

Recent Advances in Lgr5⁺ Stem Cell Research

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The discovery of leucine-rich repeat-containing G-protein-coupled receptor (Lgr5) as both a marker of adult stem cells and a critical modulator of their activity via its role as an effector of Wnt/R-spondin (Rspo) signaling has driven major advances in our understanding of stem cell biology during homeostasis, regeneration, and disease. Exciting new mouse and organoid culture models developed to study the endogenous behavior of Lgr5-expressing cells in healthy and disease settings have revealed the existence of facultative stem cell populations responsible for tissue regeneration, cancer stem cells (CSCs) driving metastasis in the gut and Lgr5⁺ and Lgr5⁺ niche cells in the lung. Here we review these recent advances and discuss their impact on efforts to harness the therapeutic potential of adult stem cells and their cancer counterparts in the clinic.

Lgr5: Past, Present, and Future

Lgr5 (a.k.a. Gpr49) first gained prominence as a marker of intestinal stem cells (ISCs) in 2007 [1]. Since then, it has been found to mark homeostatic stem cells in multiple tissues, including the antral stomach, hair follicles, mammary gland, and ovaries [2–5] (see Table 1). Lgr5 has also been documented on facultative stem cells responsible for post-injury tissue regeneration in the liver, pancreas, and stomach corpus [6–8] and on cancer stem cell and on CSCs driving tumor growth [9,10]. More recent studies have delivered advances in refining Lgr5⁺ cell behaviour refining Lgr5⁺ cell behavior during homeostasis and regeneration, delineating the mechanisms underlying Lgr5 function, elucidating the niche requirements of Lgr5⁺ stem cells and targeting Lgr5⁺ CSCs in vivo. These exciting developments bring us ever closer to realizing the therapeutic potential of Lgr5⁺ stem cells and their tumor counterparts in the clinic.

Table 1 Summary of Lgr5⁺ adult stem cells in various tissues during homeostasis and injury. [†]Adult Stem Cells in Various Tissues during Homeostasis and Injury

alt-text: Table 1					
Tissue	Lgr5 ⁺ cell type	Context	Differentiation potential	Mouse ModelReferencesSmall Intestine model	Refs
Small intestine	Crypt columnar basal cells	Homeostasis/Injury	Entire crypt	Lgr5-EGFP-Ires-Cre ^{ERT2}	[1]
Pyloric AntrumPyloric antrum	Gland base	Homeostasis	Entire gland	Lgr5-EGFP-Ires-Cre ^{ERT2}	[2]
Gastric corpusGastric corpus	Chief cells	Homeostasis Injury	None detected Entire gland	Lgr5-2A-Cre ^{ERT2}	[8]
LiverLiver	Pericentral cells	Homeostasis	None detected	Lgr5-3'UTR-IresCre ^{ERT2}	[15]
	Periportal cells	CCl ₄ injury	Hepatocytes (<i>in vitro</i>) FAH ^{-/-} <i>in vitro</i> FAH ^{+/+} mouse	Lgr5-3'UTR-IresCre ^{ERT2}	[7]

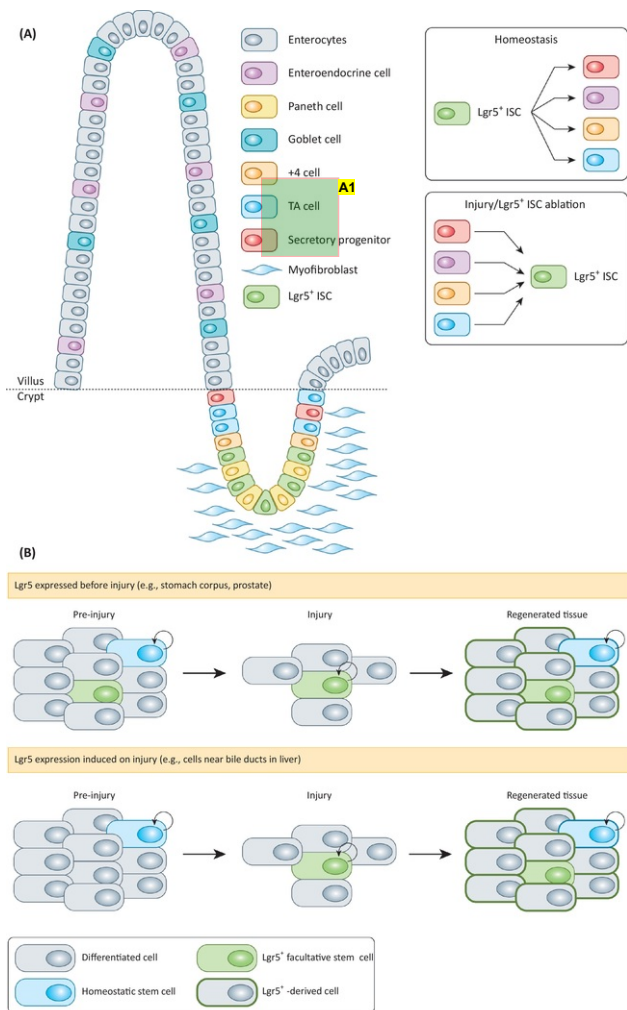
Lung Lung	Mesenchymal cells	Homeostasis	None reported	<i>Lgr5-3'UTR-IresCre^{ERT2}</i>	[64]
Mammary gland Mammary gland	Basal and myoepithelial cells	Homeostasis Injury	Basal cells and luminal cells Whole mammary gland	<i>Lgr5-EGFP-Ires-Cre^{ERT2}</i> <i>Lgr5-DTR</i>	[4,71] [4]
Prostate gland Prostate gland	Luminal cells, basal cells	Homeostasis Injury (castration)	Luminal cells Basal cells, luminal cells	<i>Lgr5-EGFP-Ires-Cre^{ERT2}</i>	[38]
Panereas Pancreas	Pancreatic ductal cells	Injury (partial duct ligation)	Endocrine and ductal cells (in vitro) (in vitro)	<i>Lgr5-lacZ</i>	[7]
Hair Follicles Hair follicles	Outer bulge cells	Homeostasis/ I njury	Entire hair follicle	<i>Lgr5^{LacZ}</i> , <i>Lgr5-EGFP-Ires-Cre^{ERT2}</i> Lgr5-DTR [3] Ovary , <i>Lgr5-DTR</i>	[3]
Ovary	Hilum ovarian surface epithelial (OSE) cells	Homeostasis	OSE cells	<i>Lgr5-EGFP-Ires-Cre^{ERT2}</i>	[5]
Eye — Retina Eye — retina	Amacrine cells	Homeostasis Injury	None Bipolar cells, Mu ller cells	<i>Lgr5-EGFP-Ires-Cre^{ERT2}</i>	[72,73]
Cochlea Cochlea	Sensory hair cells Supporting cells	Homeostasis	Sensory hair cells (organoids)	<i>Lgr5-EGFP-Ires-Cre^{ERT2}</i>	[74]
Tongue Tongue	Posterior circumvallate (CV) papillae	Homeostasis Injury	CV papillae Taste cells Perigemmal C cells CV papillae	<i>Lgr5-EGFP-Ires-Cre^{ERT2}</i>	[75]
Olfactory epithelium	Globose basal cells	Homeostasis/ I njury	Olfactory sensory neurons Bowman's glands Sustentacular cells	<i>Lgr5-EGFP-Ires-Cre^{ERT2}</i>	[76]
Olfactory bulb Olfactory bulb	Neurons	Homeostasis	None	<i>Lgr5-EGFP-Ires-Cre^{ERT2}</i>	[77]
Cerebellum Cerebellum	Cerebellar granule neurons	Homeostasis	None	<i>Lgr5-EGFP-Ires-Cre^{ERT2}</i>	[78]

