**SUMMARY**

Cell surface adhesion molecule CD44 has been identified as a gastric cancer stem cell marker. CD44 variant isoforms are expressed abundantly in epithelial-type carcinomas and are associated with initiation and progression of gastric cancer.

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Gastric cancer is the third most common cause of cancer-related death. Although the incidence of gastric cancer in the United States is relatively low, it remains significantly higher in some countries, including Japan and Korea. Interactions between cancer stem cells and the tumor microenvironment can have a substantial impact on tumor characteristics and contribute to heterogeneity. The mechanisms responsible for maintaining malignant cancer stem cells within the tumor microenvironment in human gastric cancer are largely unknown. Tumor cell and genetic heterogeneity contribute to either de novo intrinsic or the therapy-induced emergence of drug-resistant clones and eventual tumor recurrence. Although chemotherapy often is capable of inducing cell death in tumors, many cancer patients experience recurrence because of failure to effectively target the cancer stem cells, which are believed to be key tumor-initiating cells. Among the population of stem cells within the stomach that may be targeted during chronic *Helicobacter pylori* infection and altered into tumor-initiating cells are those cells marked by the cluster-of-differentiation (CD)44 cell surface receptor. CD44 variable isoforms (CD44v) have been implicated as key players in malignant transformation whereby their expression is highly restricted and specific, unlike the canonical CD44 standard isoform. Overall, CD44v, in particular CD44v9, are believed to mark the gastric cancer cells that contribute to increased resistance for chemotherapy- or radiation-induced cell death. This review focuses on the following: the alteration of the gastric stem cell during bacterial infection, and the role of CD44v in the initiation, maintenance, and growth of tumors associated with gastric cancer. *(Cell Mol Gastroenterol Hepatol 2017;4:55–63; http://dx.doi.org/10.1016/j.jcmgh.2017.03.003)*

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caused by *H pylori* infection is a trigger for the development of gastric cancer. An explanation for the causal role of *H pylori* infection in the pathogenesis of gastric cancer has been described by disruption of differentiation of epithelia as a consequence of altered gastric stem cell phenotype.\(^{18,19}\) The chronic nature of *H pylori* gastritis is critical to the carcinogenic potential of this infection. The long-term interaction of the bacteria and inflammatory mediators with gastric epithelial, progenitor, and stem cells, results in the accumulation of mutations, epigenetic modifications, and deregulation of cell function that ultimately may lead to cancer.\(^{19,21}\) Therefore, *H pylori* infection plays a critical role during the initiating steps of gastric cancer.

**Initiation of Gastric Cancer**

**Alterations in Epithelial Gastric Stem Cells**

Abnormal differentiation (metaplasia) is associated with cancer and may reflect the permanent alteration in the behavior of the stem cells, thus making the gastric stem cell a candidate *H pylori* target. It is hypothesized that tumors develop because of a rare subpopulation of cells (known as cancer stem cells [CSCs]).\(^{19}\) Although the origin of gastric cancer stem cells remains uncertain, there are a number of key studies that show the expansion of gastric stem cells during bacterial infection that may lead to their alternation and transformation into tumor-initiating cells.\(^{19,21}\) Among the populations of stem cells within the stomach that may be targeted during bacterial infection, that may lead to metaplasia or aberrant epithelial cell proliferation and differentiation, are cells expressing the leucine-rich, repeat-containing, G-protein–coupled receptor 5 (Lgr5) and the cluster-of-differentiation (CD)44 cell surface receptor.\(^{19,22}\)

Troy marks a specific subset of chief cells that are capable of replenishing entire gastric units in response to injury.\(^{23}\) In addition, the Sox2*+* stem cell compartment has been shown to be critical for normal tissue regeneration, and villin*+* is a quiescent stem cell population that becomes apparent upon cytokine stimulation.\(^{25}\) The expansion of Troy*, Sox2*, and villin*+* cell populations in response to *H pylori* infection has not been investigated thoroughly.

Lgr5, located in adult stem cells at the base of the antral glands of the stomach, are capable of long-term renewal of the epithelium.\(^{26}\) By using lineage tracing to mark cells derived from Lgr5*+* stem cells, Sigal et al.\(^{15}\) analyzed the response of these gastric stem cells to *H pylori* infection. The investigators showed that the bacteria formed distinct microcolonies deep in the stomach glands where infection accelerated Lgr5*+* stem cell proliferation.\(^{19}\) The findings show that *H pylori* can colonize and manipulate the gastric stem cell compartment and this has significant implications for *H pylori*–induced gastric disease. These studies also suggest that alterations to stem cells may be responsible for the development of gastric cancer. In support of this idea, human studies have shown that there is enhanced Lgr5 expression in patients with progressive dedifferentiation and metastasis of gastric cancer.\(^{27,28}\)

