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Logarithmic expansion of LGR5⁺ cells in human colorectal cancer

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Abstract

Stem cells of the small and large intestine are marked by expression of the Wnt target gene LGR5, a leucine-rich-repeat-containing G protein-coupled receptor. Previous studies reported increased expression of LGR5 in human colorectal cancer (CRC) compared to normal tissue either by immunohistochemistry or in situ hybridization (ISH). However, as these studies were semi-quantitative they did not provide a numerical estimate of the magnitude of this effect. While we confirm that LGR5⁺ cells are exclusively located at the base of normal human small and large intestinal crypts, representing approximately 6% of total crypt cells, we show this cell population is 10-fold expanded in all grades of CRC, representing as much as 70% of the cells of tumor crypt-like structures. This expansion of the LGR5 compartment coincides with maintenance of crypt-like glandular structure (adenomas, and well and moderately differentiated adenocarcinomas), and is reduced in poorly differentiated CRC, where crypt-like glandular architecture is lost, accompanied

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by reduced epithelial terminal differentiation. Altogether these results indicate that LGR5⁺ cell expansion is a hallmark of CRC tumorigenesis occurring during progression to adenoma, supporting CRC as a stem cell disease with implications for CRC therapy.

Keywords

LGR5⁺; stem cells; colorectal cancer; in situ hybridization

1. INTRODUCTION

Stem cells of the mouse small and large intestine are maintained through the Wnt program and can be identified by specific expression of the Wnt target gene *Lgr5*, a leucine-rich-repeat-containing G protein-coupled receptor [1]. These *Lgr5*⁺ intestinal stem cells (ISCs) are columnar cells located at the base of crypts capable of generating all cell types present in the small and large intestines [1]. Recent in situ hybridization (ISH) studies in human intestinal specimens have shown that LGR5 mRNA is located at the base of the crypt coinciding with the architecture observed in mice, suggesting that LGR5 is also a robust stem cell marker in the human intestine [2–4].

Colorectal tumorigenesis is, in most cases, driven by activating mutations in the Wnt signaling pathway [5]. Murine *Lgr5*⁺ cells with gain-of-function in the Wnt program give rise to intestinal tumors more efficiently than other intestinal epithelial cell populations, suggesting that ISCs represent the cell of origin for colorectal cancer (CRC) [6]. The cancer stem cell hypothesis suggests that cancers develop from a small subset of cells with self-renewal properties similar to normal tissue stem cells [7]. However, this hypothesis may have to be reconsidered with regard to CRC since accumulating evidence points to an expansion of the stem cell compartment in CRC [2–4, 8]. In this regard, several studies have determined a differentially high expression of LGR5⁺ cells in human CRC compared to non-tumorigenic tissue either by immunohistochemistry (IHC) [9–11] or by ISH [3, 8]. However, the significance of these findings remains unclear due to lack of reliability of the antibodies used to target LGR5 [12], and the qualitative nature in which the ISH studies were scored.

In the present study, we optimize detection of LGR5 mRNA expression by ISH in human colorectal adenomas and adenocarcinomas for direct quantitation. While we confirm that LGR5⁺ cells are exclusively located at the base of crypts in normal human small and large intestine, where they represent approximately 6% of total crypt cells, we show that this cell population is dramatically expanded in all grades of CRC, representing as much as 70% of the tumor cells. Remarkably, this observed expansion of the LGR5 compartment is associated with maintenance of crypt-like glandular structures (in adenomas, and well and moderately differentiated adenocarcinomas), and is reduced in poorly differentiated CRC, where crypt-like glandular architecture is lost.

