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Review

The MUC gene family: Their role in the diagnosis and prognosis of gastric cancer

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Summary. Early diagnosis of gastric cancer and its differential diagnosis from other non-malignant gastric diseases like gastritis is still a major clinical problem. Most patients are asymptomatic in the early stages of gastric cancer, and there is no reliable marker available for the early and specific diagnosis of gastric cancer. Many attempts have been made to define the biological profile of gastric cancer to improve the chances of its early diagnosis, prognosis and treatment. Several studies have shown the aberrant expression profile of mucins in different malignancies, suggesting that mucins have a great potential to be used as a diagnostic and prognostic marker in gastric cancer. In this review, we have briefly described the different types of gastric adenocarcinomas and the progression of gastric cancer. Furthermore, the role of mucins and their related carbohydrate epitope is discussed in the normal stomach and in the diagnosis and prognosis of gastric adenocarcinomas.

Key words: Gastric cancer, Mucin, Diagnosis, Prognosis

Introduction

Gastric cancer is the second leading cause of cancer-related deaths and the fourth most common cancer in the world. According to the National Cancer Institute (NCI), approximately 760,000 cases of stomach cancer are diagnosed worldwide and more than 24,000 cases are diagnosed in the United States each year. Although the incidence of gastric cancer is gradually decreasing, but still one of the most common causes of cancer-related deaths in Western countries. Most patients are asymptomatic in the early stages of gastric cancer. In spite of advances in diagnostic techniques like imaging, esophagogastroduodenoscopy, magnetic resonance imaging and dual phase spiral computer tomography, early diagnosis of gastric adenocarcinoma is still an ongoing challenge for clinicians. Commonly used treatment for advanced gastric adenocarcinoma includes surgery combined with other modalities such as radiotherapy and chemotherapy. Although these treatment modalities have shown promising results, the tumor frequently metastasizes and reoccurs in many patients. At the present time, no effective therapy is available for recurrent and metastatic gastric adenocarcinomas. Therefore, efforts toward early diagnosis and therapeutic interventions are needed for prevention and cure of this malignancy. In recent years, mucins have become the molecule of interest for early diagnosis, prognosis, and as therapeutic targets for various cancers. Aberrant expression profiles of mucins have been shown in different malignancies. Therefore, study of the mucin expression patterns in gastric cancer may be an effective way to understand the disease pathogenesis and to evaluate their potential use in the diagnosis and therapy of gastric cancer.

This review is a brief compilation of available information regarding different types of gastric adenocarcinomas, the progression of gastric cancer and the expression profile of mucins in normal, preneoplastic and neoplastic gastric mucosa. Further, the importance of the mucin expression pattern and its combined evaluation with other molecules in the diagnosis and prognosis of gastric cancer is also discussed.

Types of gastric adenocarcinomas

The histological classification given by Finnish pathologist Lauren divides gastric adenocarcinomas into

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intestinal and diffuse types of adenocarcinomas (Lauren, 1965). The intestinal-type gastric adenocarcinoma shows well defined glands with epithelial lining containing intestinal-type tall columnar cells, often with scattered goblet cells. The adjacent gastric mucosa often shows chronic gastritis with widespread intestinal metaplasia. The diffuse-type gastric adenocarcinoma mainly consists of scattered individual cells or clusters of cells. The majority of the cells are small and have signet-ring morphology. Unlike the intestinal type gastric adenocarcinoma, glandular morphology is uncommon in the diffuse type gastric adenocarcinoma, although it might be seen in the superficial part of the tumor. These two types of adenocarcinomas are considered to be fundamentally different in etiology and histogenesis. Intestinal-type gastric tumors are common in older patients, mostly located in gastric antrum, and are linked to chronic *Helicobacter pylori* infection accompanied with atrophic gastritis and intestinal metaplasia. The diffuse-type gastric tumors are commonly found in younger patients and are predominantly located in the gastric body. The intestinal-type gastric adenocarcinoma is more predominant in geographical areas with a high incidence of gastric cancer, but diffuse-type tumors are found uniformly throughout the world. The pattern of spread of these two types of gastric cancer is also different; hematogenous spread is usual with intestinal-type carcinomas and widespread peritoneal dissemination is predominant in diffuse-type carcinomas. A significant proportion of gastric cancers (up to 15%) show both intestinal and diffuse type patterns, and they are placed in an indeterminate category. The occurrence of intestinal-type, diffuse-type and indeterminate-type gastric cancer is 50-60%, 30-35% and 3-15%, respectively.