In another study, the investigators deleted Smad4 and PTEN in murine gastric Lgr5*+* stem cells by the inducible Cre–LoxP system. In mice with altered/mutant Lgr5*+* stem cells there was a rapid onset and progression from adenoma to invasive intestinal-type gastric cancer in the antrum.\(^{29}\) Moreover, it has been shown in an organ culture system that Lgr5*+* gastric stem cell homeostasis is regulated by the Notch signaling pathway.\(^{30}\) In this study, it also was shown that chronic activation of Notch within gastric Lgr5*+* stem cells induced the development of antral polyps in mice, implicating this pathway in gastric tumorigenesis.\(^{30}\) In addition, GLI2A is an activator form of the Hedgehog transcription factor GLI2, and its expression within Lgr5*+* gastric stem cells drives the rapid development of gastric adenocarcinoma.\(^{20}\) Thus, alterations in Lgr5*+* gastric stem cells, which potentially are induced by *H pylori* infection, may be an initiating event for the development of gastric cancer, indicating the potential of Lgr5 as an early diagnostic and prognostic biomarker.

Another host molecule that may influence carcinogenesis in conjunction with *H pylori* is CD44. CD44 is a cell surface adhesion molecule that is expressed on a variety of cells, including gastric epithelial cells, that recently was identified as a gastric cancer stem cell marker, whereby cells expressing CD44 have been shown to possess the properties of cancer stem cells.\(^{31}\) Defined as a unique subpopulation in tumors that possess the ability to initiate tumor growth and sustain tumor self-renewal, a subpopulation of CD44*+* cells showed spheroid colony formation in serum-free media in vitro as well as tumorigenic ability when injected orthotopically into stomachs of immunodeficient mice in vivo. In addition, the CD44*+* gastric cancer cells showed the stem cell properties of self-renewal, and CD44 knockdown by short hairpin RNA resulted in reduced spheroid colony formation and tumors in immunodeficient mice.\(^{31}\) In the normal stomach, CD44 labels a population of undifferentiated cells in the isthmus region where stem cells are known to reside.\(^{22}\) Atrophy of parietal cells that is induced by *Helicobacter* infection or tamoxifen treatment results in the expansion of CD44*+* cells into the base of the gastric glands.\(^{22}\)

Alternative messenger RNA splicing produces CD44 variant isoforms that are expressed abundantly in epithelial-type carcinomas, although the standard CD44 isoform is expressed predominantly in hematopoietic cells and normal epithelial cell subsets (Figure 1).\(^{32,33}\) The involvement of CD44 variant isoforms in gastric cancer has not been well studied. What is known, however, is that early studies by Heider et al.\(^{34}\) showed that the normal epithelium expresses 2 of 12 CD44 variant RNAs containing exons V5 and V6. Intestinal-type tumors express a more complex pattern of amplification products that hybridized to exons V5 and V6. In the sample of a diffuse-type tumor, expression of exon V5, but not V6, could be detected.\(^{34}\) However, CD44 variant isoform containing exon v9 (CD44v9) also has been detected as a potential predictive marker for recurrence in multiple early gastric cancers.\(^{35}\)

CD44 variant isoforms, in particular the isoform containing exon v6 (CD44v6), was identified as a marker for invasive intramucosal carcinoma and premalignant lesions.\(^{36}\) Moreover, in cases of sporadic and hereditary
diffuse gastric cancer, CD44v6 expression correlated inversely with the expression of E-cadherin.\(^\text{36}\) Another variant isoform, CD44v8–10, has been shown to be a cancer stem cell marker. Recently, CD44v9 was observed to emerge during gastric regeneration. In addition, the higher expression for CD44v9 was observed in gastric cancer tissues, with greater expression rates for CD44v9 in the intestinal type or well-differentiated gastric cancer than in the diffuse type or poorly differentiated gastric cancer.\(^\text{38,39}\) Within cancer cells, CD44v9 interacts with the glutamate-cysteine transporter SLC7A11 (xCT), stabilizes the protein, and thereby potentiates defense against reactive oxygen species that subsequently promotes...
tumor growth\(^{40}\) (Figure 2). Thus, CD44 and its splice variants are associated positively with the initiation and progression of gastric cancer and may play important roles in the diagnosis, therapy, and prognosis of this disease.

