Role and research progress of spasmolytic polypeptide-expressing metaplasia in gastric cancer (Review)

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Abstract. Gastric cancer ranks as one of the most prevalent cancers worldwide. While the incidence of gastric cancer in Western countries has notably diminished over the past century, it continues to be a leading cause of cancer-related mortality on a global scale. The majority of gastric cancers in humans are attributed to chronic *Helicobacter pylori* infection and the progression of gastric cancer is often preceded by gastritis, atrophy, metaplasia and dysplasia. However, the precise mechanisms underlying the development of gastric cancer remain ambiguous, including the formation of gastric polyps and precancerous lesions. In humans, two types of precancerous metaplasia have been identified in relation to gastric malignancies: Intestinal metaplasia and spasmolytic polypeptide-expressing metaplasia (SPEM). The role of SPEM in the induction of gastric cancer has gained recent attention and its link with early-stage human gastric cancer is increasingly evident. To gain insight into SPEM, the present study reviewed the role and research progress of SPEM in gastric cancer.

Contents

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1. Introduction

According to global cancer statistics for 2020, the incidence and mortality rates of gastric cancer were the fifth and fourth highest, respectively, among common cancers. In China, the incidence and mortality rates of gastric cancer were both the third highest among all cancers (1). However, the occurrence of gastric cancer has not been studied in depth, and its development is preceded by a series of progressive changes, namely gastritis, atrophy, metaplasia, dysplasia and carcinogenesis (2). Metaplasia is defined as the replacement of a differentiated cell type by another mature differentiated cell type that is not normally present in a given tissue (3). In humans, two types of precancerous metaplasia are associated with gastric cancer, namely intestinal metaplasia (IM) and spasmolytic polypeptide-expressing metaplasia (SPEM). SPEM, also known as pseudopyloric metaplasia or mucinous metaplasia, is represented by morphological features of the deep antral gland or Brunner's glands and expresses trefoil factor 2 (TFF2) and mucin 6 (MUC6) (4,5). IM is often considered a result of the transdifferentiation of mucosa into an intestinal phenotype, which appears to be associated with chronic inflammation (6) and is characterized by the presence of mucin-containing goblet cells, Paneth cells and absorptive cells and by the expression of TFF3 and MUC2 (7,8). Another member of the trefoil protein family, TFF1, is mainly secreted in gastric foveolar cells (9) and has a crucial role in gastrointestinal epithelium repair (10). The precise association between SPEM and IM and their origins warrant further exploration in large-scale clinical studies.

2. SPEM-discovery and overview

Gastric cancer is one of the most common cancers worldwide, but it does not occur suddenly. Gastritis progresses to malignant tumors after a series of developments and differentiation. The most common pathological type of gastric cancer is intestinal adenocarcinoma, which almost always occurs after metaplasia and is considered to occur due to the loss of acid-secreting

parietal cells in the stomach (oxyntic atrophy) (11). In 1999, Schmidt *et al* (5) reported an abnormal metaplasia cell lineage in the gastric fundic mucosa of mice infected with *Helicobacter felis*, with morphological features similar to those of Brunner's gland in the duodenum, which expressed TFF2, known as the spasmolytic polypeptide (Fig. 1). SPEM lineages have been further characterized as diastase-periodic acid Schiff-positive lineages expressing TFF2 and MUC6 and promoting GSII-agglutinin bonds, similar to mucus-secreting cells of the deep antral glands (12). Furthermore, this lineage expresses several other anal genealogical markers, including CD44 variant 9 (CD44v9) and clusterin, where CD44v9 marks the SPEM in the corpus and is involved in regeneration after gastric epithelial cell injury (7,13,14). These studies have shown a high degree of similarity between the spectra of SPEM and the deep antral glands.

3. Damage repair of the stomach

The stomach acts as an exocrine and endocrine organ involved in the digestion of food. The gastric unit consists of the glandular epithelium organized into repetitive tubular invaginations (15). Each gastric unit in the gastric body is divided into four regions: The apical pit region, which consists of mucus-producing pit cells that express MUC5AC and TFF1 (9); the isthmus region (directly below the pit region), which contains somatic stem cells expressing basic helix-loop-helix family member a15 (Bhlha15; also known as MIST1, a transcription factor) or transgenic markers driven by RUNX family transcription factor 1 enhancer elements (16,17); the neck region, which has neck cells expressing MUC6 and TFF2 and the epitope for the lectin *Griffonia simplificolia* (GS)II (9); and the bottom of the gastric somatic unit, which is filled with chief cells expressing intrinsic factors (18). In addition to the widespread distribution of acid-producing parietal cells across all four regions, it is essential to acknowledge that the emergence of SPEM is intimately connected to the observed plasticity in chief cells (18). Furthermore, there is evidence indicating that the transformation into SPEM occurs not only in the chief cell zone but also in the basal region (19,20). This highlights the intricate mechanisms at play in the development of SPEM. The mature principal cells in the isthmus region function as reserve stem cells in the metaplastic process, capable of reprogramming into various cell types. Regarding the topic of drug-induced SPEM, recent studies suggest that these mature principal cells in the isthmus are the primary contributors to the formation of SPEM cells, rather than the isthmic progenitor cells (18) (Fig. 1). This discovery challenges prior beliefs and necessitates further research for a comprehensive understanding of the mechanisms involved. However, the recognition of this novel source of SPEM cells marks a critical advancement in elucidating the process of cellular transformation within the stomach.

The human stomach undergoes daily damage from intrinsic or extrinsic sources, such as corrosion by gastric acid and damage by food, and the stomach repairs the damaged area through two mechanisms, namely superficial and glandular responses. The superficial response of the stomach requires rapid adaptation to prevent acid-induced epithelial integrity disruption. This response relies on the secretion of local protective factors to neutralize the corrosive effects of acid, regulation of mucosal blood flow to limit the duration and extent of damage and regeneration of the surface epithelium through recovery and diffusion (21). In contrast to the superficial response that occurs because of acid production, the glandular response occurs when acid production is disrupted or lost, and this response is characterized by a reduction in acid-producing mural cells (atrophy). Furthermore, the glandular response produces significant plasticity in gastric cells and this plasticity increases the risk of cancer accumulation. However, the main histological pattern observed in response to oxyntic atrophy is gland reaggregation, which depletes mature mural and principal cells but retains metaplastic cells (22). Thus, this glandular response can also be considered the initiation of SPEM. The glandular response can be transient or prolonged and the regulatory mechanisms are unclear. The available literature indicates that SPEM is an evolutionarily conserved mechanism in response to glandular injury, but cellular signals and mechanisms regulating metaplastic transitions in the stomach are still poorly understood.