The World Health Organization characterizes gastric carcinoma into five subtypes based upon the tumor architecture: papillary, tubular, mucinous, signet ring cell, and undifferentiated carcinoma, in which no definite glandular structures are present. The papillary and tubular type carcinomas fall into the Lauren's intestinal type and signet ring cell carcinoma is included in Lauren's diffuse type. Intestinal-type tumors have a better prognosis than diffuse type signet ring cell carcinomas.

Development and progression of gastric cancer

The progression of gastric cancer is a multi-step process. The most important factor responsible for gastric cancer development is *H. pylori* infection. The infection was recognized as a Class I human carcinogen by the International Agency for Research on Cancer in 1994. Recent studies on the association of *H. pylori* and gastric cancer have enhanced our understanding of the mechanisms involved in the progression of gastric cancer. Infection with *H. pylori* incites a chronic active immune response that persists for the life of the host, in the absence of antibiotic-induced eradication. The combination of *H. pylori* infection, environmental insult, and the host immune response drives the initiation and progression of mucosal atrophy, metaplasia, and dysplasia towards gastric cancer (Fig. 1) (Correa, 1988, 1992b; Censini et al., 1996). The *H. pylori* infection generates an efficient inflammatory response characterized by infiltration of neutrophils and mononuclear cells into the gastric mucosa representing gastritis. (Mannick et al., 1996; Halliwell, 2007). Additionally, simultaneous accumulation of genetic changes leads to uncontrolled growth and gastric mucosa undergoes intestinal metaplasia where the original gastric glands and the foveolar epithelium are replaced by cells with the intestinal phenotype. The metaplastic intestinal mucosa resembles the small intestinal mucosa and is lined by eosinophilic absorptive enterocytes, mucin-filled goblet cells (containing sialomucins) and Paneth cells within the crypt base. The intestinal metaplasia caused by persistent *H. pylori* infection leads to dysplasia and the development of gastric adenocarcinoma (Correa, 1992b; Correa and Shiao, 1994; Uemura et al., 2001; Correa and Houghton, 2007).

Different human and animal studies have shown the role of diverse co-factors affecting the development and progression of *H. pylori*-mediated gastric cancer. Studies suggest that diets rich in salt and nitrates or nitrites predispose to gastric cancer (Howson et al., 1986). In contrast, diets rich in vitamin C help prevent gastric cancer (Correa, 1992a). Animal studies show that chemicals like N-methyl-N'nitrosoguanidine (MNNG) in the diet induce adenocarcinoma in rat, but ascorbate decreases the incidence of gastric carcinoma induced by MNNG (Kawasaki et al., 1982). The level of gastric acids also contributes to the initiation of early stages of gastric cancer progression. Gastric ulcer patients with reduced acid production have a higher risk of getting gastric cancer than duodenal ulcer patients having high acid production (Hansson et al., 1996). Gastrin, a gastric hormone, has been proposed to play a vital role in the initiation of gastric cancer. Animal studies have shown that gastrin deficiency causes a marked change in the gastric architecture associated with a decreased number of parietal cells (Koh et al., 1997). This loss in parietal cells may lead to atrophic gastritis and may cause a progression toward gastric cancer. Interestingly, it was observed that during gastric cancer progression, once the intestinal metaplasia stage arrives, *H. pylori* eradication does not help to prevent further cancer progression (Wong et al., 2004). This observation indicates that *H. pylori* infection might be responsible for irreversible molecular changes in the gastric epithelial cells, leading to the progression of gastric cancer. Recently, Murata-Kamiya et al. 2007 showed that after *H. pylori* cagA-positive strain infection through CagA/E-cadherin interaction, MUC1 and many other genes involved in cell transformation or differentiation are up regulated. *H. pylori* infection also causes a decrease in the level of MUC5AC expression (Kocer et al., 2004). At the same time, another study showed that *H. pylori* eradication