Lgr5 is a potentiator of Wnt/ β -catenin sLgr5 is a Potentiator of Wnt/ β -Catenin Signaling

Lgr5 encodes a seven-transmembrane domain receptor belonging to the rhodopsin family of G-~~protein~~-~~protein~~-coupled receptors. Lgr5 and its closely related homologs Lgr4 and Lgr6 are high-affinity co-~~receptors~~ of the secreted R-~~spondin~~ (~~Rspo1-4~~)~~spo1-4~~ growth factors, which also bind to the transmembrane E3 ubiquitin ligases Rnf43/Znrf3. In the absence of R-~~spondin~~~~spos~~, Rnf43/Znrf3 antagonize Wnt signaling by targeting Frizzled (Fzd) receptors for degradation [11]. Binding of R-~~spondin~~~~spos~~ to Lgr4/5/6 sequesters Rnf43/Znrf3, resulting in stabilization of the Wnt/Fzd receptor complex at the cell surface and ~~a~~-concomitant amplification of canonical β -catenin-dependent Wnt signaling. As Lgr5 and Rnf43/Znrf3 are also transcriptional targets of Wnt/ β -catenin signaling [12–14], this constitutes an intricate feedback mechanism in which canonical Wnt pathway propagation necessitates the presence of both R-~~spondin~~~~spos~~ and Wnt ligands [15].

General ~~principles of homeostatic stem cell biology gleaned from Lgr5~~+Principles of Homeostatic Stem Cell Biology Gleaned from Lgr5⁺ ISCs

The intestinal crypt, one of the best-defined adult stem cell models, has long been known to drive the massive daily regeneration occurring ~~with~~in the intestinal epithelium in a Wnt/ β -catenin signaling-dependent manner. Lineage tracing with the Wnt target gene reporter Lgr5-eGFP-Ires-CreERT2 mouse model and the obligate requirement for Wnt/R-~~spondin~~~~spos~~ factors in *in vitro* organoid culture unequivocally established the Wnt requirement of ISCs. Lgr5 is expressed by a small pool of 12–15 crypt base columnar (CBC) cells intermingled with differentiated Paneth cells, which comprise an epithelial niche component. These CBC cells are long-lived stem cells ~~which~~~~that~~ undergo neutral competition to maintain the stem cell pool [16] and give rise to multiple differentiated progeny including Paneth cells, goblet cells, absorptive enterocytes, and enteroendocrine cells [1,16] (Figure 1a). ~~Single Lgr5⁺A~~. ~~Single Lgr5⁺~~ ISCs can generate *in vitro* organoids displaying the full complement of differentiated intestinal lineages in the presence of Wnt and R-~~spondin~~~~spos~~, demonstrating their stem potential [17]. Similar lineage tracing, organoid generation, and clonal dynamics analysis strategies using the Lgr5-eGFP-Ires-CreERT2 mouse model have identified Lgr5⁺ homeostatic stem cells in other tissues [2,3,18]. Nonetheless, Lgr5⁺ ISCs are the prototypical stem cell population that has been at the forefront of many stem cell-related discoveries/studies.