Notably, CD44v6 acts as the co-receptor for c-Met.\(^{31,42}\) The extracellular domain of CD44v6 is necessary for c-Met activation, which is dependent on hepatocyte growth factor binding\(^{43}\) (Figure 2). The co-receptor function of CD44v6 for c-Met is of particular interest given that studies pinpoint CD44v6 as a marker of early invasive intramucosal gastric carcinoma.\(^{36}\) The cytoxin-associated gene (Cag) pathogenicity island is a strain-specific constituent of \(H\) pylori that augments cancer risk.\(^{43,44}\) The Cag pathogenicity island encodes a type IV secretion system that mediates the translocation of bacterial factors into the host cell.\(^{43,44}\) Evidence in the literature shows that \(H\) pylori–expressed CagA accumulates in gastric cancer cells specifically expressing CD44 and showing suppression of autophagy by their resistance to reactive oxygen species (ROS), and thus suggesting that CD44+ cells are resistant to oxidative stress.\(^{45}\) In cells lacking CD44 expression, CagA is degraded by autophagy induced by the accumulation of ROS.\(^{45}\) Upon delivery into the host cells by the type IV Cag secretion system, CagA translocates into the host cell cytoplasm where it can stimulate cell signaling through interaction with several host proteins,\(^{43,46,47}\) including the tyrosine kinase c-Met receptor.\(^{48-50}\) CagA exerts effects within host cells by inducing hyperproliferation and disrupting apical-junctional complexes and cellular polarity.\(^{51-53}\) Suzuki et al.\(^{54}\) showed that CagA CM motifs interact with Met, leading to sustained PI3K-AKT signaling in response to \(H\) pylori, leading to \(\beta\)-catenin activation and cellular proliferation. We have published that CD44v6 acts as a co-receptor for the function of c-Met in response to \(H\) pylori infection and bacterial-induced epithelial proliferation.\(^{21}\) Collectively, these studies suggest that CD44 and its variant isoforms may not simply be markers of the gastric cancer stem cell, but also actively involved in the initiation and progression of disease.

**Unregulated Spasmolytic Polypeptide/Trefoil Factor 2–Expressing Metaplasia in the Initiation of Gastric Cancer**

It is accepted that loss of acid-secreting parietal cells is a prerequisite for the development of metaplasia and a mucosal lineage change associated with increased risk for gastric cancer.\(^{55-57}\) Loss of parietal cells results in the transdifferentiation of the chief cell lineage into a mucous cell metaplasia identified as spasmolytic polypeptide expressing metaplasia (SPEM).\(^{58}\) Although \(Helicobacter\) infection, tamoxifen treatment, and parietal cell–specific protonophores (DMP-777 and L635) are known inducers of SPEM,\(^{58,59}\) parietal cell loss alone is not sufficient to induce metaplasia.\(^{60}\) In a study using a mouse model expressing the diphtheria toxin receptor specifically in parietal cells to induce their death, metaplastic reprogramming of chief cells was not observed, suggesting mechanisms beyond parietal cell injury and apoptosis.\(^{60}\) Metaplastic mucous cells arising for the loss of parietal cells express trefoil factor 2, also known as spasmolytic polypeptide, thus leading to the designation of this lineage as SPEM.\(^{56,61,62}\) SPEM also is associated with increased expression of cell surface protein CD44, in particular CD44v9.\(^{63}\) SPEM also has been identified in the mucosa surrounding intestinal-type gastric cancers in human beings.\(^{62}\) Importantly, mice infected with \(Helicobacter\) felis for more than 12 months showed progression of the metaplasia to dysplasia.\(^{56,57,64}\) These reports indicated that metaplastic glands induced by parietal cell loss and chronic inflammation progress toward dysplasia. Interestingly, we reported the induction of SPEM after gastric injury.\(^{65}\) SPEM was identified in the ulcer margin in the regenerating gastric glands and disappeared when the mucosa returned to its normal compendium of cell lineages, suggesting a possible role for SPEM in ulcer repair.\(^{65}\)

Collectively, these studies suggest that in response to gastric ulceration, SPEM is a regulated mechanism that may contribute to repair. SPEM in the setting of parietal cell atrophy and chronic inflammation is an unregulated precursor lineage for the development of dysplasia associated with gastric cancer development. The stem cell marker CD44v9 also marks SPEM and may contribute to the production of metaplasia.

