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Impact of Stem Cell Genes in Gastric Cancer

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Abstract

Gastric cancer remains one of the leading causes of global cancer mortality. It has been shown that gastric cancer may originate from adult gastric stem cells and that it contains a subpopulation of cancer cells with stem cell characteristics, which are linked to *Helicobacter pylori* infection, therapy resistance and metastasis. Thus, the identification of transcription factors and related signal transduction pathways that regulate stem cell maintenance and lineage allocation is attractive from a clinical standpoint in that it may provide targets for novel cell- or drug-based therapies. This chapter summarizes the role of several important stem cell factors in gastric cancer biology.

Keywords: cancer stem cells, gastric cancer, SOX genes, *Helicobacter pylori*

1. Introduction

Gastric adenocarcinoma (GC) is the second most common cause of cancer-related mortality in the world, with developing countries being the most affected regions [1, 2]. GC is a complex disease influenced by different environmental and genetic factors. Among them, *Helicobacter pylori* (*H. pylori*) is the main etiological agent of gastrointestinal infections in children and adults and the prevalence of infection varies considerably across different geographical regions [3]. Natural acquisition of *H. pylori* infection occurs, for the most part, in childhood [4]. Infection with *Helicobacter pylori* (*H. pylori*) promotes chronic inflammation and sequential histological changes of chronic active gastritis, atrophic gastritis, intestinal metaplasia, dysplasia and ultimately invasive carcinoma [2]. Symptomatic diseases occur in approximately 10% of infected individuals, and in these cases, the risk of gastric adenocarcinoma is higher in persons carrying certain strain types as, for example, those that contain cagA or vacA alleles [1].

Gastric cancer accounts for around 10% of all new cancers (one million per year), and it is the second leading cause of cancer death globally (700,000 deaths per year). The prognosis of CG is very poor, with a survival rate below 30% at 5 years post diagnosis [1, 2]. It is usually asymptomatic or causes nonspecific symptoms at early stages. When symptoms appear, the cancer has usually reached an advanced stage and there is presence of metastasis, being this dissemination a main cause of the severe prognosis. GC-associated high mortality is the result of its silent nature and the extremely high heterogeneity exhibited between individuals and also within gastric tumors. This heterogeneity involves, at the molecular level, a broad variety of gene mutations, amplifications and/or expression alterations, diverse DNA methylation profiles and differences in the activation or inactivation of particular signaling pathways. Thus, in the last years there has been substantial progress in the elucidation of the genomic landscape of GC due to advances in high-throughput technologies and the effort of international consortiums. Consequently, gastric cancer has been recently reclassified and stratified into several distinct subtypes based on molecular and genetic/epigenetic alterations [5, 6]. In particular, GCs have been classified according to defined genetic signatures, the status of *TP53* and the presence of microsatellite instability [5, 6]. Importantly, the heterogeneity in GC involves critical consequences in terms of differential response to therapy, resistance and recurrence [6]. Nevertheless, current therapeutic strategies in GC are not adapted to GC heterogeneity and depend on the stage of the tumor. Clinically, first-line treatment consists of surgical resection (except in cases with advanced metastasis), followed by chemotherapy with cytostatic agents such as cisplatin, 5-fluorouracil (5-FU), taxanes or irinotecan, or in combinations as ECF (epirubicin, cisplatin and 5-FU) and 5-FU plus docetaxel or cisplatin (or irinotecan) [1, 7]. These treatments have been internationally and generally accepted and used since last century. In the case of metastatic dissemination, patients whose tumors exhibit high levels of HER2 receptor expression also receive Trastuzumab, a monoclonal antibody against HER2. Initially, patients respond to chemotherapy, but cancer cells eventually become resistant, facilitating the occurrence of relapses. Even with the increase in survival facilitated by the incorporation of chemotherapy, the median overall survival of patients with CG remains low, being one of the survivals associated with cancer lower.