Trends in Cell Biology

Figure 1 Leucine-Rich Repeat-Containing G Protein-Coupled Receptor 5 (Lgr5)⁺ Stem Cells during Homeostasis and Regeneration. (A) Homeostatic turnover of the intestinal epithelium is fueled by Lgr5⁺ intestinal stem cells (ISCs) intercalated between differentiated Paneth cells at the crypt base. Paneth cells, together with surrounding myofibroblasts, form the ISC niche. During homeostasis, Lgr5⁺ crypt base columnar (CBC) cells differentiate into all cell lineages in the small intestine. In the event of injury or loss of Lgr5⁺ ISCs, other cell populations can dedifferentiate to generate Lgr5⁺ ISCs to regenerate the epithelium. (b) In Lgr5⁺ In Lgr5⁺ facultative stem cell systems, Lgr5 expression can be present before injury (upper panel) or induced upon injury (lower panel). During homeostasis tissue renewal is driven by the Lgr5⁺ homeostatic stem cell. Upon injury which ablates the homeostatic stem cells, Lgr5⁺ facultative stem cells are activated to proliferate and generate homeostatic stem cells and differentiated cells to regenerate the tissue.

alt-text: Figure 1

Annotations:

A1. Please Replace TA with "Transit amplifying"

~~R-spondin-secreting mesenchymal niches drive Lgr5⁺ ISC r~~Rspo-Secreting Mesenchymal Niches Drive Lgr5⁺ ISC Renewal

Signals emanating from the local microenvironment (niche) are integral to the maintenance of stem cells. Secreted Wnts and R-spondin_{SPCs} constitute essential niche signals generally found in the vicinity of Lgr5-dependent stem cell compartments. In the

intestinal crypt, multiple sources of Wnt/R-~~spendin~~~~spo~~ signals, namely Wnt-secreting Paneth cells at the crypt base and Wnts/Rspo3-producing myofibroblasts in the underlying stroma, act redundantly to maintain the ISC population [19,20]. A possible source of Wnt2b/Rspo1 has also been reported in a CD34⁺~~Agg38⁺/gp38⁺~~ mesenchymal population within the intestinal lamina propria [21]. Similarly, in the antral stomach epithelium, Lgr5⁺ (~~superscript~~) stem cells at the gland base are maintained by Rspo3 secreted by adjacent myofibroblasts [22], and the Lgr5⁺ pericentral cells in the homeostatic liver [15] are likely to be sustained by Rspo3 expression in pericentral endothelial cells [23].

~~Though Wnts and R-spendin~~~~Although Wnts and Rspos~~ play cooperative roles in maintaining intestinal crypt homeostasis, their relative contributions and roles have proven difficult to ascertain due to their overlapping functions in Wnt/~~Agg~~-catenin signaling. A recent study teased apart the relative contributions of Wnt and R-~~spendin~~~~signaling on Lgr5⁺~~~~spo signaling to Lgr5⁺~~ ISC activity using adenoviral injections of surrogate Wnt signaling molecules [24]. They demonstrated that Wnt ligands alone were insufficient to induce ISC self-renewal and expansion beyond a certain threshold. Wnt molecules merely acted as priming factors to confer basal competency ~~to ISCs to respond to R-spendin~~~~on ISCs to respond to Rspos~~ by maintaining Lgr5 expression. Instead, R-~~spendin~~~~spos~~ played the dominant role in governing ISC self-renewal and pool size. It remains to be seen ~~if whether~~ this model can be extrapolated to Lgr5⁺ stem cells in other tissues. Nonetheless, this is consistent with the notion that Wnts are required for stem cell maintenance and R-~~spendin~~~~spos~~ for stem cell expansion. Concordant with that idea, R-~~spendin~~~~spos~~ have previously been found to exert dramatic effects on the expansion of stem cell populations in the intestinal crypt, antral stomach, and liver [15,22,25,26] and are essential for propagating ~~on of~~ Lgr5⁺ stem cell-dependent organoid cultures *in vitro* [17,27].

Heterogeneity within the Lgr5⁺ ISC populationSingle in the Lgr5⁺ ISC Population

~~Single-cell~~ profiling studies have characterized ~~Lgr5⁺ ISCs as being~~~~zed Lgr5⁺ ISCs as~~ heterogeneous, comprising both ISCs and early progenitors (bipotent progenitors [28], Paneth/enteroendocrine precursors [29], or transit amplifying cells [30]). However, there is currently no consensus on the frequency and proliferation status of the constituent subpopulations in the Lgr5⁺ ~~a population. This can likely be attributed to~~ ~~population. This is likely to be attributable~~ to differences in sequencing methodologies, analysis algorithms, and cell isolation procedures. As observed by *in vivo* live imaging, Lgr5⁺ ISCs are in constant flux between ~~the~~ crypt base and higher crypt positions, which influences their probability of remaining as ISCs or differentiating into transit amplifying cells/early progenitors [31]. ~~As such~~ ~~Thus~~, less sensitive sequencing may ascribe homogenous transcriptome profiles to these functionally equivalent Lgr5⁺ ISCs, while deeper sequencing of their transcriptomes could reveal the subtle differences between these subpopulations.