**Maintenance of Gastric Cancer: The Role of the Cancer Stem Cells**

The gastrointestinal tumor microenvironment is required for tumor initiation, progression, and metastasis. Solid tumors are heterogeneous and consist of cancer cells, cancer stem cells, and various types of stromal cells, fibroblasts, endothelial cells, and hematopoietic cells, mainly macrophages and lymphocytes.\(^{10,11,40,66}\) Poor response of gastric cancer to various existing treatment modalities may be accounted for by the cellular heterogeneity of the tumor microenvironment. In particular, chemotherapy is one of the standard methods of treatment in many cancers including gastric cancer.\(^{67}\) Although chemotherapy often is capable of inducing cell death in tumors, many cancer patients experience recurrence because of failure to effectively target the cancer stem cells, which are believed to be key tumor-initiating cells. One CSC model proposes that the growth of the tumor is driven by a population of self-sustaining cells with stem cell properties of proliferation and an ability to differentiate into the entire heterogeneous population of the tumor.\(^{13,14}\) These CSC are responsible for the formation, maintenance, and continued growth of the tumor.\(^{14,15}\) This model highlights the need to target CSCs with chemotherapy. The targeted agent eliminates the chemoresistant CSC population, preventing recurrence of the tumor, while the chemotherapy targets the differentiated cells. As discussed earlier, variant isoforms of CD44 including CD44v6 and CD44v9 have been reported to have prognostic value in gastric cancer. For example, CD44v9 expression in primary early gastric cancer is a predictive marker for recurrence,\(^{35}\) and the presence of CD44v9-positive circulating cancer cells is associated strongly with recurrence and poor survival rates in colorectal cancer.\(^{68}\) In our laboratory, gastric...
organoids derived from the tumor tissue of a patient with diffuse-type gastric cancer expressing CD44v9 were resistant to cisplatin. Interestingly, inhibition of xCT by sulfasalazine sensitized the organoids to cisplatin-induced atrophy (unpublished data). Thus, this is an example by which therapy targeted to the CD44v9–xCT system may impair the ROS defense by cancer stem cells and thereby sensitize them to currently available treatments.

Immune suppression and adaptive immune resistance are other mechanisms that contribute to the maintenance and growth of tumors. The immune system typically detects and eliminates cancer development via a process termed immune surveillance. However, during immune evasion, the tumor cells escape the immune system. For example, tumor-derived cytokines can induce the differentiation of immune effectors to a suppressive phenotype such as tumor-associated macrophages, myeloid-derived suppressor cells (MDSCs), and regulatory CD4+ T cells. Tumor-associated macrophages within the tumor microenvironment have been described as protumorigenic, supporting cancer initiation and progression. Another example is the suppressive function of MDSCs. MDSCs are a heterogeneous myeloid cell population that infiltrates tissue in response to infection, injury, autoimmune disease, and cancer. A study by the Merchant laboratory identified Schlafen 4 (Slfn4) as a GLI1 target gene and myeloid differentiation factor that correlated with the development of SPEM in mice. A recent study by the same group then showed that migration of Slfn4-expressing cells from the bone marrow to the stomach in response to Helicobacter infection showed MDSC markers, and acquired the ability to inhibit T-cell proliferation. This supports MDSC suppression of T-cell function and subsequent dampening of the immune response to create a microenvironment favoring tumor growth.

Tumor cells may evade the immune response by inducing T-cell inactivation. Dendritic cells within the tumor microenvironment present tumor-specific antigens on major histocompatibility complex class I to CD8+ T cells that then can prime an antigen-specific T-cell response. In colorectal tumors, high densities of cytotoxic and memory T cells in the tumor microenvironment are associated with reduced recurrence of disease. Tumors can evade immune surveillance by expressing molecules such as programmed cell death 1 ligand (PDL1), which interacts with PD1 and subsequently inhibits CD8+ cytotoxic T-lymphocyte proliferation, survival, and effector function. In addition, CTLA-4, which is expressed minimally on the surface of resting T lymphocytes, is highly expressed on activated T lymphocytes. Importantly, ligands B7-1/B7-2, expressed on tumor cells, bind to CTLA-4 and inhibit effector T-cell function (Figure 3). Although anti-PD1 antibodies are already in clinical trials for gastric cancer treatment, whether PD1-PDL1 or CTLA-4/B7 interactions inhibit CD8+ cytotoxic T-cell effector function within the gastric tumor microenvironment has not been well studied. Importantly, a preclinical model that predicts the efficacy of such targeted therapies in individual patients with gastric cancer does not exist. In support of this notion, however, PD1+ (B7-H1+) gastric cancer stem cells show an increased proliferative capacity. PDL1 also has been identified as an independent prognostic factor for patients.