4. Oxyntic atrophy, inflammation, metaplasia and gastric cancer

Chronic gram-negative *H. pylori* infection is a major causative agent of gastric cancer in humans (23). Chronic *H. pylori* infection causes overall changes in the gastric mucosa, which may eventually lead to gastric adenocarcinoma. These overall changes are caused by two major effects of chronic *H. pylori* infection: Mural cell loss (or oxyntic atrophy) and marked inflammation. Mural cells have an essential role in the differentiation of the entire gastric lineage, and therefore, oxyntic atrophy can have a profound effect on the entire differentiation of gastric mucosa. Mural cells are involved in the secretion of several signaling factors, including dual-regulatory proteins, transforming growth factor (TGF)-α, heparin-binding epidermal growth factor-like growth factor and hedgehog factor (24-27). With the loss of parietal cells, these signaling molecules are not secreted properly, which affects the normal differentiation of the rest of the spectrum. For instance, the loss of parietal cells, the downregulation of the chief cell by its zymogen secretory apparatus (accompanied by the loss of the master regulator of the secretory apparatus, transcription factor MIST1), and the reduction of the chief cell maturation phenotype (degeneration) lead to the reprogramming of the chief cell transcriptome to finally transdifferentiate into SPEM (11) (Fig. 1). The second major outcome of chronic *H. pylori* infection is marked inflammation throughout the mucosa, and inflammation can induce SPEM gene expression, leading to SPEM. In summary, oxyntic atrophy, as well as marked inflammation, may be prerequisites for metaplasia and gastric cancer (28).

Oxyntic atrophy, accompanied by an inflammatory response, can evolve into metaplasia, which manifests in two forms: IM and SPEM, both linked with the progression to intestinal type gastric cancer (5,29-32) (Fig. 2). Consequently, a pronounced inflammatory response and oxyntic atrophy constitute the underlying factors for the altered spectrum of gastric differentiation and the advancement toward gastric cancer. Initially, IM was perceived as a precancerous

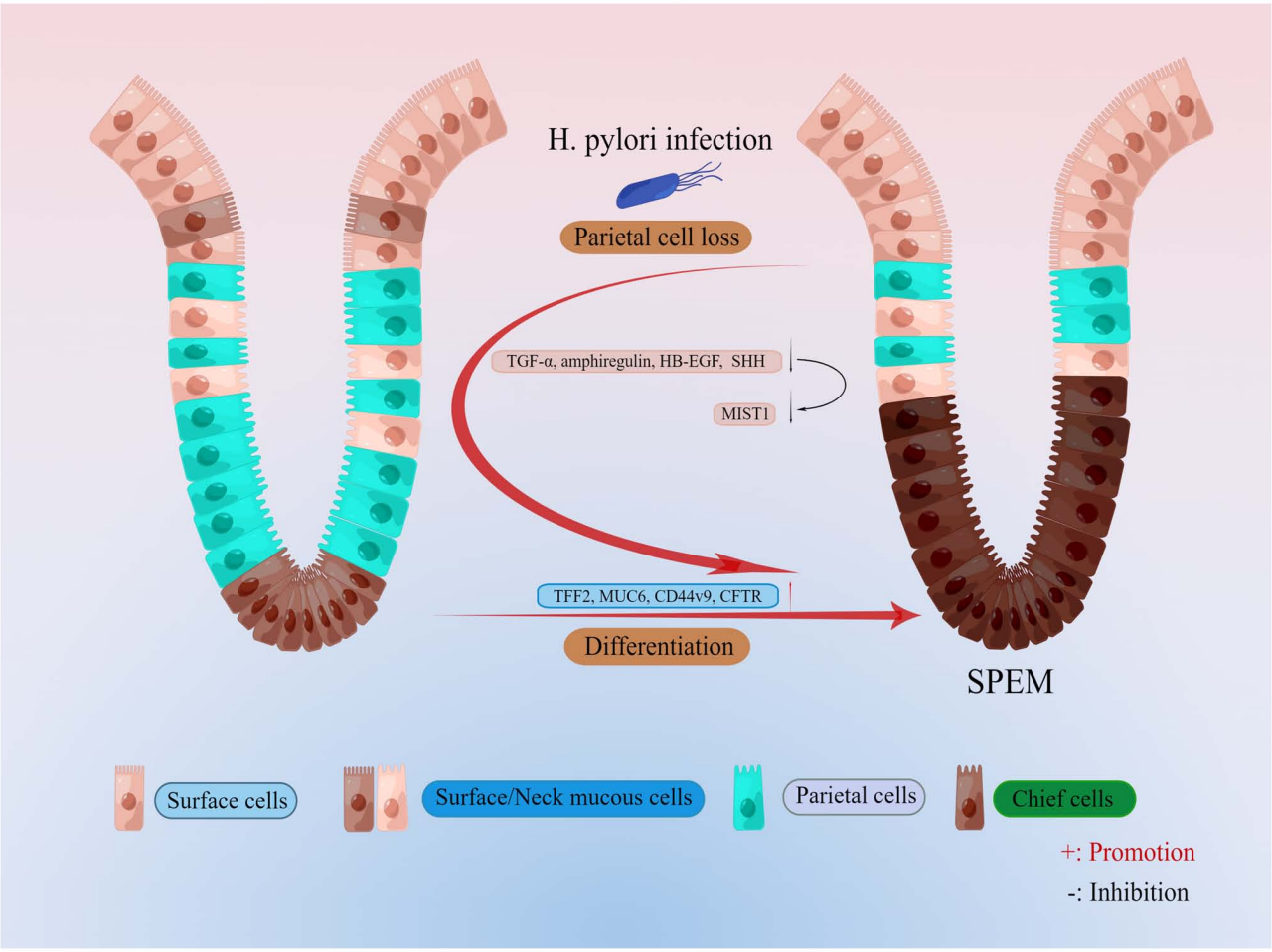

Figure 1. SPEM differentiation process. After the loss of parietal cells (caused by, e.g., *H. pylori* infection or parietal cytotoxic drugs), chief cells downregulate their zymogen secretion apparatus (accompanied by a loss of the master regulator transcription factor MIST1 of the secretion apparatus), and a reduction in the chief cell maturation phenotype (degeneration) leads to chief cell transcriptome reprogramming and finally transdifferentiation followed by SPEM formation. In addition, studies suggest that mucous neck cells and isthmus cells are involved in this process, but the available data strongly suggest that chief cell reprogramming is the main source of SPEM. SPEM, spasmolytic polypeptide-expressing metaplasia; SHH, Sonic hedgehog; MIST1, basic helix-loop-helix family member a15; HB-EGF, heparin-binding EGF-like growth factor; MUC, mucin; CD44v9, CD44 variant 9; TFF2, trefoil factor 2; CFTR, CF transmembrane conductance regulator.