Injury-Driven Stem Cell Plasticity

The intestinal epithelium displays a ~~quite~~ remarkable regenerative capacity, largely due to its ability to restore the resident stem cell pool following injury. ~~Indeed, targeted ablation of the Lgr5⁺ ISCs in Lgr5-DTR4~~ ~~Targeted ablation of Lgr5⁺ ISCs in Lgr5-DTR~~ mouse models has been shown to trigger a rapid, adaptive response from multiple crypt populations, including putative reserve stem cells at the +4 crypt position [32], specified enterocyte and secretory progenitors [33,34], Paneth cell precursors [35], and enteroendocrine cells [34] (Figure 1aA), to ensure rapid repopulation of the Lgr5⁺ stem cell pool. The impressive plasticity exhibited by these committed populations in converting to Lgr5⁺ stem cells remains poorly understood, but epigenetic mechanisms are likely to be involved [36]. The fact that reacquisition of Lgr5 expression is a common feature of the injury-driven reprogramming of the various lineages to a stem cell state implicates reactivation of a Lgr5-potentiating Wnt signaling-dependent stem cell program as a critical driver of this process. It is also likely that induction of Lgr5 expression on the reprogrammed stem cells allows them to respond to local R-~~spendin~~~~spo~~ signals to control the size and activity of the nascent population.

Such functional plasticity has also ~~been~~ observed in other tissues (Table 1). In the antral stomach, Lgr5⁺ stem cell ablation activates an independent Axin2⁺Lgr5⁺ population located in the lower isthmus region to repopulate the basal Lgr5⁺ population [22]. In hair follicles Lgr5 and CD34 mark both distinct and overlapping stem cell compartments [3]. Following ablation of Lgr5⁺ hair follicular stem cells in the lower bulge and hair germ, an inflammatory response led to ~~a~~ conversion of CD34⁺/Lgr5⁺ cells in the upper bulge to Lgr5⁺ stem cells to replenish hair germ cells and restore homeostasis [37]. The inflammatory environment, mediated by NF-κB signaling, was suggested as a possible mechanism to trigger Wnt signaling pathway activation and the reemergence of Lgr5⁺ stem cells.

These findings highlight the pivotal role played by niche-driven plasticity of local cell populations in effecting the rapid repopulation of Lgr5⁺ stem cells following injury to maintain tissue integrity and function. While the mechanistic insight into this reprogramming process remains limited, Lgr5-potentiating Wnt/~~Agg~~-catenin signaling appears to play a central role. ~~The~~ identification of other signaling pathways driving this formation of nascent Lgr5⁺ stem cells from committed progenitors will greatly aid in our understanding of cellular plasticity and ability to harness it for regeneration.

Lgr5 in facultative stem cell populations driving rLgr5 in Facultative Stem Cell Populations Driving Regeneration

Activation of stem cells in otherwise slowly dividing or quiescent cell compartments in response to injury is a common feature in the regeneration of some tissues (Figure 1bB). Such facultative stem cell populations expressing Lgr5 have been found in the stomach corpus and liver (Table 1). A novel population of Lgr5⁺ stem cells was recently reported in the adult stomach corpus using a new Lgr5^{2ACreERT2} mouse model ~~which that~~ exhibits non-variegated Cre expression, in contrast to the earliest Lgr5-eGFP-ires-CreERT2 model [1,8]. During homeostasis Lgr5 is expressed in a subpopulation of post-mitotic, differentiated chief cells in the corpus gland base. However, ablation of differentiated parietal cells led to the rapid conversion of Lgr5⁺ chief cells into proliferating, multipotent stem cells capable of long-term maintenance of the corpus epithelium. Likewise, in the adult prostate normally quiescent Lgr5⁺ basal cells exhibited bipotency following castration to contribute to prostate regeneration [38].

In the liver, studies using different Lgr5 mouse models have yielded divergent findings [6,15]. In the initial study, bipotent Lgr5⁺ cells were reported in the vicinity of Sox9⁺ biliary ductal cells following acute carbon tetrachloride-induced liver damage [6]. Of note, Lgr5⁺ cells were not detected in the liver under homeostatic conditions in this model [6]. Lgr5⁺ ductal cells gave rise to hepatocytes and biliary duct cells *in vitro* and in an *in vivo* FAH^{-/-} mouse model following engraftment of liver organoids [6]. Lgr5⁺ ductal cells were also found to exert a protective effect against fibrosis following acute liver damage [26], but the *in vivo* regenerative capability of the Lgr5⁺ population following tissue damage ~~has yet remains~~ to be demonstrated. A more recent study using a new mouse model revealed an

Lgr5⁺ population in pericentral cells during homeostasis [15]likely that is likely to be sustained by Rspo3 expression in pericentral endothelial cells [23]. These findings corroborate a recent study whichthat found pericentral cells to be labelled by another Wnt target gene, Axin2 [15]. However, contrary to their findings, the non-proliferative Lgr5⁺ population did not expand under homeostatic conditions [15,39]. Clearly, further studies are needed to clarify and define the role of Wnt-dependent populations under homeostatic and regenerative conditions.

Lgr5 in ~~cancer initiation, progression and m~~Cancer Initiation, Progression, and Metastasis

It is widely accepted that ~~cancer-stem-cells (CSCs)~~CSCs, a small tumor population with stem cell characteristics, can drive cancer initiation and/or progression by fueling tumor growth and metastases. An increasing number of studies demonstrate that these ~~cancer stem-cell~~CSCs are highly plastic and phenotypically heterogeneous and can revert readily from differentiated cells under permissive conditions [40]. Co-effectors of LGR5 (e.g., RNF43, RSPO2, RSPO3) that are involved in potentiation of Wnt signaling are common targets of mutations in human cancers of the colon [41,42], stomach [43], pancreas [44–46], liver [47] and ovary [48]. Furthermore, expression of *LGR5* is also significantly increased in subsets of colorectal, liver, pancreatic, stomach and endometrial cancers [49,50]. Hence, amplification of Wnt/~~β~~-catenin signaling due to dysregulation of the Rspo~~Lgr5/~~Lgr5-Rnf43 axis is a significant driving force in a subset of human cancers.

