# *Lgr5* joins the club of gastric stem cell markers in the corpus

Toby J. Phesse[1] and Owen J. Sansom[2,3]

[1] European Cancer Stem Cell Institute, Cardiff University, Cardiff, CF24 4HQ, UK.

[2] Cancer Research UK Beatson Institute, Garscube Estate, Bearsden, Glasgow, G61 1BD, UK.

[3] Institute of Cancer Sciences, University of Glasgow. Glasgow. G611BD

Correspondence should be addressed to either Owen Sansom, Tel: +44 0141 330 3953; Email: o.sansom@beatson.gla.ac.uk or Toby Phesse. Tel: +44 02920 874829 Fax: +44 02920 874116; Email: phesset@cardiff.ac.uk.

**Several markers of gastric stem cells have been identified in recent years. Now a new study demonstrates that *Lgr5* marks a population of reserve stem cells located at the base of the corpus glands of the gastric epithelium, which can also act as a cell of origin for gastric tumourigenesis [1].**

The gastric epithelium is replenished constantly, which strongly suggests the existence of a population of stem cells to fuel this process [2]. One of the first populations of stem cells identified in the stomach utilised the *Lgr5-EGFP-ires-CreERT2* mouse strain to lineage trace functional gastric stem cells [2]. *Lgr5* marks stem cells in several tissues, including the intestine [3] and skin [4], which are often highly proliferative. However, transgenic silencing of this mutant locus resulted in variegated expression in some tissues, such as the antrum and intestine, and in these cases the full extent of *Lgr5* as a stem cell marker has been difficult to ascertain. This limitation of the original strain led Leushacke *et al.* to develop a non-variegated mouse, *Lgr5-2A-CreERT2*, that allows further investigation of this gene [1].

Using the new *Lgr5-2A-CreERT2* mouse, the authors detect *Lgr5*+ differentiated chief cells in the stomach, located broadly at the base of the corpus glands. Lineage tracing shows that although these *Lgr5* expressing cells do not give rise to fully traced glands during homeostasis, they are activated in response to damage upon which they generate fully traced corpus glands, indicating a function as reserve stem cells.

Given the abundance of previously identified stem cell markers, it is important to place the *Lgr5*+ stem cells in context with other gastric stem cell markers (**Fig. 1**). Lineage tracing has demonstrated that *Sox2* [5], *Troy* [6], *Mist1* [6] and *Runx1* [7] all mark cells that can trace full corpus glands originating from the isthmus [5-7]. However, they have all been reported to be expressed in the base of the corpus also, with basally located *Troy*+ cells giving rise to fully labelled glands on rare occasions [6], and also acting as a reserve stem cell after injury [8]. *Mist1*+ [8] and *Runx1*+[7] cells at the base of the corpus glands can act as reserve stem cells upon tissue damage as well, but it is the *Mist1*+ [6]and *Runx1*+[7] cells residing in the isthmus that give rise to fully traced glands during homeostasis. *Sox2*+ cells can also give rise to entire corpus glands, however, as recombined cells are detected in the isthmus and the base of corpus glands, it has yet to be established which population of *Sox2*+ cells is responsible for the tracing observed [5]. Taken together, the data collected so far suggest a general model, in which stem cells with essential roles during homeostasis reside in the isthmus, whereas during regeneration, responding stem cells are located at the corpus base.[6]

The said model, however, is difficult to verify experimentally as both sets of stem cells express very similar markers. In this elegant study, the authors report that *Lgr5* exclusively labels a population of reserve stem cells at the corpus gland base and not stem cells in the isthmus. Transcriptional profiling of *Lgr5*+ cells from the corpus confirmed that they expressed *Troy*, *Mist1* and *Sox2* consistent with *in situ* hybridisation results. Previously, *Mist1*+ cells were identified in the isthmus and shown to give rise to fully traced corpus glands, even when all chief cells and *Lgr5* cells are ablated using *Mist1-CreERT2;Lgr5-DTR-GFP;R26-Tdtomato* mice [6]. Here, Leushacke *et al*. observed that a subset of *Lgr5*+ chief cells located at the gland base reconstituted whole glands in response to damage with high doses of tamoxifen [1]. *Runx1*, *Mist1* and *Troy* also mark reserve stem cells that respond during repair, suggesting these four genes could mark the same reserve stem cells at the corpus base. Stange *et al.* previously observed the occasional fully-traced corpus gland in *Troy-CreERT2* mice originating from the base of the glands [8], which is not reported by Leushacke *et al.* using their *Lgr5-2A-CreERT2* mouse model and might suggest the existence of a third smaller population of *Troy*+, *Lgr5*- stem cells that resides in the corpus base.

In the isthmus, *Troy* and *Mist1* might be expressed by the same cells and both populations are known to act as a cell of origin for gastric tumourigenesis [6]. Although *Runx1* expression was not analysed by Leushacke *et al*., a small population of *Runx1*+ cells with stem cell properties was previously found in the base of the corpus, suggesting *Lgr5* and *Runx1* could label a common reserve stem cell in this location[7]. *Runx1* and *Mist1* are both expressed in the isthmus, however, *Runx1* cells are highly proliferative, whereas *Mist1* cells are quiescent, and for this reason it is likely that these two genes label distinct populations of stem cells in the isthmus.