2. MATERIALS AND METHODS

2.1. Tissue samples

Formalin-fixed and paraffin-embedded (FFPE) colorectal tissue from 20 patients comprising 7 adenomas and 13 cancers was obtained from the Department of Pathology at Memorial Sloan-Kettering Cancer Center (MSKCC), New York. All patients underwent surgery at MSKCC and all procedures were performed with approval of the MSKCC Ethics Committee on samples obtained under informed consent. FFPE tissue blocks were prepared by the Pathology Core Facility using a standard protocol. For each sample, a hematoxylin and eosin (H&E) stained slide was reviewed by the GI attending pathologist (E. V.).

2.2. ISH, digital imaging and quantitative image analysis

ISH was performed with the following probes using the QuantiGene ViewRNA ISH Tissue 2-plex Assay kit (QVT0012, Affymetrix/Panomics) according to manufacturer's instructions. ISH probes of LGR5 (VA1-10587-01), GAPDH (VA6-10337-01) and CK20 (VA6-14373) were from Affymetrix. ISH for GAPDH was used to confirm mRNA integrity of all samples and only regions with positive GAPDH staining were used for the subsequent analysis of LGR5. Nuclei were labeled with DAPI. Slides were scanned using the Panoramic Flash 250 scanner (Perkin Elmer) and analyzed with the Panoramic Viewer software (Perkin Elmer). Pictures were captured using 10 or 20× magnification and tumor areas were identified in the H&E-stained slide and non-tumor areas were excluded from analysis. Number of LGR5⁺ cells (red) and total tumor cells (blue) per crypt or per region of interest (ROI) were counted manually.

2.3. Histology and IHC

Paraffin-embedded 5 µm sections of distal colon were deparaffinized, rehydrated through graded ethanol, and morphologically checked with H&E. For immunohistochemistry, sections were stained with the following antibodies: monoclonal mouse anti-human cytokeratin 20 (CK20, clone Ks20.8, M7019, Dako, 1:500) and monoclonal mouse anti-E-Cadherin (clone 36, 790-4497, Ventana). All immunostains were performed using Ventana Discovery XT Platform, using CC1 standard regime for CK20 and CC1 mild regime for E-cadherin. Primary antibodies were applied for 60 min followed by biotinylated anti-mouse secondary antibody also for 60 min (Vector, 1:200). Visualization was performed using Ventana DAB map kit. Quantitation of E-cadherin⁺ and CK20⁺ cells was done using Fiji software [13].

2.4. Microarray analysis

In order to confirm our ISH findings, we analyzed our previously published microarray data, deposited in the Gene Expression Omnibus database under accession number GSE41258 [14], available at: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=urgdgwwubhgxtaj&acc=GSE41258>.

We included in this analysis samples of normal mucosa, colonic adenomas and adenocarcinomas that were stratified according to pathologically confirmed grade of differentiation. Of note, there was only one sample classified as well differentiated, thus, it

was excluded from the analysis. We queried the microarray data to assess the expression of the following genes: LGR5, ASCL2, EPHB3, MYC, RNF43, CD44, SOX9, CK20 and E-cadherin.

The expression data was composed of Affymetrix GeneChip® Human Genome U133A arrays (Affymetrix, Santa Clara, CA). Data preprocessing and annotation were carried out using R and the Affy package [15] and the data was normalized using RMA [16]. Statistical analysis was done using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA) and statistical significance was determined by Kruskal-Wallis comparison of multiple pairs.

2.5. Statistical analysis

Values represent mean $\pm$ standard error of the mean (SEM) as indicated. Statistical significance of the mean difference was analyzed by Student's *t* test, using Prism 6 (GraphPad Software). A *p* value ≤ 0.05 was considered significant.