causes an increase in MUC5AC expression but does not change the distribution of MUC6 and MUC1 (Ma et al., 2003). These observations indicate that H. pylori infection in the early stage of gastric cancer progression may produce a switch in the expression of oncogenic mucins like MUC1, which may be responsible for an irreversible gastric cancer progression. The genetic events associated with the non-H. pylori-mediated transdifferentiation of normal gastric mucosa into intestinal metaplasia are poorly understood. Recently, it has been shown that the ectopic expression of Cdx2 alone, an intestinal specific transcription factor in mouse, is sufficient enough to induce gastric metaplasia (Silberg et al., 2002). During gastric cancer progression, the co-expression of CDX1 and CDX2 with MUC2 in intestinal metaplasia has been reported (Steininger et al., 2005). A recent study showed that CDX2 regulates the expression of MUC2 (Mesquita et al., 2003). This indicates the possible role of mucins like MUC2 in Cdx2-mediated gastric cancer progression.

Carbohydrate antigens in gastric adenocarcinoma

Murata et al. analyzed the expression of blood-group-related antigens in early gastric cancer, intestinal metaplasia, and normal gastric mucosa of 35 surgical specimens (Murata et al., 1992). According to their report, intestinal metaplasia and early gastric cancer were associated with frequent deletions of ABH, Le^x and Le^y antigens, which were expressed by normal gastric mucosa. Furthermore, well differentiated gastric adenocarcinomas (intestinal type) typically have more sulfomucin, whereas moderately and poorly differentiated cancers (diffuse type) have more sialomucins (characteristic of intestinal goblet cells) (Filipe, 1979; Correa, 1988). It has been shown that the transition from normal neutral mucins to sulfomucins is a marker of increased risk of gastric intestinal metaplasia (Correa, 1988; Rokkas et al., 1991). Further progression of intestinal metaplasia to gastric adenocarcinoma is associated with a qualitative and quantitative alteration of mucin-related antigens. Sialosyl-Tn, a mucin-associated carbohydrate antigen which is not expressed by normal gastric mucosal cells but over expressed in gastric cancer has been shown to directly correlate with different stages of gastric cancer and it is a marker of gastric cancer progression (Werther et al., 1996).

Mucin glycoproteins

Mucins are high molecular weight glycoproteins expressed by specialized epithelial cells lining the luminal surface of different organs like respiratory, gastrointestinal and reproductive tracts. These mucins directly or indirectly mediate different physiological functions like the maintenance of epithelial integrity, lubrication and protection of the epithelial surfaces. Based on the cellular expression pattern of mucins, they