condition leading to intestinal-type cancers, characterized by IM of cupped cells expressing specific intestinal markers such as MUC2 and TFF3 (7,8). Another form of precancerous metaplasia, SPEM, was identified, exhibiting a mucinous metaplasia profile. SPEM displays more distinct morphological characteristics akin to deep anal gland cells or Brunner's glands, expressing markers such as MUC6 and TFF2 (4,5). Numerous studies have proposed that both SPEM and IM may represent preneoplastic metaplastic conditions. Independent research conducted in the US, Japan and Iceland has identified SPEM in >90% of resected gastric cancers (5,32,33). Furthermore, SPEM is present in almost all gastric cancer types and in studies on animal models with chronic infection, transgenic and knockout genes, genetic manipulation or acute oxyntic atrophy, the findings suggest that SPEM is a crucial premalignant intermediate in the oncogenic transformation of gastric cancer (34). However, the exact mechanism by which SPEM promotes gastric carcinogenesis has remained elusive. Furthermore, a controlled study demonstrated that patients with gastric cancer infected with *H. pylori* had an increased likelihood of developing precancerous SPEM in the stomach, but SPEM regression could be achieved after the early eradication of *H. pylori* (35-37). In addition, with the production and regression of SPEM, some related tissue products, such as miR-21, miR-155 and miR-223, showed an increase and decrease in expression, respectively (36).

In humans, the origin of oxyntic atrophy and its progression into gastric cancer are difficult to understand, but in mouse experiments, these gaps can be addressed. Chronic *H. pylori* infection is one of the major causes of gastric cancer in humans, leading to oxyntic atrophy and marked inflammation. Studies have shown that the loss of mural cells, downregulation of the mature chief cell marker MIST1, and necroptosis of postmitotic cells located at the base of the gastric gland are hallmarks of SPEM (38). A mouse model of chronic *H. pylori* infection can be used to observe *H. pylori* infection in humans (39). After six months of *Helicobacter felis* infection, mural cells were markedly lost with inflammation, leading to the emergence of a proliferative SPEM lineage, almost exclusively from transdifferentiated primary cells (38), and SPEM develops into atypical hyperplasia one year after infection onset without phenotypic IM (40). It follows that

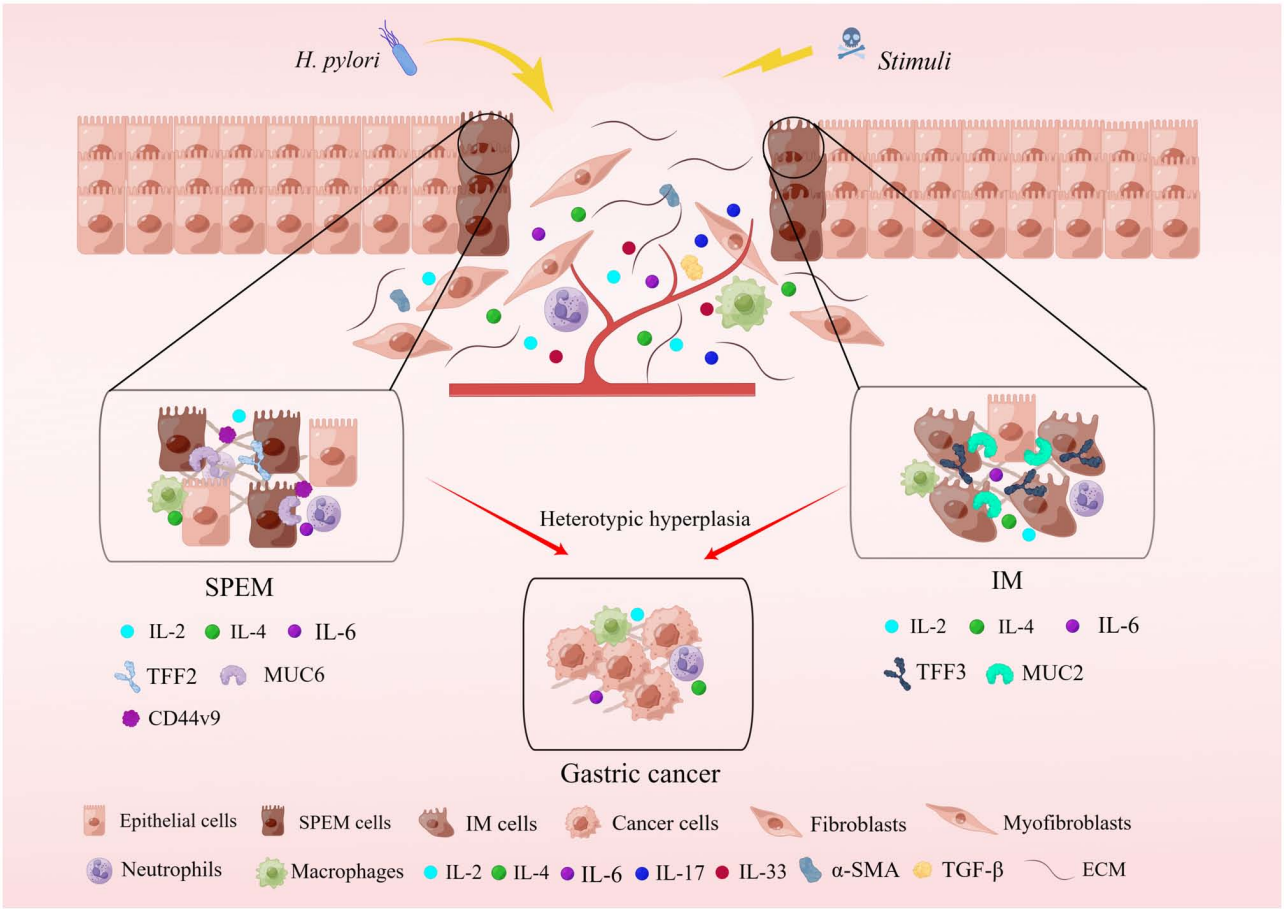

Figure 2. Expression of IM- and SPEM-specific factors associated with gastric cancer. In response to *H. pylori* or other stimuli, normal gastric mucosal cells are damaged and an inflammatory repair response is initiated. Inflammation leads to massive immune cell infiltration, gastric mucosal damage and gastric tissue repair. Cells that are repaired after injury progress in two directions, namely, IM, which is associated with gastric cancer, and SPEM. IM cells express TFF3 and MUC2, whereas SPEM cells express TFF2, MUC6 and CD44v9. They are all abnormal proliferation of gastric tissue, which eventually develops into gastric cancer. SPEM, spasmolytic polypeptide-expressing metaplasia; IM, intestinal metaplasia; CD44v9, CD44 variant 9; MUC, mucin; TFF2, trefoil factor 2; SMA, smooth muscle actin; ECM, extracellular matrix.