It has been noticed that the incidence of GC has declined over time mostly in developed countries, due to improving living standards. However, and despite increasing knowledge and improvements in the standard of care, therapy resistance and metastasis remain the main causes of treatment failure and death in GC patients and GC as a disease remains a serious and significant social concern. Consequently, identifying the major GC drivers and the molecular and cellular mechanisms responsible for the GC heterogeneity and maintenance is crucial to understand the pathobiology of GC and establish optimal therapies that able to improve the prognosis of patients.

2. Cancer stem cells in gastric cancer

Several types of solid cancers, including gastric cancers, contain phenotypically and functionally heterogeneous cancer cells [8]. These cancers present a small subpopulation of cells that display

characteristics similar to normal stem cells, including unlimited self-renewal, proliferation and multi-lineage differentiation. These cells are called cancer stem cells (CSCs), which are supposed to maintain long-term tumor growth, recurrence and chemotherapy resistance. The origin of gastric CSCs is not completely clear, but it has been observed that this subpopulation of cells can derive from the differentiated gastric epithelial cells, local progenitor cells in the gastric mucosa and bone marrow-derived cells (BMDCs) [9]. In line with this idea, chronic infection of C57BL/6 mice with *Helicobacter felis* results in chronic inflammation and injury in gastric mucosa, which leads to the loss of resident gastric stem cells, followed by hyperplasia, metaplasia, dysplasia and, ultimately, gastric cancer [10, 11]. *H. pylori* can attach and invade *Lgr5+* gastric stem cells and this residency results in more susceptibility to DNA damage and cancer initiation [12, 13]. This suggests that *H. pylori* infection directly affects epithelial stem cells in the stomach and plays an important role in transforming resident stem cells into tumor cells. In addition, *H. pylori cag*A virulence factor unveils CSC-like properties by induction of EMT-like changes in gastric epithelial cancer cells [14]. Increasing studies support the existence of these cancer cells exhibiting stem cell characteristics and involved in GC metastasis [14]. Among the underlying mechanisms of chemoresistance, CG cells resistant to 5-Fluoroacyl (5-FU) or cisplatin therapy have been identified as exhibiting high expression of stem cell markers such as BMI1, CD44, CD133 or SOX9. In addition, the inhibition of these regulators reverses the chemoresistance. This resistance is due in part to the acquisition or presence of quiescence and self-renewal characteristics by the small percentage of gCSCs. It is well known that conventional chemo and radiotherapy therapies have maximum efficacy in proliferative cells and when target events are present in all cancer cells. However, they do not affect the quiescent cells and do not take into account inter and intratumoral heterogeneity at the cellular and molecular level. Thus, identifying the major regulators of gastric CSCs is a prominent need in order to understand GC pathobiology and identify novel therapeutic targets. In this sense, in the last years, the identification of several stem cell-related genes or transcription factors has provided relevant information of the impact of gCSCs in GC initiation and progression, and how *H. pylori* or chronic inflammation affects gastric stem cells. This chapter summarizes the impact of some of the most relevant genes in gastric CSCs and gastric cancer pathobiology, including LGR5, CD133, CD44, SOX2 and SOX9.

2.1. Regulators of gastric cancer stem cells

2.1.1. LGR5

The human leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) is a member of the G protein-coupled transmembrane receptor (GPCR) superfamily. LGR5 is a receptor for R-spondins that belong to the WNT signalling complex at the membrane level [15] and is also a target gene of this pathway [16]. LGR5 is overexpressed in a variety of human cancers, including tumors of the digestive tract such as colorectal [17] or gastric cancer [18–20], wherein it has been postulated as a CSC marker. LGR5 is an established stem cell marker of the intestine, and several studies on mice and humans have shown that LGR5-positive stem cells are the cells-of-origin of intestinal and colorectal cancer [21–27]. In the stomach, there are also increasing evidence postulating LGR5 as a stem cell and CSC marker. Thereby, LGR5 expression in gastric mucosa is almost restricted to a subset of cells located at the base of the pyloric glands, distribution that fits well with the multiple sites of the gastric cancer in humans