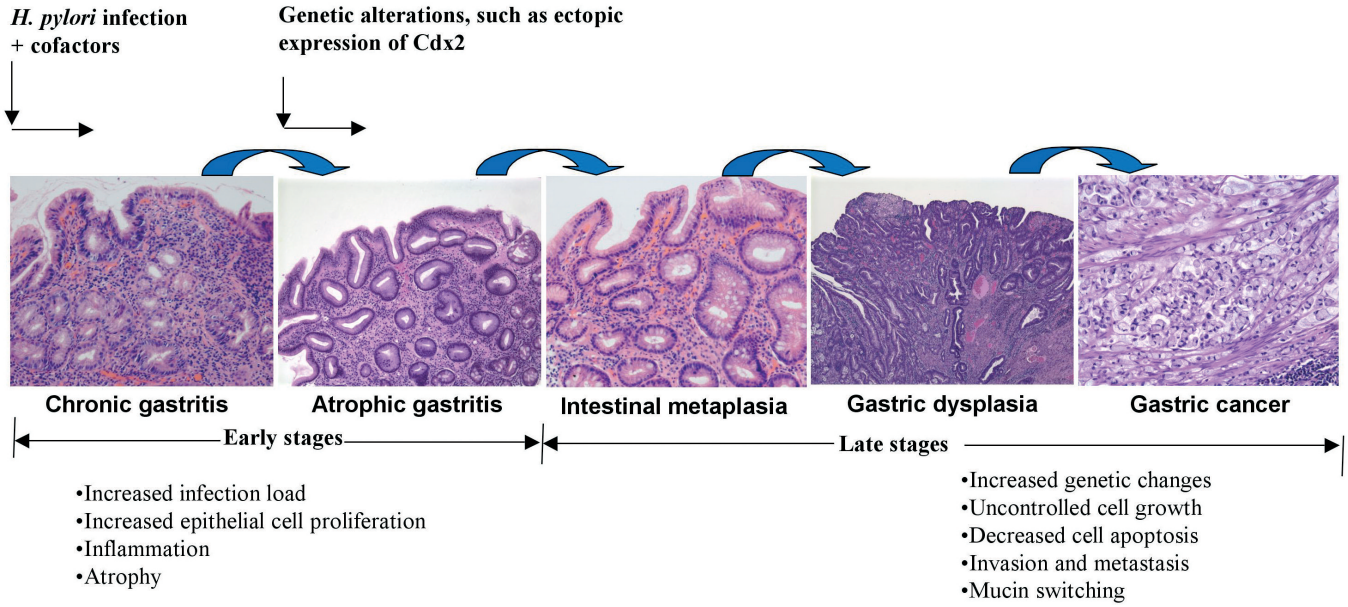

Fig. 1. Sequential pathological changes in gastric cancer progression. Chronic gastritis: Moderate to severe inflammation associated with infiltration of lymphocytes and plasma cells into the gastric mucosa, reactive epithelial changes and compensatory proliferation of the replication zone. Atrophic gastritis: Associated with complete loss of gastric secretory cells (parietal and chief cells), and chronic inflammation. Intestinal metaplasia is seen in the right half. Intestinal metaplasia: Characterized by a change in morphology of gastric mucosa to intestinal phenotype that is associated with a differential expression of mucins (mucin switching). Gastric dysplasia: Associated with marked architectural complexity and prominent cellular and nuclear atypia. Gastric cancer: A poorly differentiated signet ring cell carcinoma.

are classified as membrane-bound or secretory mucins. The secretory mucins, which include MUC2, MUC5AC, MUC5B, MUC6, MUC7, MUC8 and MUC19, lack a transmembrane domain and are secreted into the extracellular space. The membrane-bound mucins, which include MUC1, MUC12, MUC13, MUC15, MUC16, MUC17 and MUC20, are type I membrane proteins. Although mucins vary in their chromosomal location (Table 1), the primary structure of a mucin molecule consists of a protein backbone, termed "apomucin", bearing a large number of O-linked oligosaccharides and a few N-glycan chains. Many post-translational modifications, like sialylation or sulfation, are commonly observed on the mature mucin glycoprotein. The structure of apomucin typically reveals the presence of tandem repeat (TR) domains that are the characteristic feature of mucins and distinguish them from other glycoproteins (especially from other membrane bound glycoproteins/receptors) (Griffiths et al., 1990; Gendler and Spicer,1995; Moniaux et al., 2001). TRs are specifically rich in Serine, Threonine and Proline residues, which are potential sites for O-glycosylation (Corfield et al., 1995). The sugar moieties constitute up to 80% of the mucins and contribute to the rheological/viscoelastic properties of mucus secretions (Gum, Jr. 1992; Gendler and Spicer, 1995; Kim and Gum, Jr. 1995) Throughout the development of an organism, mucins exhibit a defined spatial and temporal pattern of expression (Braga et al., 1992). In various pathological conditions, this regulated expression is compromised and their expression is deregulated. It has been shown that the deregulated expression of mucins is a prominent characteristic of various types of cancers and inflammatory diseases (Devine and McKenzie, 1992; Gendler and Spicer, 1995; Hollingsworth and Swanson, 2004; Andrianifahanana et al., 2006). Aberrant expression patterns of various mucins may be used as molecular markers for the diagnosis of various malignancies.