SPEM is a direct precursor of developmental abnormalities in mice infected with *H. felis*. However, the presence of two main causative factors, namely, oxyntic atrophy and marked inflammation, make it difficult to elucidate the direct source of SPEM from this model.

The role of mural cell loss and inflammation during SPEM initiation may be distinguished using mural cell-specific plasmid DMP-777-induced acute oxyntic atrophy in mice, L635-induced acute inflammation in mice and chronic inflammation in mouse models with *H. felis* infection (41,42). The plasmid DMP-777, which is a cell-permeable inhibitor of neutrophilic leukocyte elastase, was utilized to induce mural cell loss without eliciting an inflammatory response. In mice, three days of DMP-777 treatment led to oxyntic atrophy and 14 days of DMP-777 treatment induced SPEM as a direct consequence of mural cell loss; however, even after one year of DMP-777 treatment, SPEM did not progress to developmental abnormalities (41). In addition, DMP-777 caused acute mural cell loss along with increased serum gastrin levels and altered mucosal dynamics (41,43). In another experiment, SPEM developed more rapidly when gastrin- or amphipathic regulatory protein-deficient mice were treated with DMP-777 (42,44). In comparison, L635, a proton carrier analog of DMP-777 that lacks elastase inhibition, induced a significant inflammatory response in mouse models and phenotypically induced a spectrum of SPEM similar to that of *H. felis* infection (38). However, the progression of dysplasia cannot be analyzed through the long-term administration of L635 at this time due to drug availability problems. In studies of SPEM lineages, clusterin expression was upregulated in all, with the most significant increase in MUC5AC and MUC6 proteins (45), suggesting that clusterin expression is a hallmark of SPEM regardless of the cause of SPEM differentiation or the surrounding environment. In inflamed SPEM, the upregulation of cystic fibrosis transmembrane conductance regulator (CFTR) expression was observed, whereas CFTR expression was not observed in normal gastric fundus and noninflamed SPEM models (39). By contrast, in human pathology, CFTR was not observed in SPEM, even when accompanied by significant inflammation. However, IM featured strong CFTR expression. Overall, the DMP-777 model revealed that mural cell deficiency is sufficient to trigger SPEM, but inflammation is essential for further SPEM differentiation and development. Furthermore, the combination of the mouse model and human pathology revealed an association between SPEM and inflammation in mice and IM in humans (Fig. 2).

In addition, a study revealed that endogenous glucocorticoids prevent gastric epithelial metaplastic transition through the inhibition of spontaneous inflammation and are needed to maintain gastric homeostasis (46). Glucocorticoids are steroid hormones that inhibit proinflammatory stimuli, and adrenalectomy results in the rapid onset of spontaneous gastritis, oxyntic atrophy and metaplasia, expressing spasmolytic peptides (SPEM) in mice. Regarding the process by which glucocorticoids prevent metaplasia of the gastric epithelium, three days after adrenalectomy, the proinflammatory genes monocyte chemokines C-C motif chemokine ligand 2 and C-X3-C motif chemokine ligand 1 were rapidly upregulated in the gastric body, and their expression in the gastric body was inhibited by glucocorticoids (46). In addition, dysregulation of monocytes and macrophages has been associated with the development of several cancers; furthermore, glucocorticoids regulate monocyte recruitment, regulate monocyte activity and prevent pathogenic monocyte/lymphocyte activation (47,48). Numerous clinical studies have demonstrated that patients with adrenocortical insufficiency usually exhibit autoimmune and inflammatory gastritis, but glucocorticoid replacement has been shown to suppress gastric inflammation and metaplasia in mouse models with adrenocortical insufficiency (49,50). However, a high dose of replacement therapy may lead to epithelial cell damage and further induce SPEM development (46,51,52). Thus, the disruption of glucocorticoid signaling may lay a foundation for gastric cancer development.

5. Metaplasia origin

Oxyntic atrophy may lead to SPEM, but the cellular origin of SPEM remains elusive. Of note, SPEM glands in human and mouse stomachs are similar in many ways but need to be clearly distinguished from IM because of their completely different morphology and expression markers (39). Analyses of excised human gastric body specimens showed that SPEM cells are present in the deeper regions of the gland in areas containing complex glands, and an IM lineage was observed in the luminal portion of the gland, suggesting that IM may be formed by proliferative SPEM (53). The origin of gastric epithelial metaplasia has been controversial in mouse models. Previously, studies had proposed that significant proliferation occurs in the isthmus of the stem cell zone, and therefore, metaplasia was considered to originate from isthmus stem cells or progenitor cells (53-55). Principal cells or a subset of principal cells may transdifferentiate into SPEM cells (42,43,56). However, a recent study proposed that mature chief cells are not needed for metaplasia development (57). In that study, it was proposed that the loss of chief cells is sufficient to cause short-term SPEM-like lesions that originate from chief cell precursors in the gastric neck region (57). Furthermore, certain phenotypic chief cells expressing leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5) were found at the lesser curvature of the gastric body, which failed to promote short- and long-term metaplasia, whereas isthmic stem and progenitor cells effectively promoted long-term metaplasia. Studies have implied a major role of chief cell precursors in the neck region in short-term gastric regeneration and of isthmus stem or progenitor cells in long-term metaplasia (16) (Fig. 1). It was further demonstrated that SPEM occurs not through dedifferentiation of the principal cells but through a compensatory response of neck cells to replace eliminated principal cells (58). Experiments with Lgr5-2A-CreERT, a variant of the enzyme Cre derived from *Escherichia coli*, which has high recombination efficiency and selectivity, mice revealed that Lgr5 principal cells do not contribute to normal genealogical tracking or metaplasia (59). Finally, it was concluded that the expression of Lgr5 principal cells is irrelevant to any SPEM development. Another study summarized the effect of microRNAs (miRNAs) on the transdifferentiation of primary cells to SPEM cells. Certain miRNAs were highly expressed in normal primary cells but were downregulated in SPEM cells. Among these, miR-148a was expressed >10-fold in primary cells, and in addition, miR-148a deletion resulted in the upregulation of the early SPEM marker CD44v9 and one of its target genes, DNA methyltransferase 1. These findings suggest that miR-148a is an early regulator in the reprogramming of chief cells during transdifferentiation into SPEM, and it may be involved in chief cell maturation and maintenance, as well as plasticity (60).