[28]. Through *in vivo* lineage tracing experiments, these authors found that LGR5-positive cells were self-renewing and multipotent and were responsible for the renewal of the gastric epithelium. Interestingly, they observed that the transformation of this population of stem cells could drive gastric tumorigenesis *in vivo* [28], fact that has been strongly demonstrated more recently by Li and collaborators [29]. Consistently with the observation in mice, in human gastric tissue, LGR5 expression has also been found in the bottom of gastric glands [30]. Supporting the putative role of LGR5 as a gastric CSC regulator in humans, independent studies have reported LGR5 overexpression in human gastric cancer samples respect to normal gastric mucosa in a progressively increasing manner from well-differentiated to poorly differentiated gastric carcinomas [20, 31]. Furthermore, LGR5 expression has been strongly linked to a high degree of tumor infiltration, high TNM stage, recurrence and dismal prognosis of gastric cancer patients [18–20, 31]. More recently, Wang and collaborators have shown that sphere cells derived from a gastric cancer cell line presented increased expression of some canonical stem regulators, being LGR5 particularly elevated [32]. They also showed that ectopic LGR5 overexpression potentiated the sphere cell growth and cell migration capabilities of gastric cancer cells and also their tolerance to oxaliplatin, associating LGR5 expression with the characteristic features of CSCs [32]. These findings are in concordance with previous observations showing the impairment of the invasiveness and the reduction in the expression of metalloproteinase 2 (MMP2) and β-catenin in gastric cancer cells in response to LGR5 silencing *in vitro*; and revealing a positive correlation between the expression of LGR5 and MMP2 in gastric cancer tissue samples [20]. Regarding the implications of the carcinogenic agent *H. pylori* in the LGR5-positive cells in the stomach, it has been found that this population of cells is expanded in gastric cancer tissues affected by the bacteria, indicating that LGR5 likely represents a marker of stem cells susceptible of oncogenic transformation driven by *H. pylori* [13, 33].

2.1.2. CD133

CD133 (also Prominin 1) is a pentaspan transmembrane glycoprotein present in embryonic epithelial structures, thought to function as an organizer of plasma membrane topology, and regulating the maintenance of the appropriate lipid composition within the plasma membrane [34]. CD133 has been presented as a marker of cancer stem cells in colon, pancreas, brain or lung cancer [35], yet its role in gastric CSCs is controversial. Several findings related to different aspects of gastric CSCs have been published in support of its role as a gastric CSC marker and regulator. In gastric cancer cell lines, CD133 silencing abrogates sphere formation capacity [36] and, consistently, CD133 has been found overexpressed in gastric sphere cultures [37, 38]. Noteworthy, a large number of publications show increased CD133 expression in human gastric cancer tissue respect to non-neoplastic gastric mucosa and highlight the prognostic significance of CD133, associating its overexpression with a big plethora of adverse clinic-pathological features, such as elevated cellular proliferation rates, high T stage, venous invasion, lymph node and distant metastasis, chemoresistance, recurrence, poor 5-year disease-free and overall survival and so on [37, 39–42]. According to this, studies performed in gastric cancer cell lines demonstrate that CD133-positive gastric cancer cells present a CSC phenotype, since they are more tumorigenic, more chemoresistant and exhibit higher migration or invasion capacities than CD133-negative cells [37, 38, 43]. However, some controversial findings have been published indicating that CD133 expression is not a *sine qua non* condition for gastric cancer cells

to exhibit properties of CSCs. Thus, Takaishi et al. isolated different subpopulations of cells from gastric cancer cell lines according to the expression of CD133, CD44, CD26 and other cell surface markers and showed that CD133-positive cells did not exhibit characteristics of CSCs [44]. Similar results have been obtained in other investigations using human gastric cancer specimens as source of cells, in which CD133-positive cells were not able to reproduce tumors in immunodeficient mouse models [45, 46].