3. RESULTS

3.1. LGR5⁺ cells are expanded in human colorectal adenomas

Precise identification of LGR5⁺ cells in human clinical colorectal specimens has been challenging, in part because of insufficient sensitivity and specificity of LGR5 antibodies [9–11]. To address this issue, recent studies have used RNA ISH techniques [3, 4, 17] to study LGR5 expression; however precise quantitation of LGR5⁺ cells has not been reported to date. We have recently validated use of ISH to accurately quantify Lgr5⁺ cells in murine small and large intestine [18] as well as in murine mammary glands [19], in comparison with the *Lgr5-lacZ* knock-in strain that represents an accurate reporter of Lgr5 expression [1, 20]. In the present study, we quantify LGR5 mRNA expression by ISH in human colorectal tumors. To validate our LGR5 ISH assay in human specimens, we initially showed that in the normal human small and large intestines, LGR5⁺ cells specifically reside at their known location at the base of crypts, in contrast to the control gene GAPDH, which is constitutively expressed along the entire crypt (Fig. 1A), consistent with previous reports [3, 17]. Quantitation of LGR5⁺ cells using fluorescent images determined normal human small and large intestine both display approximately 6% LGR5⁺ cells per crypt (6.1 $\pm$ 0.1% and 6.3 $\pm$ 0.1%, respectively, Fig. 1B), similar to what has been observed in mice. Quantifying brightfield images yielded highly similar data with 5.6 $\pm$ 0.3% and 5.3 $\pm$ 0.2% LGR5⁺ cells detected in the small and large intestine, respectively, indicating ISH accurately identifies LGR5⁺ cells in human FFPE specimens. For convenience, quantitation of LGR5⁺ cells in specimens in the rest of our studies reported here employed fluorescent images.

It was recently reported that the population of LGR5⁺ cells in human colorectal adenomas was expanded, although extent of expansion was not reported [2, 21]. Consistently, in the current study, LGR5⁺ cells were found to be greatly expanded in crypt-like structures both in tubulovillous and tubular adenomas (Fig. 2A). All adenomas displayed about 70% LGR5⁺ cells per crypt-like structure (73 $\pm$ 1.05%; *n*=7; Fig. 2B). Interestingly, distribution of LGR5⁺ cells was not random, but rather virtually all crypt-like structures showed expansion located

to the lower half of adenomatous glands, which dissipated in many instances towards the top of the crypt-like structure, a pattern reminiscent of normal glandular architecture.

3.2. Characterization of LGR5 expression in human colorectal adenocarcinoma according to grades of differentiation

While several reports describe global LGR5 expansion in human CRC qualitatively [2, 3, 9, 10], here we analyze incidence of LGR5 expressing cells in human CRC at different grades of differentiation as per [22]. While in well differentiated tumors >95% of tumor is occupied by crypt-like glandular structures (grade 1), moderately differentiated tumors display 50–95% glandular structures (grade 2), and poorly differentiated only 5–50% (grade 3) [22, 23]. Unexpectedly, only glandular structures were massively lined by LGR5⁺ cells within all differentiation grades (Fig. 3A). In this regard, well (n=3) and moderately differentiated (n=5) cancers present 70.6±0.8% and 76±0.5% LGR5⁺ cells per glandular structure, respectively (Fig. 3B). Similarly, in poorly differentiated colon adenocarcinomas (n=5) the sparse glandular structures present 76.7±0.9% LGR5⁺ cells, whereas non-glandular areas exhibit low frequency LGR5 expression and when combined all together, poorly differentiated cancers display only 31.1±0.1% LGR5⁺ cells. Altogether, ISH analysis of LGR5 mRNA expression reveals greater than 10-fold enrichment in LGR5⁺ cells in colon adenomas and in glandular structures in cancers when compared to normal colon, showing that expansion of LGR5⁺ compartment is sustained from adenoma to adenocarcinoma where glandular structure is maintained.

