformation, early gastric cancer and inflammation were significantly upregulated after HDT-induced injury (figure 5C,D; online supplemental figure S7C,E). An earlier study by Leushacke et al. reported upregulation of matrix metalloproteinase-7 and downregulation of sclerostin domain containing 1 after injury, which they suggested might amplify Wnt signalling to drive tissue regeneration. While we detected upregulation of various Wnt-related genes (eg, Axin2, Notch1 and Myc), there was no statistically significant trend of Wnt activation (online supplemental figure S7D,E). Instead, our data suggest that a combination of dysregulated Myc and Kras signalling—driven by injury-induced Iqgap3 expression—contributed to a less differentiated state, and a more proliferative, stem-like and neoplastic phenotype. Importantly, HDT-treated tissues showed elevated p-Erk staining labelled by tdTomato, indicating expression of Iqgap3 in chief cells (figure 4H). qPCR showed that the induction of Mki67 mRNA reflected that of Iqgap3 during tissue injury, whereas Lgr5 showed moderate increase in expression (figure 4I). There was a population of Ki67+cells near the gland bottom, in the area outside of the epithelium (figure 4G). These CD45+K6i67+E-cad-cells (online supplemental figure S6E,F) are CD45+leucocytes, most likely macrophages as described previously. To investigate whether cells with robust expression of Iqgap3 are involved in the repair of HDT-damaged tissue, Iqgap3-2A-CeER\textsubscript{2};Rosa-tdTomato mice were treated with HDT. At 14 days post HDT, the tissue morphology, confined K6i67 staining at the isthmus, and regeneration of H, K-ATPase-producing parietal cells indicated near complete repair (figure 4J,K; online supplemental figure S6G). The regenerated gastric glands showed patches of tdTomato-labelling from the bottom to the top, suggesting that Iqgap3+cells, including Lgr5+chief cells, gave rise to the regenerated parietal cells as well as multiple lineages during repair (online supplemental figure S6G).

**Iqgap3 is strongly induced following tissue injury**

We next examined the expression of Iqgap3 during tissue injury. Parietal cell protonophores such as tamoxifen, DMP-777 and L635 have been used to study the development of metaplasia. Tamoxifen was reported to cause the back wash of acid into parietal cells, resulting in parietal cell death. In addition to induction of reversible atrophy, tamoxifen treatment has been associated with progenitor cell proliferation and metaplasia in the mouse stomach. Accordingly, an intraperitoneal injection of tamoxifen results in parietal cell death. In addition to induction of reversible atrophy, tamoxifen treatment has been associated with increased p-Erk in the total cell lysate (figure 3H).

To ascertain if the Iqgap3-Ras-Erk axis contributes to organoid growth, we subjected organoids to shRNA-mediated depletion of Iqgap3 (figure 3I). Depletion of Iqgap3 using three different shRNAs (online supplemental figure S5A) resulted in drastically smaller organoids with reduced ability to be serially passaged (figure 3J; online supplemental figure S5B-D). Corpus glands were treated with two different inhibitors of the Erk pathway, U0126 and MK-88635. Immunostaining of the organoids revealed that both inhibitors resulted in severe depletion of p-Erk (online supplemental figure S5E). After 96 and 72 hours, organoid growth was strongly inhibited in a concentration-dependent manner by U0126 and MK-88335, respectively (online supplemental figure S5F,G).
Figure 4  Iqgap3-expressing cells drive corpus epithelial regeneration post-tissue damage. (A) H&E staining on untreated and high dose tamoxifen (HDT) treated WT corpus at 48 hours post-tamoxifen administration. (B) IF staining for Ki67 and E-cad on untreated and 48 hours post-HDT treated WT corpus. (C–E) ISH for Iqgap3(green) and Lgr5(red) on untreated, 24 hours and 48 hours post-HDT treated WT corpus. Boxes indicate enlarged regions. (F) IF staining for Muc5ac and Ki67 on 48 hours post-HDT treated WT corpus. (G, H) IF staining for tdTomato, Ki67 and Gif on 48 hours post-HDT treated corpus from Iqgap3-2A-tdTomato mice. (I) qPCR for Mki67, Iqgap3 and Lgr5 from isolated WT corpus tissue of untreated and 48 hours post-HDT treated mice (n=3). Error bars represent SE of mean. Data were analysed by Student’s t-test. (J) H&E staining on HDT treated corpus at 14 days post-tamoxifen administration. (K) IF staining of Ki67, tdTomato and H,K-ATPase on 14 days post-HDT treated corpus of Iqgap3-2A-CreERT2,Rosa-tdTomato mice. Scale bars=50 µm. IF, immunofluorescence; ISH, in situ hybridisation; LT, lineage tracing; qPCR, quantitative PCR; WT, wild-type.
Figure 5  HDT-induced tissue damage promotes stem cell activity and neoplastic characteristics. (A) IF staining for PGc, Ki67 and GS-II on untreated and 48 hours post-HDT treated WT corpus. (B) qPCR for Atp4b, Pgc, Gif, Muc6 and Chga from isolated corpus tissue of untreated or 48 hours post-HDT treated WT mice (n=4, expressed as Log2 scale). Data were analysed by Student’s t-test. *P<0.05. NS, not significant. (C) GSEA showing enrichment of embryonic stem cell gene signature, early gastric cancer gene signature and neoplastic transformation KRAS gene signature in 48 hours post-HDT treated WT corpus (n=2). P values determined by a weighted Kolmogorov-Smirnov-like statistic and adjusted for multiple hypothesis testing. (D) Heat MAP showing top 20 genes upregulated in (C) based on RNA-sequencing data from untreated and 48 hours post-HDT corpus tissue. (E) IF staining for p-Erk, Ki67 and E-cad on 48 hours post-HDT treated WT corpus. (F) Experimental strategy to generate corpus organoids from HDT treated mice. (G) Microscopic image of corpus organoids derived from untreated and 48 hours post-HDT-treated WT mice. (H, I) Organoid growth efficiency and diameter of corpus organoids derived from untreated or 48 hours post-HDT treated WT mice at 7 days of organoid culture (n=3). Data were analysed by Student’s t-test. Scale bars=50 µm (A), 100 µm (E), 500 µm (G). Error bars represent SEM. GSEA, gene set enrichment analysis; HDT, high dose of tamoxifen; IF, immunofluorescence; p-Erk, phosphorylated extracellular signal-regulated kinase; qPCR, quantitative PCR; SEM, SE of mean; WT, wild-type.
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at Ki67 enriched regions, thereby confirming that Ras activation is associated with proliferation during tissue repair (figure 5E). The increased efficiency of organoid formation following injury further confirmed the increased stemness and proliferative potential of the regenerating cells (figure 5F–I).