Mucins in the normal stomach

Gastrointestinal tract and accessory digestive glands (liver, gallbladder and pancreas) have a common epithelial origin. The expression of different mucins in the gastrointestinal tract begins early in human embryonic development. Buisine et al. 2000 have shown that mucin gene expression patterns in the embryonic and fetal stomach show similarities with mucin gene expression patterns in gastric adenocarcinomas in adults (Buisine et al., 2000). This indicates a possible role of mucins in different cellular mechanisms like cell proliferation, differentiation, apoptosis, and migration of cells, which are common phenomena for both embryogenesis and cancer development.

Gastric mucosal damage results from an imbalance between offensive and defensive factors. Various factors such as mucus-bicarbonate layer, prostaglandins, and growth factors help in the protection of gastric mucosa. Of these factors, mucus is the first layer of defense and

Table 1. Characteristics of human MUC genes.

MUC gene	Locus	Domains	Amino acids/TR
Secreted Gel-forming			
MUC2	11p15.5	VWD,VWC,CK,Cys-rich	23
MUC5AC	11p15.5	VWD,VWC,CK,Cys-rich	8
MUC5B	11p15.5	VWD,VWC,CK,Cys-rich	29
MUC6	11p15.5	VWD, CK	169
MUC19	12q12	VWD	ND
Secreted Non Gel-Forming			
MUC7	4q13-q21		23
MUC8	12q24		13 and 41
MUC9(OVGP1)	1p13		15
Membrane-bound			
MUC1	11q21	TM,SEA,ß-catenin	20
MUC3A and B	7q22	TM,SEA,EGF	17
MUC4	3q29	TM,NIDO,AMOP,EGF	16
MUC12	7q22	TM,SEA,EGF	28
MUC17	7q22	TM,SEA,EGF	59
MUC13	3q13	TM,SEA,EGF	27
MUC15	11p14.3	TM	None
MUC16 (CA125)	19p13.3	TM	156
Unclassified			
MUC11	7q22		28

VWD: von Willebrand factor D-like domain; VWC: von Willebrand factor C-like domain; CK: cystine knot; SEA: Sea urchin sperm protein, Enterokinase and Agrin module; EGF: epidermal growth factor-like domain; AMOP: Adhesion-associated domain in MUC4 and other proteins; NIDO: Nidogen domain; TM: transmembrane domain.

plays a major role in blocking aggressive factors (Fig. 2). The maintenance of a pH gradient between the surface of the epithelial cells and the gastric lumen is the most relevant protective property of mucus. Gastric mucus also plays a role in the transportation of pepsinogen produced by principal cells and the inhibition of its activation into pepsin (Tasman-Jones, 1985). In addition, the adherent mucus layer acts as a barrier to luminal pepsin, thereby protecting the underlying mucosa from proteolytic digestion (Allen and Flemstrom, 2005). The complex oligosaccharide structures that are tethered to the tandem repeats of the mucin core proteins help in creating an organized special structure and impart steric hindrance. Due to steric hindrance from the mucin glycoprotein, large molecules and microorganisms get excluded from the surface of the gastric epithelium. Mucins, due to their hygroscopic nature, maintain hydration above the cell surface. In addition to protecting the gastric epithelium, the mucins also play a role in other biological functions, such as sequestering growth factors, cytokines, and chemokines. Various studies have analyzed the expression profile of mucins in the human stomach under physiological and pathological conditions. For this purpose, investigators have used several methods with differences in their specificity and sensitivity, which include immunochemistry, in situ hybridization, RT-PCR and Northern blotting. Synthesis of mucins in the human stomach starts as early as between 8 to 12 weeks of gestation. MUC1, MUC3, MUC4, MUC5AC, MUC5B,