The inherent plasticity and longevity of adult stem cells is considered to render them particularly susceptible to oncogenic transformation and consequently to serve as an origin of cancer. In accordance with this, Lgr5⁺ stem cells in physiologically normal tissues have been shown to serve as a cell~~-of-origin-of~~ of origin for intestinal adenomas, colorectal cancer (CRC)~~colorectal-cancer, gastric-cancer, gastric cancer~~ and squamous cell carcinoma following oncogenic mutation ~~in-vivo~~in vivo [8,10,51–54]. Conditional knockout of the central Wnt signaling regulator Apc and/or expression of oncogenic Kras in Lgr5⁺ISCs is sufficient to drive intestinal adenoma formation [10,52], whereas targeted mutation of Pten and Smad4 in Lgr5⁺ cells in the gastric antrum leads to an invasive form of gastric cancer [53]. In the gastric corpus, oncogenic Kras activation in Lgr5⁺ chief cells was sufficient to induce metaplastic lesions, which are putative precursors ~~to~~of invasive gastric cancer [8].

Although homeostatic Lgr5⁺ stem cells are potent tumor-initiating cells in various adult tissues, the existence of tumor-resident Lgr5⁺ CSCs responsible for fueling the subsequent growth and spread of the cancer has proved more challenging to ascertain. Numerous studies to identify LGR5⁺ CSC populations and/or correlate LGR5 expression with clinical prognosis in various cancers have largely relied on poorly validated antibodies, leading to multiple, poorly substantiated claims of Lgr5⁺ CSC populations in human colon, gastric, esophageal, cervical, papillary thyroid and basaloid skin cancers [55–60]. Some indication of their CSC potential was shown when Lgr5⁺ cells in intestinal adenomas, invasive intestinal adenomas and invasive lesions of antral tumors were found to be highly proliferative, potentially correlating with a role in driving tumor expansion and invasion [51,53]. More definitive evidence of Lgr5⁺ CSCs was provided when clonal fate mapping analyses revealed that tumor resident Lgr5⁺ cells directly contribute to the growth of intestinal adenomas in mice [51]. In human ~~colorectal-cancer, purified LGR5+CRC, purified LGR5+~~ cells isolated from patient-derived organoids displayed CSC characteristics in transplantation assays [55]. However, the most compelling evidence of human LGR5⁺ ~~CSC activity in~~ ~~colorectal-cancer+~~ CSC activity in CRC was recently provided using elegant lineage tracing of LGR5⁺ cells in xenotransplanted, patient-derived CRC organoids, documenting a major contribution of the endogenous LGR5⁺ population to tumor growth *in vivo* [9,61].

Two recent studies employed a targeted ablation approach to highlight the contribution of Lgr5⁺ ~~CSCs~~ CSCs to CRC progression and to document the existence of CSC plasticity in primary tumors and their metastases (Figure 2). In the first study, targeted ablation of DTR-expressing Lgr5⁺ cells in primary mouse colorectal tumors via DT administration did not achieve the expected tumor regression, although subsequent tumor growth was impaired (~~Fig 2b~~Figure 2B) [10]. Tumor size in the absence of the Lgr5⁺ CSCs was postulated as being maintained via the acquisition of Myc-driven proliferation within the differentiated tumor bulk. Importantly, cessation of DT treatment facilitated the rapid repopulation of the Lgr5⁺ cells from the Lgr5⁺ tumor bulk cells, providing direct evidence of tumor cell plasticity reminiscent of that observed in normal tissue following injury (Figure 2eC). Similar findings were reported in a second study using a xenograft model of human colorectal cancer [9]. Using an iCaspase9 cassette knocked into the *LGR5* locus of the organoids, ablation of LGR5⁺ cells through induced apoptosis temporarily reduced tumor size (Figure 2bB). However, this also prompted differentiated tumor cells to begin proliferating. Similar to the mouse model [10], removal of the apoptotic stimulus quickly led to ~~reappearance of LGR5+~~ the ~~reappearance of LGR5+~~ cells and tumor regrowth (Figure 2eC), highlighting the plasticity of the LGR5⁺ bulk cells to convert into LGR5⁺ ~~CSCs~~ CSCs.