2.1.3. CD44

CD44 is a transmembrane glycoprotein expressed on leukocytes, endothelial cells, hepatocytes or gastric epithelial cells, which acts as a receptor for hyaluronic acid (HA) [47] and can also interact with other ligands, such as osteopontin, collagens and MMPs. CD44 is a fetal and adult hematopoietic stem cell regulator that is involved in cell-cell interactions, cell adhesion and migration and participates in a wide variety of cellular functions, including hematopoiesis and lymphocyte activation, recirculation and homing [48]. CD44 gene contains 20 exons. Ten of these exons (exons l–5 and 16–20) are expressed together on many cell types and the product is referred to as the "standard" form of CD44. Additionally, complex alternative splicing of the transcripts affecting exons from 6 to 15 (variant exons) results in many functionally distinct isoforms or variants (CD44v) [49]. The role of CD44 as a CSC marker has been broadly studied in myeloid leukemia and also in several solid tumors such as lung, brain, liver, head and neck or gastric cancer [50]. In gastric cancer, the first temptative characterization of CSCs in terms of markers was performed by Takaishi and collaborators, who found that CD44+ cells isolated from different gastric cancer cell lines presented sphere formation ability *in vitro* and tumorigenic potential when inoculated into stomach and skin of immunodeficient mice, abilities that were abrogated by CD44 silencing. Moreover, these CD44+ gastric cancer cells showed the stem cell characteristics of self-renewal and the ability to give rise to differentiated progeny [44]. In concordance, other authors have documented CD44 enrichment in spheres derived from gastric cancer cell lines [51] or have identified that CD44-positive cells derived from gastric cancer cell lines are resistant to 5-fluorouracil and cisplatin chemotherapy and also exhibit significantly more migration, invasion and anchorage-independent growth capabilities [52]. Regarding gastric cancer clinical samples, CD44 is expressed in 80% of gastric tumor resected specimens [40] and its high expression has been associated with tumor size, lymphatic vessel and intravenous invasion, moderate grade of differentiation and low response to chemotherapy [52–54]. Furthermore, the presence of CD44+ cancer cells at the invasive front of gastric tumors entails poor survival and constitutes a prognostic factor for this malignancy [55]. In relation to this aspect, Watanabe and collaborators have found that in gastric cancer patients, the frequency of circulating CD44-positive tumor cells correlates with disease stage, depth of tumors and venous invasion [56]. Moreover, it has been suggested that the emergence of gastric CSCs induced by *H. pylori* infection of gastric mucosa may rely on CD44 upregulation [57]. Nevertheless, in contraposition to these evidences, some works have not found CSC characteristics in the subpopulations of CD44-positive cells isolated from gastric tumors [45, 58]. Besides, it is being sustained the notion that some CD44 variants are more relevant for gastric CSCs than standard CD44. An example of it is the work of Lau and collaborators, who show that CD44v8-10 is the predominant CD44 variant expressed in gastric cancer cells, whose expression levels, unlike those of standard CD44, are increased in

gastric tumors respect to adjacent normal tissue. The authors also showed that ectopic expression of CD44v8-10, but not standard CD44, in gastric cancer cells potentiates their ability to initiate tumors in mice at limiting cell concentrations and that total CD44 silencing impairs tumor-initiating potential of cells, which could be rescued by restoration of CD44v8-10, but not standard CD44, expression [46].

2.1.4. CD24

CD24 encodes a cell surface sialoglycoprotein that is physiologically expressed in developing or regenerating tissues and regulates processes such as lymphocyte development [59] or neurogenesis [60]. As other stem cell genes, CD24 is expressed in hematologic malignancies and several solid tumors including gastric cancer. Suggesting a role for CD24 in gastric CSCs, some studies by using gastric cancer cell lines have shown that derived spheres are enriched in the expression of CD24 (and CD44) [51] and also that CD24 modulates positively cell migration, while its inhibition entails apoptosis [61]. However, Takaishi et al. were unable to find properties of CSCs in a CD24-positive population in terms of sphere forming capacity and tumorigenicity in mice models [44]. With regard to patients with gastric cancer, CD24 expression progressively increases in samples of normal gastric mucosa, non-atrophic chronic gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia and gastric cancer [61]. Moreover, CD24 expression has been associated with adverse clinicopathological and prognostic aspects such as depth of tumors, lymph node status, TNM stage and reduced overall survival [62], fact that underlines its relevance in the disease.

