# Sox9, a key regulator of gastric stem cell behaviour driving cancer initiation

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The transcription factor Sox9 is a versatile regulator of development, proliferation, and tumourigenesis. In gastric cancer patients, Sox9 is frequently upregulated in precancerous tissues and gastric adenocarcinomas, correlating with advanced tumour stages, metastasis, and lower disease-free survival[1,2]. These associations have made Sox9 an attractive target that could be modulated to develop more effective cancer therapies. Indeed, using *in vitro* models, Sox9 has already been shown to work in concert with the Wnt/β-catenin pathway to drive tumour cell proliferation and survival[1,3]. In this issue of Gastroenterology, Chen *et al.* build on these findings to propose a role for Sox9 in regulating cell division patterns within gastric stem cells[4]. Moreover, through the generation of novel mouse models, they highlight important contributions of $Sox9^+$ cells to gastric homeostasis and cancer.

Under homeostatic conditions, Sox9 is expressed within a heterogenous population of cells primarily located in the isthmus of corpus and antral glands, where proliferative stem cell populations are thought to reside. Lineage tracing using a Sox9-CreERT;Rosa26-tdTomato mouse model confirmed that this $Sox9^+$ population contained cells with stem potential, capable of generating tdTomato-labelled progeny that encompassed entire gastric glands. Sox9 thus joins the growing suite of markers proposed to label various stem cell populations throughout the stomach. Of note, $Sox9^+$ cells are present at the gland bases of corpus and antral glands where other presumptive stem cell populations are located, from which tracing events could also originate[4].

The accumulation of oncogenic mutations within long-lived stem cell populations is thought to be one of the major steps driving gastric tumourigenesis. This has previously been demonstrated using mouse models targeting the oncogenic transformation of stem cell populations labelled by Lgr5[5,6], Aqp5[7], and Mist1[8]. Similarly, inducing Kras hyperactivation within $Sox9^+$ cells initiated metaplastic changes throughout the gastric epithelium, although this also resulted in aggressive intestinal tumours and early lethality of the mice that precluded the development of advanced gastric tumours. To circumvent this issue, the authors generated a Sox2-CreERT;Sox9-Flp system to recombine oncogenic mutations selectively within $Sox2^+Sox9^+$ cells present only in the stomach and not in other regions of the gastrointestinal tract. This model, when used to introduce both Kras and p53 mutations within $Sox2^+Sox9^+$ cells, resulted in metastatic gastric tumours reminiscent of those from an independent mouse model where the Cldn18-CreERT2 driver was used to target $Kras^{G12D}$ and $p53^{fl/fl}$ mutations to the entire gastric epithelium[9]. Importantly, such advanced gastric cancer models offer a means to study the cascade of events leading up to primary tumour growth and metastatic spread in a physiological setting and could facilitate preclinical drug testing. This would entail a detailed

characterization of the Sox2-CreERT;Sox9-Flp cancer model, not only of histological tumour features but also accompanying genomic changes and molecular markers of inflammation, invasion, and metastasis relative to human tumour phenotypes.

Interestingly, the $Sox9^{+}$ (but not $Sox2^{+}$) cell pool expands following oncogenic activation in three independent models of gastric cancer, suggesting that Sox9 could play a role in driving disease progression. This observation was confirmed in the Sox2-CreERT;$Kras^{G12D}$ and parietal cell atrophy models, both of which recapitulate precancerous metaplastic stages without progressing to gastric cancer, and a MNU carcinogen-driven adenocarcinoma model. It remains unclear whether the increase in $Sox9^{+}$ cells is also reflected in other advanced gastric cancer models, such as the Sox2-CreERT;Sox9-Flp;$Kras^{G12D}$;$p53^{R172H}$ model generated in this paper, and whether Sox9 plays a role in these contexts. Nevertheless, when Sox9 is selectively depleted within $Sox2^{+}$ cells in the Sox2-CreERT;$Kras^{G12D}$ metaplasia model, the gastric epithelium surprisingly remains unaffected, suggesting that Sox9 is required to initiate oncogenic changes in this cancer setting. Moreover, the fact that $Sox2^{+}Sox9^{-}$ cells appear to be insufficient on their own to transform the tissue indicates that the cells co-expressing Sox2 and Sox9 could be a more specific subpopulation harbouring cycling stem cells. Indeed, a closer look into the lineage tracing events originating from the $Sox2^{+}Sox9^{+}$ putative stem cell population using the Sox2-CreERT2;Sox9-Flp system could resolve some of the confusion behind the usage of broadly-expressed stem markers encompassing cells in both the isthmus and gland bases.

In driving the early oncogenic transformation of the gastric epithelium, the authors find that Sox9 does so by regulating the balance of symmetric and asymmetric divisions within the tissue. Cell division patterns are predominantly asymmetric in the healthy gastric epithelium, with most mitotic spindles oriented in a perpendicular fashion to the basement membrane. Importantly, in the Sox2-CreERT;$Kras^{G12D}$ metaplasia model, there is a pronounced shift towards symmetric cell divisions, an effect reversed with Sox9 deletion. Thus, by favouring symmetric cell divisions, Sox9 appears to promote the expansion of the stem/progenitor compartment within the tissue, purported to be a key event driving the initial stages of tumourigenesis. Yet, how division angles translate into the actual acquisition of stem and differentiated lineage identities remains ambiguous. The authors partially address this with BrdU labeling experiments in gastric cancer cell lines, showing that individual Sox9 KO cells frequently performed asymmetric divisions with unequal segregation of BrdU. However, given that Sox9 KO cell lines already display impaired growth, it is unclear if these effects could also translate into different division patterns independent of Sox9 loss. In a similar vein, we do not yet know whether the shifts in division patterns in the Sox2-CreERT;$Kras^{G12D}$ model are a cause or consequence of the tissue-level changes arising from Sox9 deletion. Moving forward, the establishment of representative *in vitro* models that facilitate the analysis of dynamic lineage segregation events together with the documentation of oncogenic changes within native epithelial tissue would be important to piece together key insights into Sox9 functions during tumourigenesis.

In all, the present finding that Sox9 regulates the balance between stem/progenitor cell expansion and lineage differentiation highlights a promising mechanism that could be targeted for limiting the continued growth of gastric tumours. Sox9 itself marks a putative stem cell compartment capable of generating all the differentiated cell lineages in the healthy gastric epithelium. By utilizing physiologically relevant gastric cancer models, we can dissect the mechanistic functions of Sox9 to generate novel therapeutic modalities and deliver better outcomes for gastric cancer patients.

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