**Elevated Iqgap3 expression is associated with proliferation in gastric cancer**

The *Igap3-2A-CreERT2;KrasG12D/+* mouse model was used to investigate the effects of oncogenic Ras activation in Iqgap3 + cells (figure 6A,B). Three months after tamoxifen injection to induce *Kras* expression, we observed the development of pseudopyloric metaplasia, which was characterised by a massive glandular tissue (figure 6C–E). The Ki67 + proliferative cell zone was restricted to the lower neck zone (figure 6F). We observed cancer stem cell marker CD44v10 expression at the base of the metaplastic gland (figure 6G). Pdx1, frequently expressed in pseudopyloric glands and intestinal metaplasia, was also detected in corpus units with oncogenic Ras signalling (figure 6H). We also note the induction of Tif2 throughout the aberrant gland (compare figure 6L with the U-shaped Muc5ac staining pattern in figure 6E). The induced characteristics of the *Igap3-2A-CreERT2;KrasG12D/+* mouse model resembled to an extent, Menetrier’s disease. Menetrier’s disease has been attributed to TGFβ and receptor tyrosine kinase RTK EGFR. It could be that the specific expression of hyperactivated Ras in isthmus stem cells elicited features that mimic Menetrier’s disease, which has been tenuously linked to gastric cancer. Together, our findings suggest that induction of oncogenic signalling in Iqgap3 + cells can give rise to hyperproliferative disorders and perhaps, preneoplastic lesions.

We, therefore, investigated the expression of IQGAP3 in human cancer. Similar to the mouse, we detected coexpression of IQGAP3 with Ki67 at the isthmus of the normal human stomach corpus (figure 7A). Microarray and RNA-sequencing showed significantly higher IQGAP3 expression levels in gastric cancer tissues obtained from Singapore patients (figure 7B). Tissue microarray analysis of gastric tumour tissues showed that although the tumour and adjacent normal tissues showed IQGAP3 expression, IQGAP3 levels were specifically elevated in neoplastic regions, relative to the adjacent normal tissue (figure 7C; online supplemental figure S8A,B). IQGAP3 expression was elevated in intestinal and mixed gastric cancers (figure 7D). We examined the effects of depleting IQGAP3 in the undifferentiated human gastric carcinoma cell line HGC-27. siRNA-mediated knockdown of IQGAP3 in HGC-27 resulted in drastic reduction of stem cell factors such as NANOG, OCT4, SOX2 and an increase of chief cell marker PGC (see mRNA and protein levels in online supplemental figure S8C,D). Expectedly, KLF4 was significantly increased following IQGAP3 knockdown. Despite its designation as a Yamanaka factor, KLF4 is important of Erk1/2 signalling in mouse ESCs, underscoring the importance of Erk1/2 signalling in stem cells. It is, therefore, tempting to hypothesise that Iqgap3-Erk signalling influences chromatin architecture to direct stem cell-specific transcription programme.

The roles of isthmus stem cells and chief cells during tissue repair have long been mired in controversy. Two disparate models, both supported by extensive evidence, have been proposed. One model holds that chief cells acquire plasticity and contribute to repair. The other model contends that quiescent Mist1-expressing isthmus stem cells are solely responsible for repair of the entire pit. Our work not only shows that rapidly...
Figure 6  Iqgap3-2A-CreERT2;KrasG12D/+ mice present pseudopyloric metaplasia. (A) Schematic representation of the genetic construct used to establish the Iqgap3-2A-CreERT2;KrasG12D/+ mouse model. (B) Experimental strategy for inducing Iqgap3-driven active KrasG12D expression. (C) H&E staining of the lesser and greater curvature on the corpus of Iqgap3-2A-CreERT2;KrasG12D/+ mouse. (D, E) IF staining for Muc5ac and H,K-ATPase on the corpus of control and Iqgap3-2A-CreERT2;KrasG12D/+ mice. (F–I) IF staining for Muc5ac/Ki67 (F), CD44v10 (G), Pdx1/E-cad (H) and Tff2/Ki67/E-cad (I) on the corpus of Iqgap3-2A-CreERT2;KrasG12D/+ mice (n=3). Box indicates enlarged region. Scale bars=50 μm. IF, immunofluorescence; p.i, post-tamoxifen induction.
Figure 7  IQGAP3 is coexpressed with Ki67 in human gastric cancer. (A) IF staining for IQGAP3, Ki67 and E-cad on normal corpus in the human stomach (n=3). (B) Microarray and RNA-sequencing analysis for IQGAP3 expression in gastric tumour and normal stomach tissue (Singapore cohort). Error bars for microarray (tumour=185, normal=89) and RNA-sequencing (tumour=27, normal=18) represent SEM from each population. Data were analysed by two-tailed Wilcoxon RANK sum test. (C) IQGAP3 tissue microarray (TMA) of gastric cancer and paired-adjacent normal stomach tissue (n=237). (D) Box plot for comparing IQGAP3 TMA score in adjacent normal stomach, intestinal type gastric cancer, diffuse type gastric cancer and mixed type gastric cancer. Error bars represent SEM from each population. Data sets were analysed by one-way ANOVA. (E) H&E staining on human gastric tumour (n=3). (F) IF staining for IQGAP3, Ki67 and E-cad on the human gastric tumour (n=5). (G) IF staining for IQGAP3, Ki67 and CD44v9 on the human gastric tumour (n=7). Scale bar=100 µm. ANOVA, analysis of variance; IF, immunofluorescence; SEM, SE of mean.
proliferating isthmus stem cells contribute to the repair process, but also clearly supports the self-replicating and plasticity properties of chief cells. However, we cannot rule out the contribution of Mist1-expressing isthmus quiescent stem cells. We posit that expression of Iqgap3, be it in isthmus or chief cells, bestows stem-like behaviour to drive tissue repair. It is interesting that pERK was also detected in the Iqgap3-expressing cells after HDT. In view of the findings by Khurana et al.,33 it is tempting to propose that an Iqgap3-ERK-CM44 axis drives stem cell proliferation during homeostasis and tissue repair.