3.3. Relationship between LGR5, CK20 and E-cadherin expression in different types of human CRC

We next examined whether loss of glandular structure and associated reduction of LGR5 mRNA expression in poorly differentiated cancers related to morphologic and functional changes involved in de-differentiation and/or invasiveness of tumor cells. E-cadherin expression is one of the main markers of cell-cell interactions in epithelial cells and its downregulation is a phenomenon associated with de-differentiation, invasion, metastasis and poor prognosis [24]. Fig. 4 shows that tumor progression is accompanied by a decrease in E-cadherin staining intensity, consistent with reduced cell cohesion associated with loss of glandular structure. Despite this reduction in staining intensity, incidence of E-cadherin expression was mostly preserved, with more than 95% E-cadherin⁺ cells at all grades of differentiation (normal, 99.4±0.2%; adenoma 99±0.3%; MD cancer, 95±0.5%; PD cancer, 95.4±1.3%; Fig. 5), indicating that the vast majority of tumor cells still maintain an epithelial phenotype.

Tumors were also characterized with the widely-used marker of mature intestinal mucosa CK20 [25] (Fig. 4). IHC analysis of CK20 expression showed a clear reduction correlated with loss of glandular structure. In adenomatous crypt-like structures, 42±2.2% of the cells were CK20⁺ (Fig. 5), usually in regions lacking LGR5, suggesting that LGR5⁺ cells were indeed in an undifferentiated state. In a pattern highly similar to normal mucosa (46.5±2.1% of cells are CK20⁺, Fig. 4 and Fig. 5), CK20⁺ cells tended to cluster at the apex of crypt-like structures toward the “luminal” side of the adenoma, as confirmed by co-staining of LGR5 and CK20 by ISH (Fig. 6), suggesting that loss of LGR5 expression occurs mainly as a

result of terminal cell maturation. In adenocarcinomas, the number of CK20⁺ cells decreased to 24±2.2% in moderately differentiated tumors and to 17±3% in poorly differentiated tumors (Fig. 4 and 5), displaying a more disorganized distribution (Fig. 6). In fact, in poorly differentiated tumors both LGR5 and CK20 show very low-level expression. In this regard, signet ring cancer and medullary colon cancer, two types of very poorly differentiated cancers not formed through the traditional adenoma to carcinoma transition [26], present the lowest levels of LGR5⁺ cells (13±4.4% and 7±2.2%, respectively) and CK20⁺ cells (11.6±2% and 0.03±0.02%, respectively, Fig. 4 and Fig. 5). Medullary carcinoma was the only specimen showing a major reduction in E-cadherin incidence (40±3.2%, Fig. 5). Of note, reduced E-cadherin expression in medullary carcinoma has been correlated with poor clinical outcome [27].

Supporting these results, Fig. 7 shows mRNA expression of LGR5, E-cadherin and CK20 in normal human large intestine, colon adenomas and colorectal adenocarcinomas (moderately and poorly differentiated), using Affymetrix HG-U133 microarray data. While LGR5 was significantly upregulated in adenomas and moderately differentiated adenocarcinomas compared to normal large intestine ($p<0.001$ each), in poorly differentiated adenocarcinomas LGR5 positivity was similar to normal mucosa, significantly lower than moderately differentiated adenocarcinomas ($p<0.05$). Similar to our staining analysis, E-cadherin expression was maintained in all grades of tumor differentiation except in poorly differentiated tumors where it was significantly reduced, whereas CK20 expression was progressively lost as tumor grade increased ($p<0.001$). Altogether these results suggest a strong relationship between maintenance of glandular structure with epithelial phenotype and LGR5 expansion.

In addition to LGR5, we analyzed the microarray data for other well known Wnt target genes that are part of the intestinal stem cell signature [28]: ASCL2, EPHB3, MYC, RNF43, CD44 and SOX9 (Fig. 7). All of them showed the same expression pattern as LGR5, with significant upregulation in adenomas and moderately differentiated adenocarcinomas compared to normal large intestine ($p<0.001$), pointing to generic activation of the WNT signaling pathway. In poorly differentiated adenocarcinomas, as with LGR5 expression levels of ASCL2, EPHB3, MYC and RNF43 were again similar to normal mucosa, and significantly lower than moderately differentiated carcinomas, suggesting a downregulation following loss of glandular structure. Only CD44 and SOX9 remained significantly upregulated in poorly differentiated carcinomas, possibly because these genes are not only regulated by the WNT signaling pathway but also by BMP and PI3K for CD44 [29] and BMP and Notch for SOX9 [30].