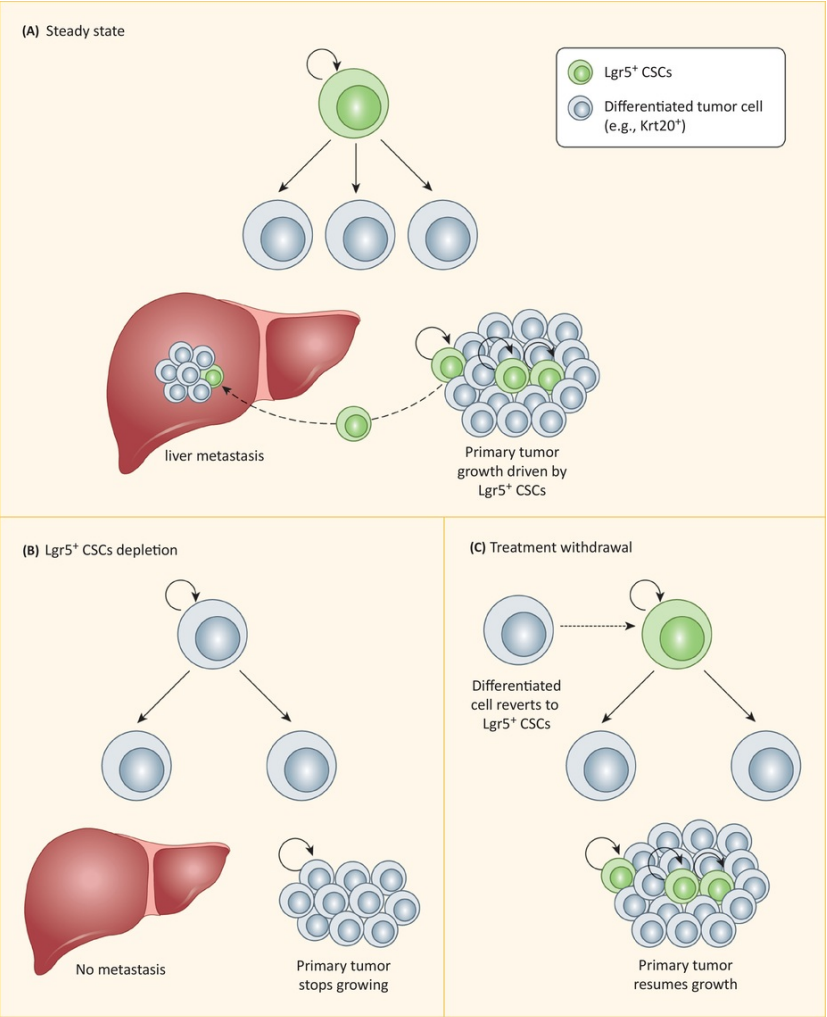


Figure 2 **Lgr5⁺ cancer stem cells in colorectal cancer drive primary tumor growth and metastasis.** (a) **Leucine-Rich Repeat-Containing G Protein-Coupled Receptor 5 (Lgr5)⁺ Cancer Stem Cells (CSCs) in Colorectal Cancer Drive Primary Tumor Growth and Metastasis.** (A) During steady state, Lgr5⁺ CSCs drive the growth of the primary tumor mass and seed metastases in liver. (b) When Lgr5⁺ CSCs are chemically ablated, differentiated tumor cells are induced to proliferate to maintain the tumor, but the size remains stagnant and no liver metastasis is observed. (c) However, on withdrawal of the chemical treatment some differentiated tumor cells revert to Lgr5⁺ CSCs, which in turn generate more daughter cells that contribute to primary tumor growth.

all-text: Figure 2

By contrast, the initiation and maintenance of liver metastases derived from the primary CRCs was found to be strictly dependent on Lgr5⁺ CSCs, as DT treatment substantially reduced the initial burden and subsequent growth of established liver metastases (Figure 2aA) [10]. These observations highlight the influence of the local tumor microenvironment on cell plasticity.

Both studies showed that while Lgr5⁺ cells are indispensable for tumor initiation and expansion of colorectal tumors, targeting Lgr5⁺ CSCs alone is insufficient to achieve complete tumor regression. Nonetheless, reduction of tumor bulk, although not its complete eradication, appears to be achievable by administering cytotoxic drugs conjugated to anti-LGR5 antibodies that selectively target LGR5⁺ CSCs, as demonstrated by two groups in xenograft rodent models [49,50]. It remains to be seen whether this approach would be efficacious in a clinical setting. Together these findings underscore the therapeutic importance of deciphering the identity of the Lgr5⁺ CSC niche responsible for the plasticity that ensures the survival of the primary tumors for complete remission. Effective

treatment regimens for primary CRCs are likely to require the development of combinatorial therapies concomitantly targeting the Lgr5⁺ CSCs and the tumor plasticity mechanism. Encouragingly, solely targeting Lgr5⁺ CSCs is likely to be useful in preventing or reducing the metastatic burden.

Emerging Themes

R-spondin-independent fspo-Independent Functions of Lgr5

Despite being one of the most widely recognized stem cell markers, there have been relatively few studies exploring the molecular function of Lgr5 in adult stem cells (see Outstanding Questions). A possible R-spondin^{spo}-independent role for Lgr5 in strengthening cell-cell adhesion in ISCs was recently suggested [62]. The study found that the cytoplasmic domain of Lgr5 phosphorylates IQ motif-containing GTPase-activating protein 1 (IQGAP1), a scaffolding protein that binds to Rac1 and Cdc42 to regulate cell adhesion and the actin cytoskeleton [62] and modulate activities of the Wnt pathway, F-actin, MAP Kinases^{kinases}, and Rho GTPases [62]. *In vivo* and *in vitro* abrogation of Lgr5 expression resulted in loss of cortical F-actin, disrupted the localization of adhesion-associated proteins, and reduced cell-cell adhesion. While it is unclear how this contributes to the stemness properties of Lgr5⁺ cells, increased cell adhesion could facilitate the anchoring of stem cells to the Wnt- and R-spondin^{spo}-rich intestinal niche to maintain ISC identity [63], whereas reduced Lgr5 expression allows for the mobilization of differentiating cells up the intestinal crypt.