Interestingly, Iqgap3 induction during tissue repair was associated with the upregulation of c-Myc and genes associated with ESC. The abilities of c-Myc to increase cancer stem cell population and induce dedifferentiation suggest its involvement in tumour initiation. Moreover, our transcriptomic analysis reveals the enrichment of gene expression signatures associated with early gastric cancer, Kras-linked neoplastic transformation in HDT treated cells. Our results suggest that Iqgap3 is not simply a proliferation factor; rather, the injury-related induction of Iqgap3 and associated gene signatures represent the initial step of cancer development. Accordingly, we found that IQGAP3 is expressed in rapidly proliferating regions of gastric cancer. Since multiple cell types respond to HDT by expressing Iqgap3, we were not able to specify the cell of origin of cancer following injury. Nevertheless, by using the mouse strain harbouring Iqgap3-2A-CreERT2 to express KrasG12D in Iqgap3-expressing cells, we observed rapid generation of pseudopyloric metaplasia. Abrupt activation of Ras in Iqgap3-expressing cells are thus likely to be responsible for induction of early stage of cancer development.

The expression of Iqgap3 in isthmus or chief cells may be necessary to trigger Ras-driven cell proliferation and subsequent metaplastic transformation. Moreover, IQGAP3 ablation has been shown to reduce proliferation of breast and gastric cancer cell lines.46 47 We propose that an IQGAP3-Ras-associated mechanism might be conserved for proliferation of cancer cells.

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**Contributors** YI, JM and ST conceived the study. YI supervised the study. YI, JM and DD designed the experiments. JM, DD, KM, AT, NNM, DLH, SC, NAM, NN and KK performed experiments. YL, SWTH, NL, HKL, JMC and HY performed bioinformatics analysis. PT, SS, TM, JBS, WYP, K-GY provided and analysed human clinical samples. ST, AT and MA provided reagents and conceptual advice. All authors commented on the results and discussed implications. YI, LSCH and JM analysed the data and wrote the manuscript.

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**Data availability statement** All data relevant to the study are included in the article or uploaded as online supplemental information. RNA-seqencing datasets generated in this study are deposited in Gene Expression Omnibus (GEO) database under accession codes GSE161443 for Iqgap3-2A-GfpTomato reporter mice and GSE161442 for HDT treatment respectively. All supporting data are available from the corresponding authors upon reasonable request.

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**REFERENCES**


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Figure S1. Expression of Iqgap1, Iqgap2 and Iqgap3 in the corpus epithelium.

A

Wildtype allele
Targeting vector
Targeted allele
Neoc deletion

Exon 1
33
34
35
36
37
38
UTR
3'

Neo
2A tdTomato UTR

SDA (Self deletion Anchor) site

B

% of cells in tdTomato cells

P < 0.001

K67+ K67-

C

16 hr p.i.

D

Iqgap1 DAPI
Iqgap1
K67 E-cad DAPI

Iqgap1
K67 E-cad DAPI

Iqgap1
K67 DAPI

Iqgap1
K67 E-cad DAPI

E

Iqgap2 DAPI
Iqgap2
K67 E-cad DAPI

Iqgap2
K67 E-cad DAPI

Iqgap2
K67 E-cad DAPI

F

Iqgap1

Figure S2. Expression of Iqgap3 marks stem cells in corpus of stomach.

A

Wildtype allele

Targeting vector

Targeted allele

Neo' deletion

SDA (Self deletion Anchor) site

B

TMX 0 mg

TMX 2 mg

1 d p.i.

C

Lumen

Neck

Transition

Gut

D

Day1

Day5

Day7

WENFRG

ENFG

E

Relative mRNA expression

Iqgap3

Muc5ac

F

WENFRG

ENFG

G

H

I

J

Figure S3. Transcriptome analysis for Iqgap3-expressing cells in isthmus.

A

lqgap3

Mki67

Stmn1

lqgap3

lqgap3

lqgap3

mRNA expression (CPM)

2

4

6

lqgap3

lqgap3

lqgap3

mRNA expression (CPM)

200

400

600

Kitl

Gpr30

lqgap3

lqgap3

lqgap3

lqgap3

lqgap3

lqgap3


B

LGR5neg GENE SIGNATURE

Slc25a5

Tubb5

Pon1

Hmgfr

Dbi

Ran

Pgf1

Nudlb

Nduv2

Pol3k

Cdk6

Pamec3

Cald30

Fadd

Crb2

Erih

Birc5

Mki67

Kapr

Pansa1

Tymr

Hape1

Ect2

Rmpi

Nmc1

Bax

Ndufb1

Mtk2

Stmn1

Nes = 2.02

P < 0.001

C

Enrichment plot:

ST-HSC SIGNATURE

Nes = 1.06

P = 0.246

D

HALLMARK_HEDGEHOG_SIGNALING

Nes = -1.23

P = 0.0173

E

HALLMARK_WNT_BETA_CATENIN_SIGNALING

Nes = 1.06

P = 0.360

F

HALLMARK_NOTCH_SIGNALING

Nes = 1.17

P = 0.246

Enrichment plot:

HALLMARK_SELF_RENEWAL_ASSOCIATED

Nes = 2.16

P < 0.001

Enrichment plot:

GOASYMMETRIC_CELL_DIVISION

Nes = 1.63

P < 0.001

Enrichment plot:

HALLMARK_HEDGEHOG_SIGNALING

Nes = -1.23

P = 0.0173

Enrichment plot:

HALLMARK_WNT_BETA_CATENIN_SIGNALING

Nes = 1.06

P = 0.360

Enrichment plot:

HALLMARK_HEDGEHOG_SIGNALING

Nes = 1.06

P = 0.360

Enrichment profile

Hits

Nes = 1.63

P < 0.001

Nes = 1.06

P = 0.246

Nes = -1.23

P = 0.0173
Figure S4. Ras-Erk pathway regulators are expressed in the isthmus.
Figure S5. Iqgap3 regulates stem cell proliferation via Ras-Erk pathway.