4. DISCUSSION

The major finding of these studies is the greater than 1 log increase in LGR5⁺ stem cell-like cells in human colorectal adenomas and differentiated carcinomas. Previous studies reported LGR5 overexpression in several human epithelial neoplasms including gastric, esophageal and colorectal cancer by techniques such as IHC and RT-PCR [31–33]. Because these techniques are semi-quantitative, however, these studies do not provide a numerical estimate of the magnitude of this tumor biology. Effectively the current study identifies CRC as a

stem cell tumor, with the proviso that a generic increase in WNT program *per se* does not necessarily define stemness. Nonetheless, there is precedent for human cancers manifesting as pure or near pure stem cell diseases. In this context, blast crisis in chronic myelogenous leukemia and medulloblastoma can be considered such disease processes [34, 35]. Additional substantive investigation will be required to determine whether the amplification of the WNT program effectively renders CRC a stem cell disease. In this context, other groups have shown that overexpression of LGR5 in human CRC is accompanied by increase of other well-known WNT target genes such as ASCL2, EPHB3, MYC and RNF43 by PCR [3, 4], a profile confirmed in our microarray data in a subset of 150 colorectal tumors.

A related finding of the current work is the observation that the massive expansion of the stem cell compartment occurs during adenoma formation. These data suggest that, in CRC, malignant transformation is dependent on prior benign transformation of colonic epithelium characterized by dysregulated growth, accumulation of stem-like tumor cells, and loss of terminally differentiated cells. These observations imply the widely recognized adenoma-carcinoma progression of colorectal tumorigenesis is explained by step wise genetic alterations within a progressively expanded intestinal stem cell compartment, producing tumor cells that retain varying degrees of stem-cell properties [36]. The increase in radioresistance that occurs upon transition from normal colon to adenoma (Paty, unpublished observation) may reflect a stem cell phenotype, as LGR5⁺ crypt base columnar stem cells in the small intestines are more radioresistant than other cell types in the crypt or villus due to enhanced utilization of homologous recombination for DNA repair [20]. A similar result has been obtained in the colon as the colon epithelial stem cell represents the most radioresistant component of the colonic crypt [18].

The World Health Organization histologic grading system for colorectal adenocarcinoma in well, moderately and poorly differentiated grades, is based on the proportion of the tumor composed of crypt-like gland formation and structural resemblance to normal crypts [22, 23]. The current study demonstrates that, irrespective of the tumor grade, individual crypt-like structures show a phenotypic pattern with massive expansion of the LGR5 compartment at the base and CK20 expression in cells at the apex. Thus, high levels of LGR5 expression and CK20 expression are direct measures of crypt-like content of CRC. In addition, these data indicate that tumor crypts retain a structural polarity that resembles normal crypts. Whether this crypt polarity phenotype is driven by the LGR5⁺ cells themselves or is enforced by physical constraints of crypt architecture cannot be known from the current data set. In contrast, in poorly differentiated colorectal cancers LGR5 and CK20 expression are low and crypt-like morphology is reduced. While the process that underlines the loss of LGR5 and CK20 positivity during de-differentiation of CRC is not known, we speculate that it might be attributable to rapid evolution of CRC cells and emergence of new clones dissimilar to the cell of origin and not dependent on the WNT program for tumor maintenance and/or growth. A correlative finding is that poorly differentiated adenocarcinomas such as signet ring adenocarcinoma and medullary carcinoma, which usually display microsatellite instability and generally do not obey the usual adenoma to carcinoma progression paradigm, also show minimal LGR5 and CK20 positivity [37–39].