Figure 3. Illustration for the proposed development of gastric cancer initiated by chronic inflammation. During H pylori infection, CD44v9 expression emerges, a marker of the gastric cancer stem cell. Despite loss of H pylori infection over time, CD44v9 expression is maintained in cancer stem cells within the tumor. We may predict that tumor antigen secreted by the CD44v9+ cells activate dendritic cells that subsequently activate CD8+ cytotoxic T cells to express PD1 and CTLA-4, a mechanism by which cancer stem cells can evade the immune response via inactivation of T-cell effector function.
with stage II/III gastric cancer, suggesting that patients with stage II/III gastric cancer might be appropriate for PD1/PDL1-targeted therapy. Furthermore, selective expression of the PDL1 was observed on CD44(+) cells in squamous cell carcinoma of the head and neck and breast cancer cells. In addition, PDL1 expression was associated with worse overall survival in patients with stage II/III gastric cancer, suggesting that these patients might be appropriate for PD1/PDL1-targeted therapy.

Future Directions

Given the poor response of gastric cancer to various existing treatment modalities, there is an unmet need for approaches to predict individual therapy responses. Although cancer cell lines and patient-derived tumor xenografts (PDTXs) have proven very valuable in fundamental cancer research, both models have disadvantages. Cell lines lack the tumor heterogeneity found in tumor tissues, and although PDTXs bear promise as preclinical models for human cancer, there are several limitations. For example, similarities between PDTX and parental tumors cannot be assumed until rigorously tested, tumor-host interactions are not always conserved across species and tumor immunity is entirely absent. As mentioned earlier, a preclinical model that predicts the efficacy of such targeted therapies in individual patients with gastric cancer does not exist. We and others have shown that gastric organoids not only have the tissue architecture and physiological function of the human stomach, but are also an experimentally tractable system allowing for cell and genetic manipulation. Moreover, patient-derived gastric tumor organoids can be transplanted orthotopically into NOD.Cg-Rag1[tmMom]/L2rg[tmWjl]Tg(CMV-IL3, CSF2, KITLG)/Eav/J (NRGS) (NRG-SGM3) mice expressing human-derived immune cells so that the role of the immune response within the tumor microenvironment may be identified in vivo (unpublished data). Understanding the immune response within the tumor microenvironment is crucial not only to addressing important biological questions, but also for the success of current and future immunotherapy. Potential studies that use organoids include the following: (1) in vitro and in vivo organoid-based approaches for the study of the interaction between cancer stem cells and the immune microenvironment using organoid/immune cell cultures; (2) a preclinical organoid-based platform for anticancer drug evaluation; and (3) in vitro and in vivo preclinical models that will allow us to effectively evaluate novel cancer therapeutics as well as to identify predictive biomarkers for gastric cancer. Although organoids have been widely used for biological and clinical studies, this system does show key challenges, as follows: (1) the lack of the native microenvironment; (2) organoids within the same culture may be phenotypically heterogeneous; and (3) maintenance of epithelial heterogeneity within the organoid culture over time is unclear. Despite these challenges, the development of cutting edge in vitro and in vivo organoid-based approaches from a common patient sample is a critical first step for personalized medicine providing a preclinical approach to prevent, diagnose, or treat gastric cancer.

References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136:E359–E386.
2. Piazuelo MB, Epplen M, Correa P. Gastric cancer: an infectious disease. Infect Dis Clin North Am 2010; 24:853–869.
3. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11–30.
4. Neugut AI, Hayek M, Howe G. Epidemiology of gastric cancer. Semin Oncol 1998;23:281–291.
5. Covacci A, Telford JL, Del Giudice G, et al. Helicobacter pylori virulence and genetic geography. Science 1999; 284:1328–1333.
6. Parsonnet J, Friedman GD, Vandersteen DP, et al. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127–1131.
7. Joossens JV, Hill MJ, Elliott P, et al. Dietary salt, nitrate and stomach cancer mortality in 24 countries. European Cancer Prevention (ECP) and the INTERSALT Cooperative Research Group. Int J Epidemiol 1996;25:494–504.
8. El-Omar E. The importance of interleukin 1beta in Helicobacter pylori associated disease. Gut 2001; 48:743–747.
9. El-Omar EM, Chow WH, Rabkin CS. Gastric cancer and H. pylori: host genetics open the way. Gastroenterology 2001;121:1002–1005.
10. Quante M, Wang TC. Inflammation and stem cells in gastrointestinal carcinogenesis. Physiology 2008; 23:350–359.
11. Quante M, Varga J, Wang TC, et al. The gastrointestinal tumor microenvironment. Gastroenterology 2013; 145:63–78.
12. Liu S, Cong Y, Wang D, et al. Breast cancer stem cells transition between epithelial and mesenchymal states reflective of their normal counterparts. Stem Cell Reports 2014;2:78–91.
13. O’Connor ML, Xiang D, Shigdar S, et al. Cancer stem cells: a contentious hypothesis now moving forward. Cancer Lett 2014;344:180–187.
14. Brungs D, Aghmesheh M, Vine KL, et al. Gastric cancer stem cells: evidence, potential markers, and clinical implications. J Gastroenterol 2016;51:313–326.
15. Dewi DL, Ishii H, Kano Y, et al. Cancer stem cell theory in gastrointestinal malignancies: recent progress and upcoming challenges. J Gastroenterol 2011;46:1145–1157.
16. Correa P, Haenszel W, Cuello C, et al. A model for gastric cancer epidemiology. Lancet 1975;2:58–60.
17. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984;1:1311–1315.
18. Bessède E, Staedel C, Acuña Amador L, et al. Helicobacter pylori generates cells with cancer stem cell properties via epithelial-mesenchymal transition-like changes. Oncogene 2014;33:4123–4131.
19. Sigal M, Rothenberg ME, Logan CY, et al. Helicobacter pylori activates and expands Lgr5(+) stem cells through direct colonization of the gastric glands. Gastroenterology 2015;148:1392–1404 e21.