A

Control shRNA  Iqgap3 shRNA1  Iqgap3 shRNA2  Iqgap3 shRNA3

lqgap3

Gapdh

HSP90

B

0 passage (6 days after Dox+)

C

1st passage (8 days after passage)

D

2nd passage (7 days after passage)

E

DMSO  U0126 (50 μM)  MK-8353 (1 μM)

pErk (red)

DAP (green)

F

G

DMSO  U0126  MK-8353

0 hr

96 hr

10 μM

50 μM

0.5 μM

1 μM
Figure S6. Iqgap3-expressing corpus cells proliferate in response to tissue damage.

A) Untreated and HDT 48 hr

B) Untreated, HDT 24 hr, HDT 48 hr, and HDT 14 days

C) Iqgap3 and Lgr5

D) HDT 48 hr

E) CD45, Ki67, and E-cad

F) Graph showing Ki67 positive cells per CD45 positive cells (%)

G) HDT 14 days
Figure S7. HDT-induced tissue damage promotes stem cell activity.

A. Enrichment profile for HALLMARK_MITOTIC_SPINDLE and HALLMARK_MYTHER_V1

B. Enrichment profile for MITOTIC_SPINDLE, MYC_TARGETS_V1, E2F_TARGETS, and G2M_CHECKPOINT

C. Enrichment profile for HALLMARK_INFLAMMATORY_RESPONSE

D. Enrichment profile for HALLMARK_WNT_BETA_CATENIN_SIGNALING
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B. TMA score high in tumor

C. Relative mRNA expression

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A

B

C
ONLINE SUPPLEMENTARY MATERIAL

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Supplementary Methods

Organoid generation, culture, differentiation and drug treatment

Details of gastric epithelial cell isolation and organoid culture were described previously[1]. Gastric epithelial single cells were mixed with ice-cold Matrigel (Corning) and plated on 24-well plates (Thermo Fisher Scientific), and mouse gastric culture medium (Advanced Dulbecco’s Modified Eagle Medium/F12 supplemented with 10 mM of HEPES (Thermo Fisher Scientific), GlutaMAX (Thermo Fisher Scientific), penicillin/streptomycin (Thermo Fisher Scientific), N2 (Thermo Fisher Scientific), B27 (Thermo Fisher Scientific), 10 mM Y27632, 1 mM of N-acetylcysteine (Merck) and 1% BSA) was overlaid. The cells were also cultured with growth factors such as 100 ng/ml of Noggin (Miltenyi Biotec), 50 ng/ml of EGF (Merck), 100 ng/ml of FGF10 (Peprotech), 10 mM of gastrin I (Merck) and R-spondin 1-conditioned medium and Wnt3a-conditioned medium (WENFRG). The medium supplemented with growth factors was replaced every 3-4 days. All organoids were maintained in the condition of 5% CO$_2$ and 37 $^\circ$C in a humidified incubator. To induce tissue stem cell differentiation in organoid, Wnt3a and R-spondin 1 were removed from day 5 organoids (ENFG). The organoids in ENFG culture condition were maintained for 2 days and analysed by qPCR. Ras-Erk pathway inhibitors, U0126 (Selleckchem) and MK-8353 (Selleckchem), were added at day 0 of organoid culture. Organoids were cultured until day 4 (96 h).

The SMARTvector Inducible shRNA with tRFP (Horizon Discovery) was used to suppress Iqgap3 expression in mouse organoid. Three different targets of shRNA were prepared. The target of shRNA1 is ATGTTGGCTTTTGCATCAA. The target of shRNA2 is TCAGAGTTCTTGTCTTGCC. The target of shRNA3 is AGATTGGTCTGCTCGTCAA. Details of organoid transfection was described previously [2]. The single cells from organoids were mixed with BTXpress electroporation buffer (BTX) and shRNA plasmids. Electroporation was performed with NEPA21 Super Electroporator (NEPA gene). The cells were cultured in the
WENFRG-culture condition to generate organoids. Next, 4 µg/ml of puromycin (Thermo Fisher Scientific) was added to the culture medium. After 4 days of puromycin selection, organoids were cultured in WENFRG-culture condition with 2 µg/ml of puromycin (Thermo Fisher Scientific) and 4 µg/ml of doxycycline (Merck) to induce shRNA. Passage of organoids was performed every 7-10 days. Organoids were visualized by Eclipse TS100 (Nikon) and counted at days 5-8.

**Cell culture and shRNA/siRNA knock-down**

NTERA-2, human malignant pluripotent embryonal carcinoma cell line, obtained from ATCC (NTERA-2 cl.D1) was cultured in DMEM (Dulbecco’s Modified Medium, Nacalai Tesque) with 10% FBS (fetal bovine serum) at 37 °C in a humidified 5% CO2 incubator according to the manufacturer’s instructions. Cells were maintained at high density and the medium was changed every 2-3 days. HGC-27, human gastric cancer cell line, obtained from CellBank Australia, the European Collection of Cell Cultures (Cat#94042256), was cultured in RPMI 1640 (Nacalai Tesque) with 10% FBS 37 °C in a humidified 5% CO2 incubator according to the manufacturer’s instructions. Cells were passaged when the confluency reached 80-90%. ON-TARGET plus SMARTpool siRNAs targeting human IQGAP3 (Dharmacon, Cat# L-009077-00-0005), and a non-targeting siRNA pool (Dharmacon, Cat#D001810-10-50) as the control siRNA were used for the knockdown experiments. At 24 h after seeding in a 6-well culture plate, cells were transfected with 35 nM of siRNAs using jetPRIME (Polypus Transfection) according to the manufacturer’s protocol. At 48 h after transfection, cells were reseeded into a 6-well plate and a 6 cm dish which were then transfected in a similar manner at 6-8 h after seeding, and harvesting the cells at 72 h later for RNA and protein quantification.

HEK293T cells and NIH3T3 cells were cultured with DMEM (Nacalai Tesque) supplemented with 10% FBS and 1% Penicillin/streptomycin. Cells were maintained at 5% CO2 and 37 °C in a humidified incubator. For attenuation of Iqgap3 mRNA expression in NIH3T3 cells, the SMARTvector Inducible shRNA with tRFP (Horizon Discovery) was used. The shRNA plasmids were transfected into NIH3T3 cells using jetPRIME (Polypus Transfection). Proteins were extracted from knockdown cells to perform immunoblot.