In summary, LGR5⁺ cell expansion is a hallmark of CRC tumorigenesis occurring during adenomatous progression that supports CRC as arguably a stem cell disease. This observation is in contradistinction to the most common conceptualization of the cancer stem cell hypothesis, which implies that a small cadre of cancer stem cells maintain tumors, as in the case of normal tissues [40–42]. If CRC is in fact a stem cell disease there are broad implications for treatment as stem cells may be radioresistant based on preferential use of homologous recombination for DNA repair [20]. However, this may also represent a vulnerability, as agents exist, such as the Mre11/Rad50/Nbs1 (MRN) inhibitor Mirin that preferentially inactivate homology-directed repair [43, 44]. Further, one can easily conceive of strategies designed to attack the WNT program in general or LGR5 cell surface presentation specifically [45–47]. Additional work is needed to address all of these possibilities.

5. CONCLUSIONS

To conclude, LGR5⁺ cell expansion is a hallmark of CRC tumorigenesis occurring in the first stage of benign transformation from normal intestinal mucosa to adenoma. This expansion is maintained in individual crypt-like glandular structures across all tumor grades and is accompanied by a generic increase in the WNT program. With the loss of glandular structure and tumor progression, both LGR5⁺ cells and CK20⁺ cells are reduced, possibly implying emergence of new de-differentiated cells. These results point to CRC as a stem cell disease with implications for CRC therapy.

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Highlights

- LGR5⁺ cells are 10-fold expanded in human colorectal cancer (CRC)
- LGR5⁺ cell expansion relates to maintenance of crypt-like glandular structure
- LGR5⁺ cells decrease in poorly differentiated CRC with loss of glandular structure
- With tumor progression, glandular structure becomes less organized
- Loss of glandular structure is accompanied by reduction of CK20 expression

Figure 1. ISH analysis of LGR5 expression in normal human small and large intestine

(A) Brightfield ISH images of LGR5⁺ cells in normal human small and large intestinal crypts. Representative images at low magnification (left panels) and at high magnification of the boxed area (middle panels) demonstrate specific LGR5 expression (red) at the base of normal human small and large intestinal crypts (black arrows delineate individual cells). An adjacent section showing constitutive GAPDH expression in all small and large intestinal crypt cells serves as control for RNA quality (right panels). Nuclei were counterstained with hematoxylin. (B) Quantitation of LGR5⁺ cells in normal human small and large intestinal crypts by fluorescent ISH. White arrows indicate LGR5⁺ cells at the crypt base. Nuclei are labeled with DAPI (blue). Data (mean $\pm$ SEM) represent percent of total crypt cells that are LGR5⁺ positive. Each dot represents a wild type crypt in human normal small (n=64, from 1 sample) or large intestinal (n=220 from 3 samples) tissue.

Figure 2. Logarithmic increase in LGR5 expression in human colon adenomas

(A) Representative fluorescent ISH images show expansion of LGR5⁺ cells (red) in both human colon tubulovillus and tubular adenomas. Nuclei are labeled with DAPI (blue). Co-staining with GAPDH (green) serves as control for RNA quality (left lower panels each). High magnification of boxed area is shown in right panels. (B) Quantitation of LGR5⁺ cells per crypt-like structure in seven human colon adenomas. Data are presented as mean $\pm$ SEM. Each dot represents an individual crypt-like structure in a human adenoma specimen (specimen 1, n=110; specimen 2, n=38; specimen 3, n=289; specimen 4, n=195; specimen 5, n=146; specimen 6, n=91; specimen 7, n=82).