2.1.5. SOX transcription factors

SOX factors are a family of transcription factors that are emerging as potent regulators of stem cell maintenance and cell fate decisions in multiple organ systems including the gastrointestinal tract [63]. There are at least 20 members divided into eight groups (from A to H), based on their HMG sequence identity in humans. Members within a group preserve higher than 80% identity in their HMG-domain and share other well-conserved regions. In addition, they share biochemical properties, have overlapping expression patterns and perform synergistic or redundant functions [63]. SOX proteins play critical roles during the development of several cell types and tissues in the embryo. They are also essential for stem cell types in the adult through the regulation of the cell fate determination, differentiation and proliferation [63]. SOX members fulfill their role by activating or repressing transcription and their action on target genes is context dependent, relying on other transcription factors with which they may directly interact for specificity. Dysfunction of SOX factors has been implicated in several human diseases. Such diseases are consistent with SOX function and expression pattern during embryonic development. A growing number of evidences are demonstrating that the expression and function of SOX factors are altered in a variety of cancers, and their roles in these malignancies are related to their stemness feature [64].

2.1.5.1. SOX2

SOX2 belongs to the SOXB1 subgroup along with the closely related SOX1 and SOX3. SOX2 is required for establishing embryonic stem cells and the maintenance of the early embryo [65]. It is also one of the factors necessary for reprogramming terminally differentiated cells into induced pluripotent stem cells [66]. Furthermore, SOX2 belongs to the core transcriptionally circuitry found on the regulatory regions of many genes with embryonic stem cell-specific expression [67]. This evidence demonstrates that SOX2 is a key factor in the control of embryonic stem cells fate and activity. SOX2 has additional functions during development, thus emerging as a critical regulator of stem cell maintenance and cell fate decisions. Furthermore, SOX2 also plays a relevant role during adulthood controlling tissue homeostasis and regeneration. Its expression is elevated in different populations of stem cells [68–71], and its high levels can be used to identify quiescent stem cells and distinguish them from transient amplifying progenitors [72, 73]. SOX2 is a regulator of gastric stem cells highly relevant for gastric patterning during development [74] and involved in the physiological renewal of the gastric epithelium in the adulthood [71, 75]. SOX2 displays several roles in cancer as an oncogenic driver, prognostic factor or a marker and regulator of CSCs [76–80]. In GC, its action is controversial. Several authors observed that SOX2 is frequently downregulated in gastric cancer [81–86]. Furthermore, low SOX2 expression is associated with shorter survival time [82] and also with worse prognosis [84]. In contrast, higher SOX2 levels are found among patients who have better prognosis [84]. In a large set of patients, Wang and coworkers demonstrated that SOX2 expression is progressively reduced during gastric carcinogenesis, from normal into invasive cancers including a series of premalignant states, supporting the role of SOX2 decrease as a robust predictor of disease outcome [85]. Similarly, SOX2 downregulation is linked with diffuse type of cancer with SOX2 expression becoming a good biomarker to discriminate between tumor (negative) and non-tumor (positive expression) and also high/low grades of tumor malignancy [86]. The regulation of SOX2 expression in GC has been mostly associated to epigenetic changes. Thus, aberrant DNA methylation has been shown as a key mechanism underlying SOX2 downregulation in a set of primary gastric carcinoma samples [82]. Besides promoter methylation, miR-126 overexpression also decreases SOX2 levels and therefore acts as a tumor suppressor [83]. Recently, it has been shown that SOX2 has an important role in gastric differentiation [87]. It is known that during gastric carcinogenesis, the homeobox transcription factor CDX2 is critical for intestinal differentiation driving the onset of intestinal metaplasia (IM) [88, 89]. Thereafter, Camilo and coworkers showed that SOX2 is associated with gastric differentiation in incomplete IM and is lost in the progression to dysplasia, whereas CDX2 is acquired *de novo* in IM and maintained in dysplasia [87]. Taken it into account, the authors hypothesized that balance between gastric and intestinal differentiation programs might interfere on the gastric carcinogenesis progression [87]. Since SOX2 and CDX2 expression were found in about half of the cases, the interaction of both transcription factors in gastric carcinogenesis remains to be investigated. Functional characterization performed in gastric epithelial cell lines showed that SOX2 ectopic activation inhibits cell proliferation through G1 cell-cycle arrest and induces apoptosis by decreasing cyclin D1 and phosphorylated Rb and increasing p27Kip1 protein levels [82]. Overall, the authors observed that SOX2 performs a critical part in gastric carcinogenesis, operating as a tumor suppressor.