As *H. pylori* infection in mouse experiments does not lead to IM (61), numerous studies over the past few years have used the Mongolian gerbil model to analyze gastric carcinoma development caused by *H. pylori* infection. Studies have demonstrated that *H. pylori*-infected Mongolian gerbils develop IM and can further develop gastric cancer (62). SPEM has been reported to be more frequently associated with early gastric cancer than IM in humans, and SPEM is also a precancerous lesion of the stomach (32,63). In *H. pylori*-infected Mongolian gerbils, SPEM developed along with oxyntic atrophy after only 3 weeks of infection, and IM appeared at weeks 24 and 39 of infection, suggesting that SPEM precedes the development of cupped cell IM. Localized regions of IM develop within preexisting SPEM regions (64). This pattern of IM development has been reported in humans (65). These studies suggest that oxyntic atrophy leads to SPEM, followed by IM. To date, however, no definitive evidence exists regarding the exact sequence of these processes.

Of note, SPEM was found to always appear in the intermediate zone and then spread along the border between the gastric sinus and the basal zone, with structural distortion and concave proliferation of the gland also being the most prominent in the intermediate zone along the smaller curvature and expanding continuously toward the larger curvature over a longer infection period (64). This pattern is similar to that observed in human saprophytic development (66). In addition, *H. pylori* colonizes first at smaller curvatures in both mouse models and humans. This localization of metaplasia initiation in rodents and humans may be a consequence of the colonization pattern of *H. pylori* infection.

6. Effects of cytokines on metaplasia

In the metagenesis process, inflammatory monocytes can differentiate into M2 macrophage subsets under cytokine stimulation, which can inhibit the immune response and promote tissue remodeling through exposure to interleukin (IL)-4 and IL-13 and inactivated cytokines IL-10 and TGF-β (67). The M2 subpopulation of macrophages is needed for metaplasia to develop into a more advanced SPEM

phenotype, and it penetrates the stomach following parietal cell loss, thereby facilitating metaplasia (7). M2 macrophages are characterized as anti-inflammatory, tumor-associated inflammatory cells driven by type 2 T-helper (Th2) cytokines (68). Studies in mouse models and human metaplasia have shown that M2 macrophages promote SPEM progression in the context of inflammation and parietal cell atrophy (69). After RNA sequencing of macrophages related to DMP-777 and L635 treatment in mice, IL-33 was found to be significantly upregulated in late metaplasia M2 macrophages. The IL-33/ST2 axis has a crucial role in the development of gastric metaplasia and carcinogenesis. IL-33 is a key mediator in SPEM, effectively stimulating epithelial cell proliferation and metaplasia, and inducing the maintenance of Th2-driven chronic inflammation, thereby increasing cancer risk (70,71). To demonstrate the necessity of IL-33 and the IL-33 receptor (ST2) for SPEM induction, wild-type mice were compared with IL-33-knockout (IL33KO) mice. It was demonstrated that acute parietal cell loss in wild-type mice results in the loss of MIST1, a mature chief cell transcription factor, in chief cells located at the base of the gland (43). The results of the final experiment showed that, compared with wild-type L635-treated mice, L635-treated IL33KO mice expressed less MUC6 (GSII-lectin), CD44v9 and gastric intrinsic factor (GIF). Furthermore, in IL-33 receptor (ST2) KO mice, SPEM deletion and proliferative SPEM decreased, and the expression of MIST1 was reduced (7,70). In addition, a study found that IL-13 has a central role in supporting cytokines and producing immunomodulatory responses to acute injury (70). Besides these cytokines regulating the differentiation and development of macrophages, deoxycholic acid has recently been documented to promote SPEM development by altering macrophage secretion in mice and modulating communication between macrophages and gastric organs, thereby promoting SPEM development (72) (Fig. 3).

In addition to the two aforementioned ILs, numerous other related ILs have been found to affect the occurrence and development of SPEM, e.g., the proinflammatory cytokine IL-17A secreted by CD4+ Th cells and other immune cells, such as CD8+ T cells, natural killer cells and γ-δT cells (73-75). Related experiments have demonstrated that parietal cells respond to IL-17A by undergoing apoptosis, and excess IL-17A is produced during chronic inflammation, thus promoting atrophy and metaplasia (76). In addition, IL-2 is a proinflammatory cytokine that promotes the introduction and spread of inflammatory immune responses, including *H. pylori*-induced gastric inflammation (77). IL-4 is an anti-inflammatory cytokine that inhibits gastric mucosa inflammation and atrophy by reducing interferon-γ. IL-6, as a multifunctional cytokine, regulates inflammatory mediators and endocrine responses and acts as a messenger between the innate and adaptive systems in host defense mechanisms. Its expression was found to increase in *H. pylori*-associated gastritis and decrease after eradication (78). Furthermore, IL-8 induces cell proliferation, migration and angiogenesis (79). In the tamoxifen-induced SPEM model, decreased IL-10 was found to be closely related to SPEM occurrence. IL-10 may regulate gastric mucosa homeostasis, inhibit mucogenesis development and have a potential therapeutic role in the inhibition of SPEM development in early gastric cancer (80). Further research into the role of IL-10 in the epithelium may shed light on the mechanism by which SPEM is activated in gastric tissue.

Bone morphogenetic protein (BMP) signaling in the gastrointestinal tract is essential for the correct specification of epithelial cell lineages and gastric endocrine cells (81). Some scientists have observed that BMP signaling has a role in gastric tumorigenesis (82,83). However, in these experimental models, the possible role of mesenchymal BMP signaling could not be ruled out in their phenotypes. Further studies have speculated that when BMP signaling is lost, induction of an inflammatory response may lead to a series of drastic changes that increase sensitivity to tumorigenesis (81). Studies have suggested that BMP signaling may be involved in the terminal differentiation of certain subsets of intestinal epithelial cells (84). Furthermore, BMP signaling may have a specific role in gastric epithelial cells. In the stomach, BMP signaling negatively regulates endocrine cell production. It also has a role in the control of gastric epithelial cell proliferation, glandular morphogenesis and gastric cancer development (81). In addition, Wnt and prostaglandin E2 (PGE2) signaling pathways have been studied. When activated, Wnt signaling leads to the development of pretumor lesions. *H. pylori* infection induces the expression of cyclooxygenase 2 and microsomal PGE synthase-1, which induces PGE2 synthesis and leads to SPEM development. Experimental data demonstrate that simultaneous activation of these two signaling pathways leads to gastric tumor development through metaplastic transitions (SPEM) and that the three signaling pathways are correlated. Wnt signaling alone is not sufficient to promote tumor development. Relevant experimental results indicated that tumor formation in BMP-inhibited gastric mucosa also requires the induction of the PGE2 pathway (85). In addition, huntingtin interacting protein 1-related has a crucial role in SPEM formation in response to gastric inflammation. It changes the metagenetic lineage of gastric mucosa by affecting the hypertrophy and proliferation of mucosal cells in the enzymatic lineage (86) (Fig. 3).