**Human clinical samples**
Primary healthy or cancer stomach tissues and clinical information were collected with patient consent in the GASCADII (Gastric Cancer Biomarker Discovery II) study, which was conducted at National University Hospital (Singapore) and approved by the National Healthcare Group Domain Specific Review Board (ref. no. 2005/00440). Gastritis tissues were collected with patient consent from the National University Hospital tissue repositories or pathology archives and with the approval of the respective Institutional Research Ethics Review Committees in accordance with local regulations and legislations. Clinical information was collected with the approval of the Institutional Review Board.

**Immunofluorescence staining**

To prepare paraffin tissue sections, mice or human stomachs were washed with PBS containing 10% FBS and fixed with PBS containing 4% paraformaldehyde (PFA). Fixed tissues were embedded in paraffin after incubation with ethanol and butanol. For preparing frozen tissue sections, mice or human stomach were washed with PBS containing 10% FBS and fixed with PBS containing 1% or 4% PFA. Fixed tissues were embedded in OCT compound (Leica Biosystems) after incubation with sucrose in PBS. Organoids were embedded in Histogel (Thermo Fisher Scientific) and fixed with PBS containing 4% PFA. Fixed organoids were embedded in paraffin after incubation with ethanol and butanol. 5-µm paraffin embedded tissue sections or 5-µm OCT frozen tissue sections were processed using a standard histological protocol.

To stain paraffin or frozen tissue slides, blocking was performed with Dako protein Block (Agilent, Santa Clara, CA), 3% BSA (Merck) in PBST, 2% Goat serum (Merck) in PBST or 5% skim milk in PBS. Primary antibodies were diluted in the blocking reagent and incubated with the tissue slides overnight at 4 °C. Secondary antibodies were diluted in 5% skim milk in PBST and incubated with the slides for 1 h at room temperature.

The antibodies used to stain mouse IgG-family proteins, namely anti-IQGAP3 (1:200), anti-IQGAP1 (1:200), and anti-IQGAP2 antibodies (1:200), were as described previously [3]. The other primary antibodies for immunofluorescence staining are as follows: anti-IQGAP3 (PA5-56363, Thermo Fisher Scientific, 1:100), anti-IQGAP1 (sc-376021, Santa Cruz Biotechnology, 1:200), anti-IQGAP2 (sc-17835, Santa Cruz Biotechnology, 1:200), anti-GFP (598, MBL, 1:500), anti-RFP (PM005, MBL, 1:500), anti-RFP (MBS448122, MyBioSource.com, 1:200), anti-Ki67 (14-5698-82, Thermo Fisher Scientific, 1:2000), anti-Ki67 (ab16667, Abcam, 1:500), anti-Ki67 (14-5698-82, Thermo Fisher Scientific, 1:2000), anti-Ki67 (ab16667, Abcam, 1:500),
(M7240, Agilent, 1:100), anti-E-cadherin Alexa488/555/647-conjugated (560061/64/62, BD Bioscience, 1:200), anti-Nras (10724-1-AP, Proteintech, 1:200), anti-Hras (sc-35, Santa Cruz Biotechnology, 1:100), anti-Kras (12063-1-AP, Proteintech, 1:200), anti-phosphorylated ERK (4370, Cell Signaling Technologies, 1:100), anti-HER2 (2165, Cell Signaling Technologies, 1:200), anti-CD44v10 (LKG-M002, Cosmo Bio, 1:500), anti-CD44v9 (LKG-M001, Cosmo Bio, 1:500), anti-PDX1 (ab47267, Abcam, 1:200), anti-Tff2 (13681-1-AP, Proteintech, 1:100), anti-CD45 (14-0451-82, Thermo Fisher Scientific, 1:500), anti-Muc5ac (sc-16903, Santa Cruz Biotechnology, 1:200), anti-H,K-ATPase (D031-3, MBL, 1:1000), anti-Pepsinogen C (ab31464, Abcam, 1:200), anti-GIF (provided by D. H. Alpers, 1:1000), Lectin GS-II Alexa647-conjugated (L-32451, Thermo Fisher Scientific, 1:1000). Following primary antibody incubation, slides were washed in PBST then incubated with Alexa Fluor 488-, 546/555- or 633/647-conjugated secondary antibody (Thermo Fisher Scientific, 1:200) for 1 h at room temperature. Tissue slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI) and visualized by Eclipse Ti (Nikon) or LSM880 with Airyscan (Zeiss) confocal microscope.

**Flow cytometry**

Detail of gastric glands isolation was described previously [1]. Isolated mouse stomach was chopped into 2 mm² pieces and incubated with chelating buffer (5 mM EDTA and 0.52 mM DTT in PBS) for 2 h at 4 °C on a shaking platform, then gastric units were released by vigorous shaking in cold dissociation buffer (54.8 mM D-sorbitol and 44 mM Sucrose in PBS). Isolated mouse gastric units were incubated in TrypLE Express (Thermo Fisher Scientific) with Y-27632 (Merck) for 12 minutes at 37 °C with repetitive pipetting for digestion into single cells. The cells were passed through a 30 µm cell strainer (Miltenyi Biotech) and labeled with antibodies in HBSS with 2% FBS. Antibodies for the flow cytometry are as follows: anti-EpCAM APC/Cy7-conjugated (BioLegend) and anti-ErbB2 PE-conjugated (R&D Systems). Dead cells were excluded by DAPI staining, and labeled cells were sorted by using FACSaria II (BD Bioscience). Data were analyzed with FlowJo 10.6 (BD Bioscience).

**Quantitative PCR (qPCR)**

Total RNA from the cell lines was extracted using RNeasy Kit (Qiagen) with the removal of genomic DNA using the RNase-free DNase Set (Qiagen) according to the manufacturer’s
instructions. Complementary DNA (cDNA) was prepared from 1 µg of total RNA extracted using iScript reverse transcription supermix for RT-qPCR (Bio-Rad). qPCR was performed using SYBR Green qPCR kit (Bio-Rad) and QuantStudio3 Real-Time PCR systems (Thermo Fisher Scientific). RNA in FAC-sorted Iqgap3high and Iqgap3low/neg cells of Iqgap3-2A-tdTomato mice was extracted using NucleoSpin RNA XS (MACHEREY-NAGEL) by following the manufacturer’s manual. RNA from Iqgap3high/Iqgap3low/neg cells was used for cDNA synthesis with RT-RamDA cDNA Synthesis Kit (Toyobo). RNA in HDT-treated mice stomach was extracted using the RNeasy Mini Kit (QIAGEN) with on-column genomic DNA digestion using the RNase-free DNase Set (QIAGEN); cDNA synthesis was done using PrimeScript RT-PCR kit (Takara Bio). RNA in organoids and FAC-sorted corpus epithelial cells was extracted using NucleoSpin RNA XS (MACHEREY-NAGEL); cDNA synthesis was performed with High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). All were done according to the manufacturers’ instructions. qPCR was performed with KAPA SYBR FAST (Merck) and QuantStudio3 Real-Time PCR systems (Thermo Fisher Scientific). Gene expression levels in each sample were normalized by glyceraldehyde-3-phosphate dehydrogenase (Gapdh) expression. All experiments were performed in triplicate, and primer sequences are listed in Table S1.