20. Syu LJ, Zhao X, Zhang Y, et al. Invasive mouse gastric adenocarcinomas arising from Lgr5(+) stem cells are dependent on crosstalk between the Hedgehog/GLI2 and mTOR pathways. Oncotarget 2016;7:10255–10270.

21. Bertaux-Skeirik N, Feng R, Schumacher MA, et al. CD44 plays a functional role in Helicobacter pylori-induced epithelial cell proliferation. PLoS Pathog 2015;11:e1004663.

22. Khurana SS, Riehl TE, Moore BD, et al. The hyaluronic acid receptor CD44 coordinates normal and metaplastic gastric epithelial progenitor cell proliferation. J Biol Chem 2013;288:16085–16097.

23. Stange DE, Koo BK, Huch M, et al. Differentiated Troy(+) chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. Cell 2013;155:357–368.

24. Arnold K, Sarkar A, Yram MA, et al. Sox2(+) adult stem and progenitor cells are important for tissue regeneration and survival of mice. Cell Stem Cell 2011;9:317–329.

25. Qiao XT, Ziel JW, McKimpson W, et al. Prospective identification of a multilineage progenitor in murine stomach epithelium. Gastroenterology 2007;133:1989–1998.

26. Barker N, Huch M, Kujala P, et al. Lgr5(+) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell 2010;6:25–36.

27. Zheng ZX, Sun Y, Bu ZD, et al. Intestinal stem cell marker LGR5 expression during gastric carcinogenesis. World J Gastroenterol 2013;19:8714–8721.

28. Bu Z, Zheng Z, Zhang L, et al. LGR5 is a promising biomarker for patients with stage I and II gastric cancer. Chin J Cancer Res 2013;25:79–89.

29. Li XB, Yang G, Zhu L, et al. Gastric Lgr5(+) stem cells are the cellular origin of invasive intestinal-type gastric cancer in mice. Cell Res 2016;26:838–849.

30. Demitrack ES, Gifford GB, Keeley TM, et al. Notch signaling regulates gastric antral LGR5 stem cell function. EMBO J 2015;34:2522–2536.

31. Takaishi S, Okumura T, Tu S, et al. Identification of gastric cancer stem cells using the cell surface marker CD44. Stem Cells 2009;27:1006–1020.

32. Orian-Rousseau V. CD44 Acts as a signaling platform controlling tumor progression and metastasis. Front Immunol 2015;6:154.

33. Orian-Rousseau V, Ponta H. Perspectives of CD44 targeting therapies. Arch Toxicol 2015;89:3–14.

34. Heider KH, Dammrich J, Skroch-Angel P, et al. Differential expression of CD44 splice variants in intestinal- and diffuse-type human gastric carcinomas and normal gastric mucosa. Cancer Res 1993;53:4197–4203.

35. Hirata K, Suzuki H, Imaeda H, et al. CD44 variant 9 expression in primary early gastric cancer as a predictive marker for recurrence. Br J Cancer 2013;109:379–386.

36. da Cunha CB, Oliveira C, Wen X, et al. De novo expression of CD44 variants in sporadic and hereditary gastric cancer. Lab Invest 2010;90:1604–1614.

37. Lau WM, Teng E, Chong HS, et al. CD44v8-10 is a cancer-specific marker for gastric cancer stem cells. Cancer Res 2014;74:2630–2641.

38. Jang BI, Li Y, Graham DY, et al. The role of CD44 in the pathogenesis, diagnosis, and therapy of gastric cancer. Gut Liver 2011;5:397–405.

39. Go SI, Ko GH, Lee WS, et al. CD44 variant 9 serves as a poor prognostic marker in early gastric cancer, but not in advanced gastric cancer. Cancer Res Treat 2016;48:142–152.