7. Metaplasia and other factors may further lead to gastric cancer

Gastric cancer may occur under specific conditions after atrophy and metaplasia from *H. pylori* infection. Among them, the role of inflammation is indispensable. It is now suggested that the development of acute SPEM precedes oxyntic atrophy and that the development of SPEM depends on gastric infiltration of C-X3-C motif chemokine receptor 1 + monocytes (46). These results provide novel insight into the normal physiology of the stomach and the mechanisms that regulate pathogenic gastric inflammation and metaplastic transitions.

In mouse experiments, SPEM is produced after drug-affected parietal cell loss but cannot be further cancerous because of the lack of inflammatory stimulation. After a significant inflammatory response, *H. pylori*-infected mice further differentiate abnormally (87). Furthermore, a study of the inflammatory component of mice revealed that the progression of SPEM into developmental abnormalities requires a Th1-dominated inflammatory response (88,89). In addition, a study showed that bone marrow-derived cells (BMDCs) have a role in the progression of metaplasia to

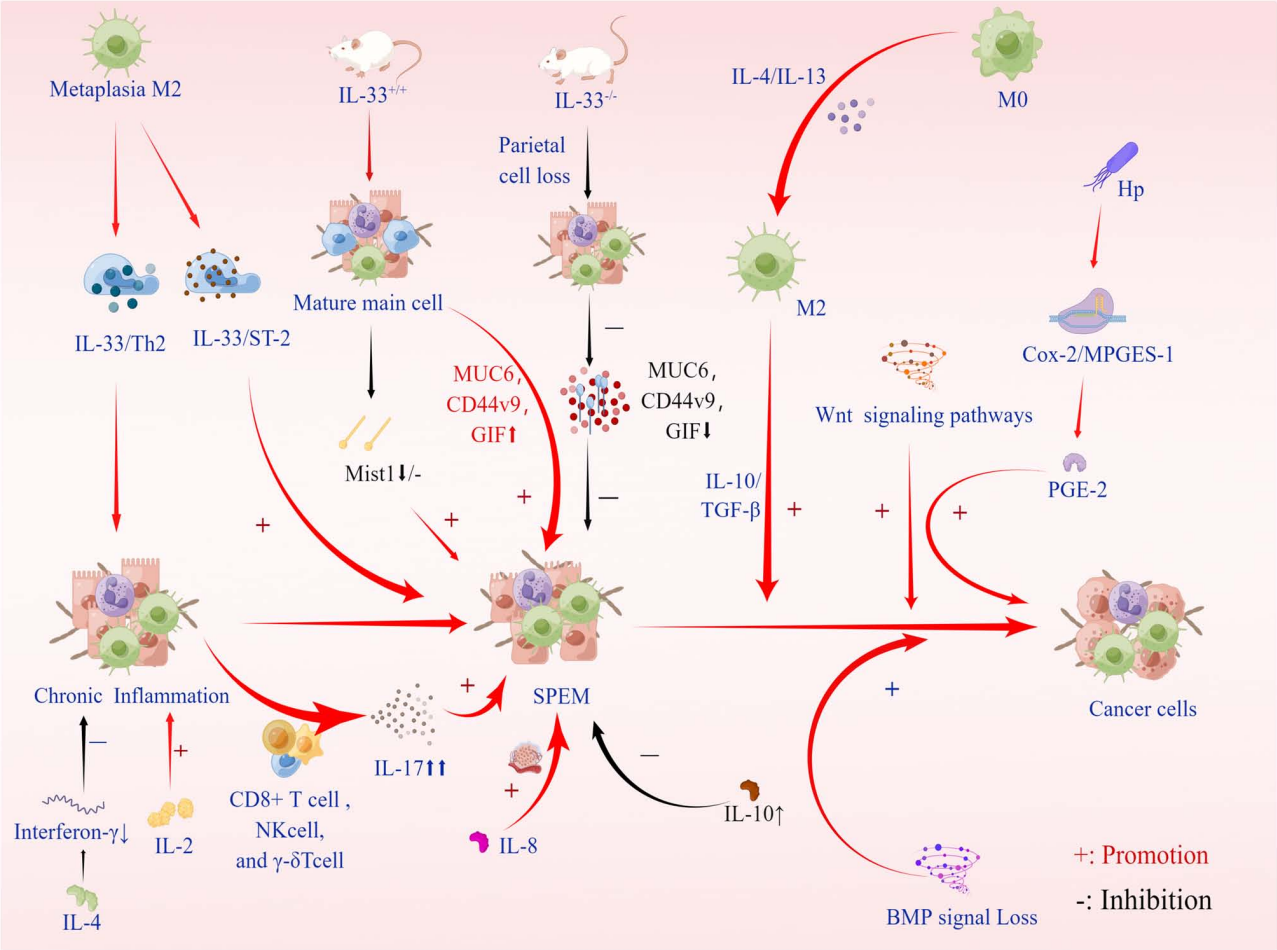

Figure 3. Role of cytokines in chemotaxis. M2 macrophages promote SPEM progression in the context of inflammation and mural cell atrophy, which is characterized by anti-inflammatory, tumor-associated inflammatory cells driven by Th2 cytokines. RNA sequencing of macrophages associated with DMP-777- and L635-treated mice revealed significant upregulation of IL-33 in M2 macrophages with advanced chemosis, a crucial role of the IL-33/ST2 axis in the development of gastric chemosis and carcinogenesis, and the necessity of IL-33 and IL-33 receptor (ST2) for SPEM induction. L635-treated IL33KO mice express less of the shared spectrum of SPEM characteristics, such as MUC6 (GSII-lectin), CD44v9 and GIF, and have reduced SPEM and proliferative SPEM. Several ILs influence SPEM development, such as the proinflammatory cytokine IL-17A secreted by CD4+ Th cells and other immune cells, such as CD8+ T cells, NK cells, and γ-δ T cells. Parietal cells respond to IL-17A by undergoing apoptosis, and during chronic inflammation, IL-17A is overproduced, thereby promoting atrophy and chemotaxis development. In addition, IL-2, IL-4, IL-8 and IL-10 have different roles in inflammatory and atrophic processes. The M2 subpopulation is needed for the progression of chemosis to an advanced SPEM phenotype. Inflammatory monocytes differentiate into the M2 macrophage subpopulation in response to cytokine stimulation, which suppresses the immune response through exposure to IL-4, IL-13 and the inactivating cytokines IL-10 and TGF-β, promoting tissue remodeling and, consequently, chemosis development. Gastric epithelial cell proliferation, glandular morphogenesis and gastric cancer development are controlled by bone morphogenetic protein. When infected with *H. pylori*, it induces the expression of COX-2 and MPGES-1, which in turn induces PGE2 synthesis, which then leads to SPEM development. When these two signaling pathways are activated simultaneously, gastric tumors develop through the chemotaxis (SPEM) cancer sequence. SPEM, spasmolytic polypeptide-expressing metaplasia; COX, cyclooxygenase; IL, interleukin; PGE, prostaglandin E; MPGES-1, microsomal PGE synthase-1; MIST1, basic helix-loop-helix family member a15; NK, natural killer; CD44v9, CD44 variant 9; BMP, bone morphogenetic protein; HP, *H. pylori*, Th2, type 2 T-helper cells; MUC6, mucin 6.

developmental abnormalities, and during chronic *H. felis* infection, BMDCs are recruited to the stomach, and these recruited BMDCs appear to be transplanted into SPEM glands and progress to deep cystic gastritis. Currently, it is uncertain whether BMDC implantation into SPEM specifically targets *H. felis* infection, and there is no strong evidence to support the role of BMDCs in human metaplasia or carcinogenesis (40).

Recent experimental studies have shown that SPEM development is accompanied by mutations in the MUC5AC, KRAS, BRAF and enhancer of zeste 2 polycomb repressive complex 2 subunit genes and that SPEM and gastric cancer are genomically similar (90). Furthermore, experimental studies have shown that chronic *H. pylori* infection in both mice and humans leads to the expression of Toll-like receptor 9 (TLR9), which is associated with immunosuppression and an increased incidence of gastric tumors in patients with TLR9 polymorphisms and *H. pylori* infection (91-95). Studies of the target genes miR30a and integrin α2 (ITGA2) revealed that miR30a expression was downregulated in pretumor and early gastric cancer tissues but maintained at a certain level in advanced gastric cancer tissues and that miR30a had a significant tumor-suppressive effect, whereas ITGA2 levels were significantly increased in gastric cancer tissues (96). A pathological study of metaplasia revealed that upregulation of trophoblast antigen 2 (TROP2) expression occurred during the transition from gastric mucosal metaplasia to heteroplasia, and it promoted heteroplasia (97) (Fig. 4).