Lgr5 as supporting niche eLgr5 Supporting Niche C(Change to "Lgr5+ Cells as Supporting Niche Cells")ells

It was recently discovered that Lgr5⁺ cells serve as niche cells during lung homeostasis and regeneration [64], expanding the functional repertoire of cells marked by Lgr5 (Table 1). Lgr5 and Lgr6 were expressed in distinct mesenchymal cell populations in the lung (Figure 3), with Lgr5 marking a small population of alveolar mesenchymal cells and Lgr6 labeling airway smooth muscle cells (aSMCs). Upon damage to club cells in the bronchiolar epithelium, secretion of Fgf10 from Lgr6⁺ cells enhanced club cell expansion. Co-culture of club cell-derived organoids with Lgr5⁺ mesenchymal cells also promoted organoid formation and increased the alveolar differentiation of club cells, and supported the expansion of alveolar type 2 (AT2) cells, mainly through secretion of Wnt ligands by Lgr5⁺ cells. Depletion of Lgr5⁺ cells through *in vivo* ablation studies would further aid in defining its role in lung homeostasis and regeneration.

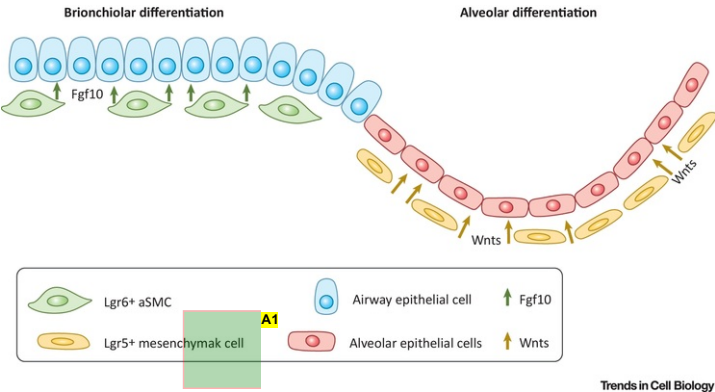


Figure 3 Lgr5 and Lgr6 serve as niche signals in different compartments of the lung mesenchyme. Leucine-Rich Repeat-Containing G Protein-Coupled Receptor 5 (Lgr5) and Lgr6 Serve as Niche Signals in Different Compartments of the Lung Mesenchyme. In the adult lung, Lgr5 is expressed by mesenchymal cells adjacent to alveolar epithelial cells. Lgr5⁺ cells secrete Wnt ligands (e.g. Wnt3a, Wnt5b and Wnt3a, Wnt5b, Wnt7b) to promote alveolar lineage differentiation from epithelial progenitor cells. Lgr6 is expressed by airway smooth muscle cells (aSMCs) lining the bronchiolar epithelium. Secretion of Fgf10 by Lgr6⁺ aSMCs promotes differentiation of airway epithelial cells.

alt-text: Figure 3

Annotations:

A1. Mesenchymal

Interaction with mInteraction with Microbiome

The effects of host-microbial interactions on our physiology are undisputed. However, any effect of these interactions on the adult stem cells remains poorly characterized. *Helicobacter pylori*-infected individuals have a higher risk of developing gastric cancer owing to increased inflammation in the mucosa [65]. In the gastric antrum, *H. pylori*-induced chronic inflammation causes upregulation of stromal R-spondin^{spo}3 in underlying myofibroblasts, resulting in the expansion of Axin2⁺Lgr5⁺ proliferating cells in the lower isthmus region of the gland. Surprisingly, unlike in intestines and liver, and liver, the proliferation and population size of Lgr5⁺ stem cells in the gland base were unaffected by the increased Rspo3 from the myofibroblasts or ectopic injection of exogenous Rspo1 [22]. In another

recent study, the bacteria *Fusobacterium nucleatum* has been reported to be closely physically associated with colorectal tumors and metastatic lesions, promoting tumor growth [66]. While the link with CSCs was not explored, it is conceivable that *F. nucleatum* could influence CSC behavior since Lgr5⁺ CSCs are responsible for seeding metastases [10]. Given the increasing attention to microbiome studies, their interaction with host stem cells in homeostatic and cancer contexts and the effect on signaling pathways like Wnt signaling is poised to garner more momentum in research.

Stem cell metabolism

Recent studies using Lgr5⁺ ISC as a model have revealed the major influence of metabolism in stem cell maintenance and differentiation [67,68]. In a groundbreaking study, ISC and Paneth cells were found to have distinct metabolomes, with higher pyruvate:lactate ratio and respiration detected in ISC than in Paneth cells [67], a reflection of their high energy demand owing to a high turnover rate. The enhanced mitochondrial oxidative phosphorylation (OXPHOS) in ISC was fueled by lactate produced from Paneth cells, once again highlighting the dependence of crypt stem cells on neighboring niche cells. As a result of increased OXPHOS, mitochondrial-derived reactive oxygen species produced in ISC activated the p38 MAP kinase pathway, thereby promoting cell differentiation and crypt formation *in vitro* [67]. These findings provide evidence of crosstalk between energy metabolism and signaling pathways in homeostatic stem cell maintenance and differentiation and underscores the orchestration of multiple cellular processes in stem cell regulation.