**Single-molecule RNA in situ hybridization**

Tissue sections (5 µm thickness) were processed for RNA in situ detection using RNAscope 2.5 HD Reagent Kit according to the manufacturer’s instructions (Advanced Cell Diagnostics). All probes, including controls, were designed by the manufacturer (Advanced Cell Diagnostics). Probes for ISH are the following: Iqgap1 (542081), Iqgap2 (542101), Iqgap3 (539811), Mki67 (416771-C2), Lgr5 (312171-C2), Duplex positive control (321651) and negative control (320751). Images were acquired by TissueFAXS (TissueGnostics).

**Immunoprecipitation and immunoblot**

The plasmids (pCAG-Venus-Iqgap3, pCAG-Myc-Hras and pCAG-Myc-HrasG12V) were transfected into HEK293T cells by using TransIT reagent (Mirus Bio) (7). Cell lysates were extracted by using lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 5 mM MgCl2, Halt phosphatase inhibitor (Thermo Fisher Scientific), 1% Triton-X-100, 2 mM Dithiothreitol, Complete protease inhibitor (Merck)). Cell lysates were incubated with GFP-Trap (ChromoTek). The cell lysates or
GFP-Trap-affinity-purified proteins were analyzed by immunoblot with 4–20% Mini-PROTEAN TGX Precast Protein Gels (Bio-Rad). After electrophoresis, the proteins were transferred to PVDF membrane (Merck) and detected by primary antibodies and HRP-conjugated secondary antibodies. Antibodies for immunoblots are as follows: anti-Myc (sc-40, Santa Cruz Biotechnology), anti-GFP (D153-3, MBL, 1:1000), anti-phosphorylated ERK (4370, Cell Signaling Technologies, 1:1000), anti-ERK (9102, Cell Signaling Technologies, 1:1000), anti-α-tubulin (T9026, Merck, 1:1000), anti-IQGAP3 (25930-1-AP, Proteintech, 1:1000), anti-Rabbit IgG HRP-conjugate (GE Healthcare Life Sciences) and anti-Mouse IgG HRP-conjugate (GE Healthcare Life Sciences). Blots were visualized by Immobilon Western Chemiluminescent HRP Substrate (Merck) and ImageQuant LAS500 (GE Healthcare Life Sciences).

The siRNA transfected NTERA-2 and HGC-27 cell pellets were first lysed by resuspending of the cell pellets with the lysis buffer (50 mM Tris pH 8, 150 mM NaCl, 0.1% SDS, 0.5% Sodium Deoxycholate and 1% NP-40) supplemented with Halt phosphatase inhibitor (Thermo Fisher Scientific), Complete protease inhibitor (Merck) and PMSF (Sigma-Aldrich), and incubating at 4 °C for 45 minutes. After centrifugation of the lysates at 13500 rpm for 15 minutes at 4 °C, the supernatant of each lysate was collected and the protein concentrations were measured using the Pierce BCA Protein Assay kit (Thermo Fisher Scientific) according to the manufacturer’s protocol. 40 µg of protein was loaded into each well of 8% SDS/PAGE. The antibodies used were as follow: anti-IQGAP3 (25930-1-AP, Proteintech, 1:1000), anti-NANOG (4903, Cell Signaling Technology, 1:1000), anti-OCT4 (ab19857, Abcam, 1:1000), anti-KLF4 (ab106629, Abcam, 1:1000), anti-CD44v9 (LKG-M001, Cosmo Bio, 1:1000), anti-GFAP (16825-1-AP, Proteintech, 1:1000), anti-PGC (ab93886, Abcam, 1:1000) and anti-GAPDH (2118, Cell Signalling Technology, 1:1000). Blots were visualized by Immobilon Western Chemiluminescent HRP Substrate (Merck) and ImageQuant LAS500 (GE Healthcare Life Sciences).

RNA-sequencing and data analysis
RNA extracted from FACS-sorted Iqgap3\textsuperscript{high} and Iqgap3\textsuperscript{low/neg} cells of Iqgap3-2A-tdTomato mice were sent to Beijing Genomics Institute for PCR amplification, transcriptome library preparation and sequencing. Raw reads were aligned to mouse reference genome version GRCm38 gencode M23 (downloaded from https://www.gencodegenes.org/) using STAR aligner version 2.7.1a with default parameters [4]. Read counts per gene were generated using featureCounts function in the
subread package version 2.0.0. [5]. Counts per million (cpm) values were calculated using cpm function of the edgeR R package [6]. Normalized gene expression data were subjected to Gene Set Enrichment Analysis (GSEA) using the broad institute GSEA tool (http://software.broadinstitute.org/gsea/index.jsp) with the Molecular Signature Database v6.0, FAC-sorted Lgr5\textsuperscript{high} or Lgr5\textsuperscript{neg} corpus epithelial cells public datasets (GSE86603) and HSC gene signature [7] to identify enriched gene sets/pathways in Iqgap3\textsuperscript{high} and Iqgap3\textsuperscript{low/neg} cells.

RNA extracted from the HDT-treated stomach was sent to BGI for transcriptome library preparation and sequencing. Sequenced reads were aligned with the STAR software to mm10, and mapped counts were employed to generate the raw expression counts using the FeatureCounts with GENCODE transcriptome annotation. The raw expression counts were then further normalized using the Cross-Correlation method [8]. Normalized gene expression data were subjected to GSEA using the broad institute GSEA tool with the Molecular Signature Database v6.0 to identify enriched gene sets/pathways in HDT versus untreated.