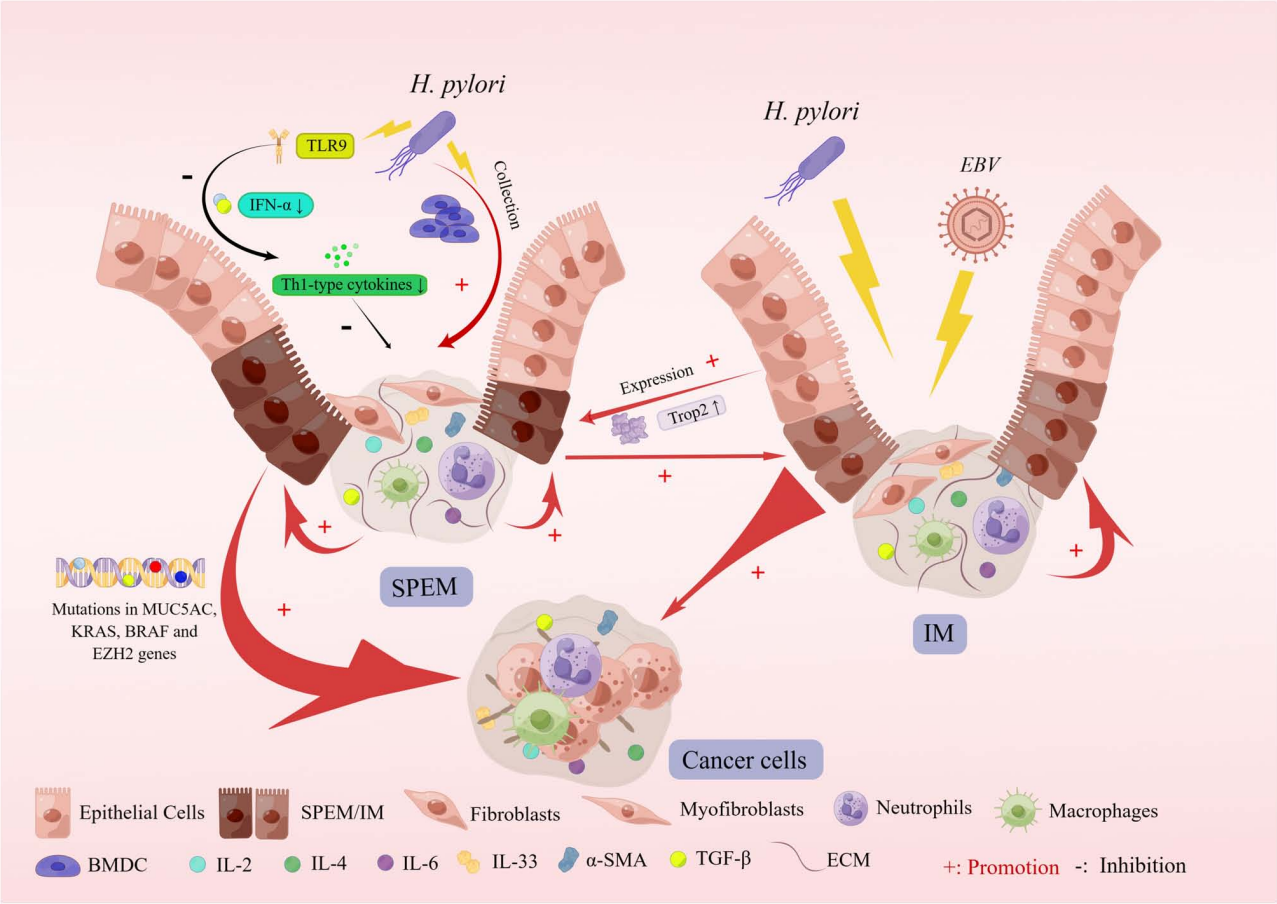

Figure 4. Chronic inflammation based on *H. pylori* infection is associated with gastric cancer development. TLR9 signaling has a crucial role in the early suppression of *H. pylori*-induced gastritis through the downregulation of Th1-type cytokines regulated by interferon α. Mutations in the MUC5AC, KRAS, BRAF and EZH2 genes lead to SPEM formation. Potentially, BMDCs are recruited into the stomach, transplanted into SPEM glands and progress to deep cystic gastritis. TROP2 is a transmembrane glycoprotein encoded by the tumor-associated signal transduction 2 gene, also known as gastrointestinal antigen 733-1. TROP2 is upregulated during the transition to dysplasia in the stomach and promotes dysplastic cell behaviors. Early SPEM is common in patients with EBVaGC, whereas late SPEM is common in patients with EBVnGC. The incidence of SPEM spectrum chemosis is higher than the incidence of IM in patients with EBVaGC and EBVnGC. In addition, inflammatory cells are a key factor in the development and progression of SPEM to IM. However, whether SPEM is induced by EBV alone or by *H. pylori* infection remains elusive. SPEM, spasmolytic polypeptide-expressing metaplasia; BDMCs, bone marrow-derived cells; EBV, Epstein-Barr virus; MUC5AC, mucin 5 subtype AC; KRAS, Kirsten rat sarcoma viral oncogene homolog; BRAF, v-raf murine sarcoma viral oncogene homolog B1; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; TLR9, Toll-like receptor 9; EBVaGC, EBV-associated gastric cancer; EBVnGC, EBV-negative gastric cancer; Th1, type 1 T-helper cells; IM, intestinal metaplasia; SMA, smooth muscle actin; ECM, extracellular matrix; TROP2, trophoblast antigen 2.

In the proposed multistep pathway of Correa carcinogenesis, mediated chronic gastritis progresses over the years to atrophic gastritis, SPEM and IM, developmental abnormalities and ultimately gastric cancer (98). Chronic *H. pylori* infection is considered the underlying cause of IM and intestinal-type gastric cancer (99). The Epstein-Barr virus (EBV), the first virus associated with human malignancy, is another powerful risk factor for gastric cancer (100). Statistically, EBV-associated gastric cancer (EBVaGC) accounts for ~9% of all cases worldwide (101,102). In addition, *H. pylori* infection is a crucial risk factor for gastric cancer, unlike EBVaGC, suggesting that *H. pylori* and EBV are involved in different carcinogenic pathways (103). The study was divided into two clinical control studies-EBVaGC and EBV-negative gastric cancer (EBVnGC)- and pathological sections of both types of patients were subjected to laboratory tests, which ultimately revealed that both EBVaGC and EBVnGC patients had a higher complication rate with SPEM than with IM and that IM occurred more frequently in EBVnGC patients, suggesting that the association between gastric cancer and SPEM is stronger than IM. Furthermore, this study found that early SPEM was more common in patients with EBVaGC, whereas late SPEM was more common in patients with EBVnGC, and the different distribution patterns of SPEM in these two groups of patients with gastric cancer may be due to different pathogenic microbial infections during gastric cancer (104).

8. Conclusions and future outlook

In recent years, significant breakthroughs have been achieved regarding the knowledge of gastric cancer development and a deeper understanding was gained in this field. Studies of metaplasia-associated genes have identified an independent prognostic biomarker, calcium adhesion protein 17 (CDH17), which is a structurally unique member of the calcium adhesion protein superfamily and is a functional Ca^{2+}-dependent homologous cell adhesion molecule (105). A study reported that CDH17 was markedly increased in SPEM and was