Although *Lgr5+* cells do not exhibit detectable stem cell properties during homeostasis of the corpus epithelium, they nevertheless seem to be functionally important to maintain normal homeostasis. Continuous depletion of *Lgr5+* cells for three weeks in mice resulted in weight loss and a marked reduction of chief cells and mucous cells that was accompanied by lesions in the gastric pits and surface epithelium [1]. One explanation that Leushacke *et al*. explored was that *Lgr5* cells in the antrum were also depleted in this experiment, resulting in a 65% reduction of Gastrin-producing cells, which might have a profound impact on cell activity in the corpus. Another reason for this phenotype could be the function of *Lgr5* as a co-Wnt receptor. An important objective for future studies will be to further elucidate the importance of Wnt signalling in *Lgr5* corpus cells; particularly the Wnt receptor Fzd7, which was recently identified as the predominant Wnt receptor regulating *Lgr5*+ stem cell activity in the intestine [9].

Leushacke *et al.* further establish that *Lgr5*+ chief cells represent the cell of origin for tumours in the corpus base [1]. To date, several studies have suggested transdifferentiation of zymogenic chief cells, which re-express progenitor genes to become metaplastic cells [10]. This process is referred to as spasmolytic polypeptide expressing metaplasia (SPEM), which is regarded as a precursor of gastric cancer [10]. Consistent with this, Leushacke *et al*. report that *Lgr5-2A-CreERT2*; $Kras^{G12D}$ mice develop SPEM/ pseudopyloric metaplastic lesions in the corpus [1]. They also observe similar properties in human corpus gastric tumours, and suggest a mechanism requiring deregulation of Wnt signalling via *Mmp7* and *Sostdc1*, which may provide easier therapeutic targets than the stem cells themselves [1].

*Mist1*[6], *Runx1*[7], *Troy*[6] and *Sox2* [11] have also been shown to mark cells of origin for tumorigenesis in the corpus, illustrating that several different cells can induce gastric cancer (**Fig. 2**). *Mist1* and *Troy* expressing cells located in the isthmus are both able to form intestinal type tumours when *Apc* is deleted together with $Kras^{G12D}$ expression or *Notch1* overexpression [6]. Diffuse-type tumours are also

observed when E-cadherin is deleted in the *Mist1*+ cells together with *Helicobacter felis* infection [6]. *Runx1* expressing cells are the origin of different types of lesions in the corpus depending on the location of the *Runx1*+ cells [7]. Expression of $Kras^{G12D}$ in the isthmus-located *Runx1*+ cells results in antral-like gland-like structures, whereas in basal *Runx1*+ cells, $Kras^{G12D}$ expression induces metaplastic lesions similar to SPEM [7]. It is tempting to speculate whether the metaplastic lesions in the *Runx1Cre;* $Kras^{G12D}$ mice at 2 months would have progressed to tumours similar to those observed by Leushacke *et al.* in the *Lgr5-2A-Cre;* $Kras^{G12D}$ mice at 4 months, suggesting that *Runx1* and *Lgr5* may mark the same cell of origin in the corpus base. Likewise, *Sox2Cre*; $Apc^{fl/fl}$mice develop intestinal metaplasia[11]. In this case *Sox2*+ expressing cells are detected in the base of the corpus gland, which supports the observations by Leushacke *et al*. that the cell of origin can be outside the highly proliferative isthmus. Interestingly, the *Mist1+* cells in the corpus base, as noted by Hayakawa *et al.* [6], may not be the same cells as the basal *Lgr5+* population identified by Leushacke *et al.* Both sets of experiments used $Kras^{G12D}$ as their oncogenic driving mutation, however, Hayakawa *et al.* [6] observed tumour formation exclusively from the isthmus, while Leushacke *et al.* showed that *Lgr5+* corpus base cells can be the cell of origin [1]. Together these studies suggest that there may be marked phenotypic differences in tumorigenesis dependent on both the cell of origin and the driving oncogenic mutation, and that there are distinct sub-sets of chief cells with different capacities to act as cells of origin for gastric tumorigenesis.

The work by Leushacke *et al*. not only provides a new tool for researchers to investigate the role of *Lgr5*, but also indicates that *Lgr5* marks an additional population of reserve stem cells in the corpus base and is yet another cell of origin for metaplastic tumorigenesis in this tissue [1]. Going forward, it will be important to further delineate the relationships between these cell populations, taking into account shared expression patterns and their properties as cell of origin.

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**Figure 1. Corpus gland stem cell function.** The corpus gland is divided into four sections: the pit, which opens onto the surface of the stomach, the isthmus, in which several stem cells have been identified, the neck, and finally the base, which houses reserve stem cells. The location and function of each of the stem cells identified to date is indicated along with the associated reference.

**Figure 2. Corpus gland tumourigenesis cell of origin.** Different tumour types develop in the corpus, depending on the cell of origin and the driving oncogenic mutations. The stem cell marker that drives Cre recombinase is indicated along with the oncogenic mutations used, the location of the observed phenotype and the associated reference.