The RNA-seq datasets (Figures 3 and S3) revealed that Iqgap3 mRNA was unexpectedly low in the tdTomato\textsuperscript{high} fraction – only its 3’UTR was detected. Because of the Iqgap3-2A-CreERT2;Rosa-tdTomato cassette design, the presence of tdTomato protein and the enrichment of Ki67 and Stmn1, Iqgap3 mRNA expression in the tdTomato\textsuperscript{high} fraction can be inferred. Of note, Iqgap3 mRNA in Iqgap3\textsuperscript{high} fraction was detected by qPCR analysis (figure 1G). As to why the Iqgap3 mRNA was poorly detected by the RNA sequencing technology, we posit that mRNA transcribed from an artificially manipulated genome might be more susceptible to nonsense-mediated mRNA decay [9] during stem cell isolation and lysis. Moreover, different RNA amplification methods, such as SMART-seqII and RT-RamDA were used for RNA-seq and qPCR respectively. This might also have affected Iqgap3 mRNA detection.

**Transcriptomic analysis for human gastric cancer and normal tissue samples**

Microarray gene expression data for 185 primary gastric cancer (GC) and 89 normal gastric samples were generated as described previously [10]. RNA-seq for 27 primary GC and 18 matched normal gastric samples were performed and analyzed as described previously [11]. For each cohort, IQGAP3 expression was compared between primary GC and matched normal samples using the two-sided Wilcoxon’s rank-sum test (p < 0.05).
**Tissue-microarray for human gastric cancer**

Tissue microarrays (TMAs) were constructed from the archival formalin fixed paraffin embedded (FFPE) gastric cancer tissue specimens (between the year 2000-2013) obtained from the Department of Pathology, National University Hospital, Singapore. TMAs with 2 mm cores were constructed from the tumor and the adjacent normal area. Consecutive 4 µm sections were obtained from each TMA and mounted on coated glass slides for immunohistochemical staining. Ethics approval was obtained from the Domain Specific Review Board of the hospital (2015/0209).

**Database analysis**

*IQGAP3, MKI67* and *PCNA* expression dataset in human gastric cancer were obtained by using eBioportal for cancer genomics (https://www.ebioportal.org/) [12]. RNA-sequencing dataset from The Cancer Genome Atlas (TCGA)[13] was used. Annotated data was visualized by GraphPad Prism7 (GraphPad software).

**Quantification and statistical analysis**

All statistical analyses including cell number counts, organoid sizes, qPCR and gene expression in cancer patients were shown with standard deviation (SD) or standard error of mean (SEM). Two-tailed student’s t-test, One-way ANOVA or two-sided Wilcoxon’s rank sum test were used for statistical analysis. P-values less than 0.05 were considered statistically significant.

**Data and code availability**

All data relevant to the study are included in the article or uploaded as online supplemental information. RNA-sequencing datasets generated in this study are deposited in Gene Expression Omnibus (GEO) database under accession codes GSE161443 for *Iqgap3-2A-TdTomato* reporter mice and GSE161442 for HDT treatment respectively. All supporting data are available from the corresponding authors upon reasonable request.

**References for Methods**

Supplementary Figure Legends

Figure S1. Expression of Iqgap1, Iqgap2 and Iqgap3 in the corpus epithelium.

(A) Knock-in strategy for generation of Iqgap3-2A-tdTomato mouse model.

(B) Quantification of Ki67+ cells in the tdTomato+ (Iqgap3+) population of Iqgap3-2A-tdTomato mice (n=3). Error bar represents standard deviation (SD) from 796 cells of 3 mice. Data were analyzed by Student’s t-test.

(C) IF staining for Iqgap3 and tdTomato on the corpus of eR1-CreERT2;Rosa-tdTomato mice at 16 h post tamoxifen induction (p.i.) (n=3). eR1-LT, eR1-derived cell lineage tracing. Arrow, eR1+ cell.

(D-E) IF staining for Iqgap1, Iqgap2, Ki67 and E-cad on the corpus of wild type (WT) mice (n=3).

(F-G) ISH for Iqgap1, Iqgap2 and Mki67 on corpus gastric units of WT mice (n=2).

Scale bar = 100 µm.

Figure S2. Expression of Iqgap3 marks stem cells in corpus of stomach.

(A) Knock-in strategy for generation of Iqgap3-2A-CreERT2 mouse model.

(B) IF staining for tdTomato on corpus of Iqgap3-2A-CreERT2;Rosa-tdTomato mice at 1 day (1 d) post tamoxifen treatment (TMX 2 mg) and untreated control (TMX 0 mg) (n=3). Dashed box, Co-IF staining for tdTomato and Ki67 is shown in Fig.2. LT, Lineage tracing.

(C) IF staining for tdTomato, GS-II and Gif on the corpus of Iqgap3-2A-CreERT2;Rosa-tdTomato mice at 1 year post tamoxifen induction (n=3). Image were shown as X-Z plane of neck (#1: GS-II+ and Gif-), neck-base transition (#2: GS-II+ and Gif+) and base (#3: GS-II- and Gif+).

(D) Microscopic image for induction of differentiation in organoids from WT mouse. The organoids were generated with WENFRG (Wnt3a, Egf, Noggin, Fgf10, R-spondin1, Gastrin) culture condition. On day 5, the organoids were cultured with ENFG (Egf, Noggin, Fgf10, Gastrin) for 2 days to induce differentiation (n=3).
(E) qPCR analysis for \textit{Iqgap3} and \textit{Muc5ac} mRNA on organoids with WENFRG condition and organoids with ENFG condition (n=3). The mRNA expression was normalized by \textit{Gapdh} expression. Error bars represent SD from each population. Data sets were analyzed by Student’s t-test.

(F) IF staining for tdTomato and Muc5ac on WENFRG- and ENFG-cultured organoids from Iqgap3+ cells of \textit{Iqgap3-2A-CreERT2;Rosa-tdTomato} mice (n=2).

(G-J) IF staining for tdTomato, PgC, Gif, GS-II, Ki67 and HK-ATPase in organoids from Iqgap3+ cells of \textit{Iqgap3-2A-CreERT2;Rosa-tdTomato} mice (n=2). Arrow, PgC+/GS-II- or Gif+/GS-II- cell. Arrow head, PgC+/GS-II+ or Gif+/GS-II+ cell. Open arrow head, PgC-/GS-II+ cell. Scale bar, 100 µm.