expressed in 61-65% of human gastric cancers. Although the relationship between CDH17 expression and cancer stage or patient survival is inconclusive, it is now considered an independent prognostic factor in patients with stage I or lymph node-negative gastric cancer (106). SPEM has a crucial role in the entire process of gastric cancer progression. The appearance of SPEM has been found to precede intestinal epithelial hyperplasia in numerous studies; therefore, the detection of early SPEM has a clear role in gastric cancer diagnosis. Therefore, the identification of markers of epithelial hyperplasia and hyperplasia progression to atypical hyperplasia is necessary for the development of effective screening methods that can identify preproliferation. Several researchers have started investigating the relationship between biomarkers and cancer. For instance, a previously identified SPEM-specific marker, human epididymis protein 4 (HE4), was initially used as a serum marker for ovarian cancer, but as research progressed, is was found that HE4 is not present in normal gastric sinuses but can significantly increase in metaplasia and carcinoma, and this study identified secreted whey acidic protein structural domain protein HE4, which may be used as a putative biomarker (43,107). Furthermore, HE4 was expressed in all SPEM and IM samples. Two other early marker proteins of SPEM and IM have been recently identified, lactotransferrin (LTF) and deleted in malignant brain tumor 1 (DMBT1), both of which are associated with the inflammatory response and cell differentiation. The expression patterns of LTF and other SPEM markers, as well as DMBT1 support the notion that human SPEM evolves into IM (108). Multiple types of BMDCs are involved during SPEM development and the ability to track these cell types in the preneoplastic state expands the options for more effective screening of subjects susceptible to the eventual occurrence of gastric cancer and the development of atrophic gastritis when prophylactic treatment options, including mTOR antagonists, are available (109). Gastrokine 3 (GKN3) mRNA can accurately assess SPEM in the analysis of mouse and human chronic inflammatory gastric tissues, mainly because it is absent in normal gastric tissues. Furthermore, GKN3 mRNA and GKN3-positive cells were detected in the gastric body during SPEM (110). In addition, human SPEM cells in TROP2-labeled incomplete IM glands at the base strongly express aquaporin 5 (AQP5) but not in intact IM glands, and AQP5-expressing SPEM cells are present in pyloric metaplasia and TROP2-positive incomplete IM, which may be an essential component of metaplasia and can predict a higher risk of gastric cancer development (111). Therefore, GKN3 mRNA and AQP5 may be used as specific markers for SPEM diagnosis.

In summary, the present study described the role of various cytokines and expression products in SPEM formation, and with the discovery of specific markers, the understanding of the origin of SPEM and cancer progression will improve, which may aid in the easy and accurate diagnosis of early developmental abnormalities. These results provide research directions on SPEM pathogenesis and new opportunities for future diagnosis and treatment.

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Availability of data and materials

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Authors' contributions

YC, DY, ZL and FN conceived and designed the study. YC, DY and ZL performed the literature search. YC and DY drafted the manuscript. YC, DY and FN designed and drew the figures. YC critically revised the manuscript. All the authors were involved in revising the paper critically. All authors have read and agreed to the published version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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