Similarly, Wang and coworkers verified that enforced SOX2 expression inhibited proliferation, increased apoptosis and reduced invasion and motility, both in vitro and in vivo [85]. Mechanistically, SOX2 directly transactivates PTEN. Therefore, this SOX2-dependent PTEN upregulation may directly orchestrate downstream phospho-Akt dephosphorylation, affecting diverse cellular phenotypes such as survival, growth, proliferation and migration [85]. These studies show that SOX2 plays important roles in gastric epithelial cells growth inhibition through cell-cycle arrest and apoptosis [90]. Regarding its relationship with *H. pylori*, SOX2 expression is decreased by the bacteria, and this inhibition leads to an upregulation of CDX2 expression [75, 91, 92]. Additionally, *in vitro* and in a mice model infected with *Helicobacter* spp. demonstrated that CDX2 and SOX2 are downstream targets of the BMP (bone morphogenetic protein) pathway in gastric carcinogenesis. The authors showed that *H. pylori* upregulates BMP pathway, through an increase in BMP2, SMAD4 and pSMAD1/5/8 expression. Thus, SOX2 expression was downregulated by *H. pylori* and the BMP pathway [93]. From a mechanistic perspective, it was postulated that the activation of an intestinal differentiation program may occur concomitantly with the silencing of a gastric differentiation, induced or controlled by SOX2 [93]. Another recent study identified that the bacteria might trigger its pro-carcinogenic activity through a blockage of SOX2 [85]. However, other authors verified that overexpression of SOX2 is associated with tumor invasion, lymph node metastasis and chemoresistance [94–97]. Tian and coworkers were able to show that SOX2 enhances the tumorigenicity and chemoresistance of cancer stem-like cells derived from gastric cancer, suggesting an oncogenic effect of SOX2 in the stomach [94]. In addition, it has been demonstrated that SOX2 overexpression was significantly correlated with lymph node metastasis and the stage of tumor invasion in gastric cancer indicating that SOX2 might be a predictive prognostic factor [95]. Hutz and coworkers proved that high levels of SOX2 are involved in gastric carcinogenesis by regulating the expression of genes associated with proliferation, apoptosis and cell cycle regulation, *in vitro* and *in vivo* [96]. Functionally, the SOX2 suppression induced a decrease in cell proliferation, which coincided with an increase of apoptosis in AZ-521 cells. Similarly, blocking of SOX2 in a xenograft mouse model resulted in reduced tumor growth [96]. Moreover, the expression of SOX2 in human gastric tumor samples was observed at high proliferation rate sites [96]. Likewise, SOX2 overexpression in gastric cancer has been recently observed in other study, where the surge in the expression is attributed to SOX2 locus copy number variation, being related as well with the presence of regional lymph node metastases [98].