40. Ishimoto T, Nagano O, Yae T, et al. CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc(-) and thereby promotes tumor growth. Cancer Cell 2011;19:387–400.

41. Orian-Rousseau V, Chen L, Sleeman JP, et al. CD44 is required for two consecutive steps in HGF/c-Met signaling. Genes Dev 2002;16:3074–3086.

42. Orian-Rousseau V, Morrison H, Matzke A, et al. Hepatocyte growth factor-induced Ras activation requires ERM proteins linked to both CD44v6 and F-actin. Mol Biol Cell 2007;18:76–83.

43. Odenbreit S, Puls J, Sedmaier B, et al. Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. Science 2000;287:1497–1500.

44. Segal ED, Falkow S, Tompkins LS. Helicobacter pylori attachment to gastric cells induces cytoskeletal rearrangements and tyrosine phosphorylation of host cell proteins. Proc Natl Acad Sci U S A 1996;93:1259–1264.

45. Tsugawa H, Suzuki H, Saya H, et al. Reactive oxygen species-induced autophagic degradation of Helicobacter pylori CagA is specifically suppressed in cancer stem-like cells. Cell Host Microbe 2012;12:764–777.

46. Higashi H, Tsutsumi R, Muto S, et al. SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA. Science 2002;295:683–686.

47. Selbach. The Helicobacter pylori CagA protein induces tyrosine dephosphorylation of ezrin. Proteomics 2004;4:2961–2968.

48. Oliveira MJ, Costa AC, Costa AM, et al. Helicobacter pylori induces gastric epithelial cell invasion in a c-Met and type IV secretion system-dependent manner. J Biol Chem 2006;281:34888–34896.

49. Oliveira MJ, Costa AM, Costa AC, et al. CagA associates with c-Met, E-cadherin, and p120-catenin in a multi-protein complex that suppresses Helicobacter pylori-induced cell-invasive phenotype. J Infect Dis 2009;200:745–755.

50. Churin Y, Al-Ghoul L, Kepp O, et al. Helicobacter pylori CagA protein targets the c-Met receptor and enhances the motogenic response. J Cell Biol 2003;161:249–255.

51. Amieva MR, Vogelmann R, Covacci A, et al. Disruption of the epithelial apical-junctional complex by Helicobacter pylori CagA. Science 2003;300:1430–1434.

52. Franco AT, Israel DA, Washington MK, et al. Activation of beta-catenin by carcinogenic Helicobacter pylori. Proc Natl Acad Sci U S A 2005;102:10646–10651.