Another recent study explored pyruvate metabolism as a mechanism for stem cell regulation and differentiation in ISC [68]. Similar to the above study [67], a comparison between Lgr5⁺ ISC and Lgr5⁺ ISC and their progeny cells also unveiled metabolic differences between different cell populations. Higher metabolic activity was found in progeny cells compared to ISC, owing to higher mitochondrial pyruvate carrier (MPC) expression in differentiating cells [68]. MPCs are molecules that transport pyruvate into the mitochondria and thereby play a pivotal role by directing energy metabolism to occur in either the cytoplasm (glycolysis) or mitochondria (OXPHOS). Loss of mitochondrial pyruvate genes inhibited differentiation and allowed the expansion of the stem cell compartment in *Drosophila* and mouse intestines, *Drosophila* and mouse intestine as well as in *in vitro* organoids. It was suggested that Myc could be involved in suppressing MPC gene expression, although it remains unclear how differences in MPC activity can lead to changes in cell fate. Nevertheless, this study provides a significant step in addressing the differences in energy metabolism among different stem cell populations. Such metabolic differences have been postulated to apply to cancer stem cell CSCs and the more differentiated tumor cells as well [reviewed in 69] [reviewed in [69]], revealing potentially new avenues for targeting stem cell metabolism in the clinic.

Concluding remarks

Recent studies have provided significant advances in our understanding of stem cell hierarchies and plasticity during homeostasis, regeneration and cancer. Studies into, and cancer. Studies of the intestinal stem cell hierarchy have placed Lgr5⁺ ISC at the apex of the hierarchy in crypt homeostasis and regeneration. However, while the dynamic nature of stem cell plasticity is advantageous for regeneration, it also poses a challenge for therapeutic intervention in tumor progression and metastasis. A thorough understanding of niche requirements, cellular metabolic pathways, and factors that drive stem cell and CSC plasticity will be essential for developing the development of more effective cancer therapies. Additionally, little is known about the molecular mechanisms regulating Lgr5 expression in homeostatic stem cells and CSCs. Delineating of such regulatory mechanisms could also propel efforts towards Lgr5-targeted therapies.

While the field has made great strides in stem cell and disease modeling using *in vitro* organoid cultures, much work is still needed to fully harness these advances for clinical applications. By combining genome editing technologies with organoid cultures, it is now possible to model disease mutations more closely than ever before and track the behaviour of LGR5⁺ and to track the behavior of LGR5⁺ stem cells in physiologically normal and cancer contexts [9,10,61]. A recent breakthrough in the culture of organoids using a synthetic hydrogel network and mechanically dynamic matrix as an extracellular matrix substrate [70] in place of undefined, animal-derived Matrigel has cleared a major obstacle for its use in clinical settings and paves the way for the possibility of autologous transplantation in regenerative medicine. In light of the roles of Lgr5 as a stem cell marker and potentiator of Wnt signaling in numerous stem cell contexts, techniques such as labeling LGR5⁺ stem cells and modulating LGR5 levels in patient-derived organoids can be applied to disease modeling, drug testing, transplantation, and etc. (see Outstanding Questions). These advances will continue to provide exciting areas and avenues for future research in stem cell and tumor biology.

Outstanding questions

Does Lgr5 have other molecular functions besides potentiating Wnt/β-catenin signaling? How do these other molecular functions contribute to stem cell maintenance?

Due to conflicting findings, the role of Lgr5⁺ central vein hepatocytes in the liver during homeostasis and regeneration needs to be clarifiedDoes LGR5 mark cancer stem cell?

Does LGR5 mark CSCs of other cancers besides human colorectal cancer? Is LGR5 function required for the initiation, maintenance, and/or proliferation of cancer stem cell CSCs?

How can niches of Lgr5⁺ cancer stem cell CSCs be therapeutically targeted to prevent dedifferentiation of non-stem tumor cells in order to achieve complete eradication of tumors?

Extend techniques for labeling or genetically modifying LGR5⁺ modification of LGR5⁺ stem cells in patient-derived organoids for personalized drug testing, disease modeling, and transplantation in clinical settingsUnlited-reference[69].

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Footnotes

*In the Lgr5-DTR mouse model, the *Diphtheria Toxin-R* diphtheria toxin receptor (DTR) cassette is knocked into the endogenous *Lgr5* locus. This induces apoptosis in Lgr5⁺ cells upon administration of Diphtheria toxin (DT).

Highlights

Lgr5 potentiates Wnt/ β -catenin signaling with R-spondin *Znrf3* (Rspo), *Znrf3*, and Rnf43.

Lgr5 marks homeostatic and facultative stem cells in numerous vertebrate tissues.

Lgr5⁺ stem cells depend on the R-spondin Rspo ligands from the stem cell niche, a key determinant of stem cell identity. The niche can reprogram more differentiated cells to replenish lost stem cells during regeneration, accounting for stem cell plasticity.

LGR5 is expressed in some human cancers and colorectal cancer stem cells (CSCs).

Recent advances in lineage tracing and modulation of LGR5⁺ cells in human and murine organoids established their roles in tumor initiation, growth and metastasis, and metastasis but not tumor survival. Lgr5⁺ CSCs can potentially be targeted by drugs conjugated to anti-LGR5 antibodies.

Other emerging themes of Lgr5 include cell adhesion functions of Lgr5 protein, the Lgr5 protein and its expression in supporting niche cells.

Queries and Answers

Query: The author names have been tagged as given names and surnames (surnames are highlighted in teal color). Please confirm if they have been identified correctly.

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Answer: Fig 1A: Please replace "TA" with "Transit amplifying" Fig 3: Spelling error in legend within the figure - Lgr5+ mesenchymal cell not mesenchymak

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