**Figure S3. Transcriptome analysis for Iqgap3-expressing cells in isthmus.**

(A) The average gene expression level of \textit{Iqgap3}, \textit{Mki67}, \textit{Stmn1}, \textit{Kitl}, \textit{PgC} and \textit{Gpr30} from RNA sequencing of tdTomato\textsuperscript{high} (Iqgap3\textsuperscript{high}) and tdTomato\textsuperscript{low/neg} (Iqgap3\textsuperscript{low/neg}) cell fractions from \textit{Iqgap3-2A-tdTomato} mice. Iqgap3 mRNA reads comprised mainly 3'-UTR (See RNA-sequencing and data analysis in Supplementary Methods). CPM, counts per million.

(B) Heat map showing the top 30 genes from Iqgap3\textsuperscript{high} cell fractions upregulated in the Lgr5-negative (LGR5neg) gene signature.

(C) GSEA showing enrichment of the short term hematopoietic stem cell (ST-HSC) gene signature in Iqgap3\textsuperscript{high} and Iqgap3\textsuperscript{low/neg} cell fractions.

(D) Heat map showing the top 30 genes from Iqgap3\textsuperscript{high} cell fractions upregulated in the ST-HSC gene signature.

(E-F) GSEA showing enrichment of the HSC self-renewal associated gene signature, GO asymmetric cell division gene signature, Hallmark Notch signaling gene signature, Hallmark Hedgehog gene signature and Hallmark Wnt-β-catenin signaling gene signature in Iqgap3\textsuperscript{high} and Iqgap3\textsuperscript{low/neg} cell fractions.
Figure S4. Ras-Erk pathway regulators are expressed in the isthmus.

(A-B) IF staining for Kras, Nras, Ki67 and E-cad on the corpus of wild type mice (n=3).

(C) FACSort to isolate Her2\textsuperscript{high} and Her2\textsuperscript{low} gastric epithelial cell fractions (n=3).

(D-E) qPCR analysis for Her2 (Erbb2), Iqgap3, Mki67, Lgr5, Mist1, Hras, Kras and Nras mRNA in Her2\textsuperscript{high} and Her2\textsuperscript{low} fractions of gastric epithelial cells (n=3). Error bars represent SD from each population. Data sets were analyzed by Student’s t-test.

Scale bar, 100 µm.

Figure S5. Iqgap3 regulates stem cell proliferation via Ras-Erk pathway.

(A) Iqgap3 shRNA knock-down in NIH3T3 cells (n=2).

(B-D) Representative microscopic image of Iqgap3 shRNA knock-down (KD) organoids generated from WT mice (n=2). Organoids from 0 passage (no passage), 1\textsuperscript{st} passage and 2\textsuperscript{nd} passage are shown. RFP expression indicated shRNA plasmid transfected organoids.

(E) IF staining for p-Erk and E-cad on U0126 and MK-8353 treated organoids from corpus of WT mice (n=2).

(F-G) Microscopic images of U0126 and MK-8353 treated organoids from corpus of WT mice (n=2).

Figure S6. Iqgap3-expressing corpus cells proliferate in response to tissue damage.

(A) IF staining for E-cad and H,K-ATPase on untreated and 48 h post-HDT treated WT corpus.

(B) IF staining for Ki67 on untreated, 24 h, 48 h and 14 days post-HDT treated WT corpus.

(C) ISH for Iqgap3(green) and Lgr5(red) on 48 h post-HDT treated WT corpus (X-Z plane). Boxes indicate enlarged regions.

(D) IF staining for tdTomato and Ki67 on 48 h post-HDT treated corpus from Iqgap3-2A-tdTomato mice.
(E) IF staining for Ki67, CD45 and E-cad on the bottom part of corpus gastric units from untreated and 48 h post-HDT treated WT mice.

(F) Quantification of CD45+ cells and Ki67+/CD45+ cells per 20× fields (n=3). Data were analyzed by Student’s t-test.

(G) IF staining for tdTomato, H,K-ATPase and Ki67 on 14 days post-HDT treated corpus from Iqgap3-2A-CreERT2;Rosa-tdTomato mice.

Scale bars = 100 μm.

Figure S7. HDT-induced tissue damage promotes stem cell activity.

(A) GSEA showing enrichment of Mitotic spindle, Myc targets V1, E2F targets and G2M checkpoint gene signatures in 48 h post-HDT corpus (n=2).

(B) Heat map showing top 20 genes upregulated in (A) based on RNA-seq data from untreated and 48 h post-HDT corpus tissue.

(C-D) GSEA showing enrichment of Inflammatory response and Wnt/β-catenin signaling gene signatures in 48 h post-HDT corpus (n=2).

(E) Heat map showing top 20 genes upregulated in (C-D) based on RNA-seq data from untreated and 48 h post-HDT corpus tissue.

P values determined by a weighted Kolmogorov-Smirnov-like statistic and adjusted for multiple hypothesis testing.

Figure S8. Robust expression of IQGAP3 in human cancer tissue.

(A) Difference of IQGAP3 Tissue Microarray (TMA) score in gastric cancer and paired-adjacent normal stomach tissue.

(B) Representative image of IQGAP3 expression in adjacent normal stomach tissue from the TMA
(C) qPCR for $IQGAP3$, $NANOG$, $OCT4$, $KLF4$, $cMYC$, $SOX2$, $CD44v9$ and $PGC$ mRNA from $IQGAP3$ knockdown gastric cancer cell line HGC-27. The mRNA expression was normalized by $GAPDH$ expression ($n=3$). Error bars represent SD. Data sets were analyzed by Student’s t-test. * $P < 0.05$, ** $P < 0.01$. n.s., not significant.

(D) Immunoblot for $IQGAP3$, $CD44v9$, $PGC$ and $GAPDH$ from $IQGAP3$ knockdown HGC-27 cells ($n=3$).

(E) Correlation of expression between $IQGAP3$ and proliferating markers, $MKI67$ and $PCNA$ in human gastric cancer. Gene expression dataset was obtained from the TCGA database.

Scale bar, 100 µm.

**Figure S9. IQGAP3 is expressed in proliferative CD44v9+ gastric cancer cells.**

(A-C) IF staining for IQGAP3, Ki67 and CD44v9 in the human gastric tumor ($n=7$). Scale bar, 100 µm.
**Supplementary Table 1. Primer sequencing for qPCR**

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