53. Saadat I, Higashi H, Obuse C, et al. Helicobacter pylori CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. Nature 2007;447:330–333.
54. Suzuki M, Mimuro H, Kiga K, et al. Helicobacter pylori CagA phosphorylation-independent function in epithelial proliferation and inflammation. Cell Host Microbe 2009; 5:23–34.
55. El-Zimaity HM, Ota H, Graham DY, et al. Patterns of gastric atrophy in intestinal type gastric carcinoma. Cancer 2002;94:1429–1436.
56. Wang TC, Goldenring JR, Dangler C, et al. Mice lacking secretory phospholipase A2 show altered apoptosis and differentiation with Helicobacter felis infection. Gastroenterology 1998;114:675–689.
57. Fox JG, Li X, Cahill RJ, et al. Hypertrophic gastropathy in Helicobacter felis-infected wild-type C57BL/6 mice and p53 hemizygous transgenic mice. Gastroenterology 1996;110:155–166.
58. Nam KT, Lee HJ, Sousa JF, et al. Mature chief cells are cryptic progenitors for metaplasia in the stomach. Gastroenterology 2010;139:2028–2037.
59. Weis VG, Sousa JF, LaFleur BJ, et al. Heterogeneity in mouse spasmodic polypeptide-expressing metaplasia lineages identifies markers of metaplastic progression. Gut 2013;62:1270–1279.
60. Burclaff J, Osaki LH, Liu D, et al. Targeted apoptosis of parietal cells is insufficient to induce metaplasia in stomach. Gastroenterology 2017;152:762–766.
61. Wang TC, Dangler CA, Chen D, et al. Synergistic interaction between hypergastrinemia and Helicobacter infection in a mouse model of gastric cancer. Gastroenterology 2000;118:36–47.
62. Schmidt PH, Lee JR, Joshi V, et al. Identification of a metaplastic cell lineage associated with human gastric adenocarcinoma. Lab Invest 1999; 79:639–646.
63. Wada T, Ishimoto T, Seishima R, et al. Functional role of CD44v-xCT system in the development of spasmodic polypeptide-expressing metaplasia. Cancer Sci 2013; 104:1323–1329.
64. Nomura S, Baxter T, Yamaguchi H, et al. Spasmodic polypeptide expressing metaplasia to preneoplasia in H. felis-infected mice. Gastroenterology 2004;127:582–594.
65. Engevik AC, Feng R, Choi E, et al. The development of spasmodic polypeptide/TF2-expressing metaplasia (SPEM) during gastric repair is absent in the aged stomach. Cell Mol Gastroenterol Hepatol 2016; 2:606–624.
66. Quante M, Tu SP, Tomita H, et al. Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. Cancer Cell 2011; 19:257–272.
67. Ahmad SA, Xia BT, Bailey CE, et al. An update on gastric cancer. Curr Probl Surg 2016;53:449–490.
68. Katoh S, Goi T, Naruse T, et al. Cancer stem cell marker in circulating tumor cells: expression of CD44 variant exon 9 is strongly correlated to treatment refractoriness, recurrence and prognosis of human colorectal cancer. Anticancer Res 2015;35:239–244.
69. Matsueda S, Graham DY. Immunotherapy in gastric cancer. World J Gastroenterol 2014;20:1657–1666.
70. Burnet FM. The concept of immunological surveillance. Prog Exp Tumor Res 1970;13:1–27.
71. Wang M, Busuttill RA, Pattison S, et al. Immunological battlefield in gastric cancer and role of immunotherapies. World J Gastroenterol 2016;22:6373–6384.
72. Lee K, Hwang H, Nam KT. Immune response and the tumor microenvironment: how they communicate to regulate gastric cancer. Gut Liver 2014;8:131–139.
73. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell 2010; 141:39–51.
74. Murray PJ, Allen JE, Biswas SK, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity 2014;41:14–20.
75. Yan Y, Zhang J, Li JH, et al. High tumor-associated macrophages infiltration is associated with poor prognosis and may contribute to the phenomenon of epithelial-mesenchymal transition in gastric cancer. Onco Targets Ther 2016;9:3975–3983.
76. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009;9:162–174.
77. El-Zaatari M, Kao JY, Tessier A, et al. Gli1 deletion prevents Helicobacter-induced gastric metaplasia and expansion of myeloid cell subsets. PLoS One 2013; 8:e58935.
78. Ding L, Hayes MM, Photenhauer A, et al. Schlafen 4-expressing myeloid-derived suppressor cells are induced during murine gastric metaplasia. J Clin Invest 2016;126:2867–2880.
79. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 2012;12:253–268.
80. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245–252.
81. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006; 313:1960–1964.
82. Ahmadzadeh M, Johnson LA, Heemskerk B, et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood 2009;114:1537–1544.
83. Chen X, Fosco D, Kline DE, et al. PD-1 regulates extrathymic regulatory T-cell differentiation. Eur J Immunol 2014;44:2603–2616.
84. Reissfelder C, Stamova S, Gossmann C, et al. Tumor-specific cytotoxic T lymphocyte activity determines colorectal cancer patient prognosis. J Clin Invest 2015; 125:739–751.
85. McCoy KD, Hermans IF, Fraser JH, et al. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) can regulate dendritic cell-induced activation and cytotoxicity of CD8(+) T cells independently of CD4(+) T cell help. J Exp Med 1999;189:1157–1162.
86. McCoy KD, Le Gros G. The role of CTLA-4 in the regulation of T cell immune responses. Immunol Cell Biol 1999;77:1–10.
87. Muro K, Chung HC, Shankaran V, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. Lancet Oncol 2016;17:717–726.
88. Yang Y, Wu KE, Zhao E, et al. B7-H1 enhances proliferation ability of gastric cancer stem-like cells as a receptor. Oncol Lett 2015;9:1833–1838.

89. Tamura T, Ohira M, Tanaka H, et al. Programmed death-1 ligand-1 (PDL1) expression is associated with the prognosis of patients with stage II/III gastric cancer. Anticancer Res 2015;35:5369–5376.

90. Lee Y, Shin JH, Longmire M, et al. CD44+ cells in head and neck squamous cell carcinoma suppress T-cell-mediated immunity by selective constitutive and inducible expression of PD-L1. Clin Cancer Res 2016;22:3571–3581.

91. Alsuliman A, Colak D, Al-Harazi O, et al. Bidirectional crosstalk between PD-L1 expression and epithelial to mesenchymal transition: significance in claudin-low breast cancer cells. Mol Cancer 2015;14:149.

92. McCracken KW, Catà EM, Crawford CM, et al. Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. Nature 2014;516:400–404.

93. McCracken KW, Aihara E, Martin B, et al. Wnt/beta-catenin promotes gastric fundus specification in mice and humans. Nature 2017;541:182–187.