2.1.5.2. SOX9

SOX9 is overexpressed in a variety of human cancers, being its high levels correlated with malignant character and progression in prostate, lung, breast and brain tumors [80, 99, 100]. SOX9 expression is also elevated in tumors of the digestive system such as esophageal, colorectal and pancreatic cancers [101, 102]. In esophageal and pancreatic tumors, SOX9 stimulate self-renewal properties [102, 103]. However, in colorectal cancer, there are contradictory results between functional studies and clinical samples, suggesting a context-dependent activity of SOX9 [100, 104]. Remarkably, several studies have reported clinical implications of SOX9 in GC. Thereby, in GC patients, high tumoral SOX9 expression has been observed and associated with advanced TNM stages and lower overall patient survival [105]. Interestingly, in clinical

samples, high levels of SOX9 correlate with elevated expression of the carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) [106], which facilitates GC metastasis, and are positively associated with lymph nodes metastasis and advanced TNM stage [107]. In samples from patients, there is also an inverse relation between SOX9 and the tumor suppressor gastrokine 1 (GKN1), relationship also observed in GC cell lines, wherein GKN1 negatively regulates SOX9 expression [106, 108]. Furthermore, elevated SOX9 expression in gastric tumors is associated with the activation of the WNT canonical oncogenic pathway, with whom it establishes a feedback regulatory loop [105].

Noteworthy, SOX9 is a critical executor of the carcinogenic action of *H. pylori*. According to this notion, the bacterium induces SOX9 expression in pre-tumorigenic gastric mouse cells [109] and also in GC cells [105], being the induction more pronounced in response to specimens of *H. pylori* containing the pathogenically significant *CagA* virulence factor. Notably, SOX9 is required for bacteria-induced GC cell proliferation, induction of β-catenin and acquisition of stem cell-like properties. Mechanistically, it has been found that TNFα and IL-1β cytokines, involved in the inflammatory response to *H. pylori*, induce the expression of SOX9 in mouse models and GC cells [105, 109], being probably the action of TNFα in human GC cells stronger and more extensive. In fact, TNFα high levels correlated with SOX9 upregulation in *H. pylori*-positive GC samples, and there was a positive association between them in two independent large cohorts of GC samples [TCGA and ACRG] [105]. Overall, these results identified a novel association between SOX9 and *IL1-β* and *TNFα* cytokines, which links *H. pylori* infection with SOX9 and GC outcome in patients, evidence supported by other studies [110–114]. SOX9 represents a key driver of GC and given the importance of its strong clinical implications, elucidating the molecular mechanism of its action in GC has constituted an important challenge to identify novel and suitable therapeutic targets. With respect to that, it is known that SOX9 establishes a feedback regulatory loop with WNT/β-catenin signaling pathway. Consistently, SOX9 abrogation in GC cells diminishes *CYCLIN D1* and *c-MYC* expression, and there is a positive correlation between these genes and SOX9 in patient samples [105]. Functionally, SOX9 silencing in GC cells promotes apoptosis and senescence through BMI1 decline and the consequent upregulation of p21$^{\text{CIP}}$ [105]. SOX9 silencing also supposes detrimental effects on the subpopulation of gCSCs, reflected by a reduction in tumorsphere self-renewal and decreased tumor initiating capability [105]. Paralleling these effects, and likely due to its functions in gCSCs, SOX9 mediates cisplatin chemoresistance [105, 114], fact that might explain the reduced disease-free survival of patients presenting tumors with high *SOX9* expression levels [105]. Additionally, there are other SOX members associated to GC. Thus, SOX4 has been shown to display pro-oncogenic activities and become upregulated with gastric cancer progression, in the population of gCSCs and in response to *H. pylori* infection [115–117]. Finally, SOX18 mRNA levels are increased in gastric cancer tissues compared to normal tissue, and the frequencies of both lymphovascular invasion and lymph node metastases are higher in SOX18 positive than in the negative group. Furthermore, both the 5-year survival and the recurrence-free survival were shorter for SOX18-positive cancers suggesting that SOX18 expression might be a prognostic tumor marker and a potential therapeutic target in gastric cancer [118].

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