Figure 3. Logarithmic increase in LGR5 expression in well differentiated CRC is attenuated in poorly differentiated CRC

(A) Fluorescent images show expansion of LGR5⁺ cells (red) in crypt-like glandular structures across all grades of cancer differentiation. However, in poorly differentiated cancers overall LGR5 expression is greatly reduced coincident with loss of glandular pattern. Nuclei are labeled with DAPI (blue). In each tumor, a low magnification fluorescent image is accompanied by a high magnification fluorescent image of the boxed area and an adjacent H&E cut. Note in the poorly differentiated specimen high magnification images are displayed from a region of residual gland like structure (upper box) and de-differentiation

(lower box). (B) Quantitation of LGR5⁺ cells in human CRC. Data are presented as mean $\pm$ SEM and represent percent of LGR5⁺ cells per glandular structure or per region of interest (ROI) in the case of poorly differentiated tumors [well differentiated CRC (specimen 1, n=194; specimen 2, n=82; specimen 3, n=61); moderately differentiated CRC (specimen 1, n=90; specimen 2, n=202; specimen 3, n=103; specimen 4, n=57; specimen 5, n=79); poorly differentiated CRC (specimen 1, n=43; specimen 2, n=105; specimen 3, n=95; specimen 4, n=141; specimen 5, n=224)].

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Figure 4. Relationship between LGR5, CK20 and E-Cadherin expression in different types of human CRC

Representative images of H&E, LGR5 ISH (fluorescent red), and CK20 and E-Cadherin IHC stained human colon specimens illustrating the complexity of stem cell architecture. The intensity of both CK20 and E-cadherin staining decreases with increasing loss of glandular structure. MD, moderately differentiated; PD, poorly differentiated.

Figure 5. Quantitation of CK20⁺ and E-cadherin⁺ cells in normal large intestine, adenomas and colorectal adenocarcinomas

Representative regions of interest (ROIs) from CK20 and E-Cadherin IHC stained human colon specimens in Fig. 4 were quantified. Data (mean $\pm$ SEM) represent percent of E-cadherin⁺ and CK20⁺ cells per glandular structure or per ROI in the case of poorly differentiated tumors [1 normal large intestine (n=12 for E-cadherin and n=10 for CK20), 3 adenomas (n=18 for E-cadherin and n=28 for CK20), 3 moderately differentiated CRCs (n=20 for E-cadherin and n=35 for CK20); 3 poorly differentiated CRCs (n=4 for E-cadherin and n=17 for CK20), 1 signet ring carcinoma (n=4 for E-cadherin and n=11 for CK20) and 1 medullary carcinoma (n=5 for E-cadherin and CK20)]. MD, moderately differentiated; PD, poorly differentiated.

Figure 6. Distribution of LGR5⁺ and CK20⁺ cells by co-staining ISH

Co-staining of LGR5 (red) and CK20 (green) by fluorescence ISH in a representative human colorectal adenoma and poorly differentiated carcinoma (PD). Nuclei are labeled with DAPI (blue). Adenoma crypt-like structures (upper panels) retain normal crypt polarity with LGR5⁺ cells located toward the base (upper left panel) and CK20⁺ cells toward the apex of the crypts (upper middle panel), displaying no co-localization of LGR5 and CK20 (upper right panel). In contrast, PD cancer presents a more disorganized pattern of expression of LGR5 (lower left panel) and CK20 (lower middle panel), also showing no co-localization (lower right panel).

Figure 7. Gene expression profile of Affimetryx HG-U133 microarray data in normal human large intestine, colon adenomas and colorectal adenocarcinomas
 Microarray analysis of LGR5, E-cadherin, CK20, ASCL2, EPHB3, MYC, RNF43, CD44 and SOX9 mRNA expression. Box-whisker plots showing relative mRNA expression (Log₂) of each gene in normal human large intestine, colon adenomas and colorectal adenocarcinomas (MD and PD), using Affimetryx HG-U133 microarray data. Kruskal-Wallis comparison of multiple pairs: * = $p < 0.0001$ vs. normal. The box indicates the interquartile range with the median; whiskers indicate the full range of data. Data are derived from 31 normal colon mucosa, 49 colonic polyps and 101 CRCs (93 MD and 8 PD). MD, moderately differentiated; PD, poorly differentiated.