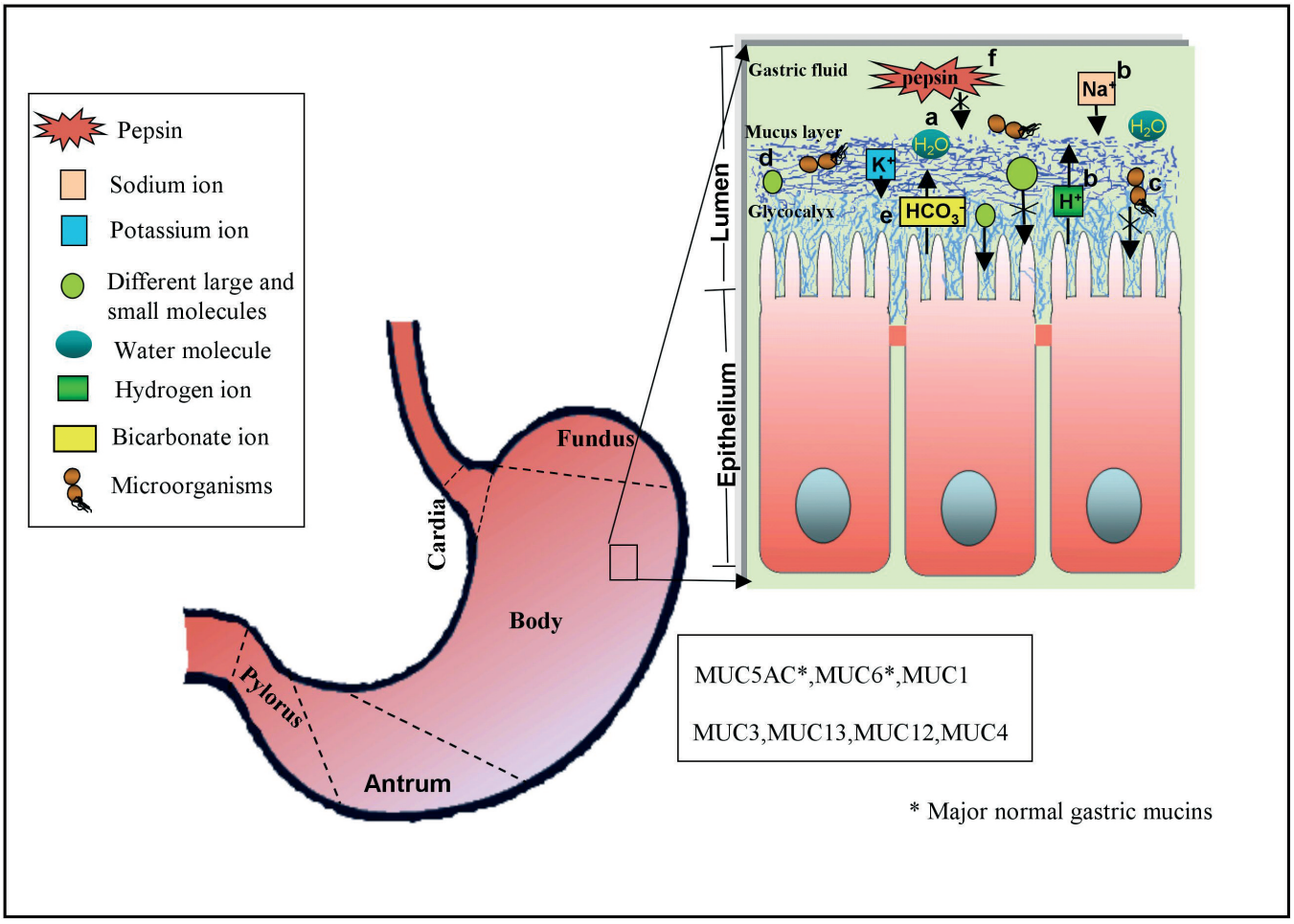

Fig. 2. Mucins of the normal stomach and their protective function. **a.** Mucins, due to their hygroscopic nature, maintain hydration above the cell surface. **b.** Mucus has the ability to direct hydrogen ions from the epithelial cell surface toward the gastric lumen and allows the flow of other cations (potassium,sodium) from the lumen to the mucosa, thus avoiding damage to adjacent cells. **c**. Steric hindrance from the mucus glycoprotein excludes large molecules from gastric epithelium. However, smaller molecules can pass through the mucus glycoprotein. **d.** The mucus layer segregates microorganisms and protects the epithelium. e. In the mucin layer above the mucosa, stable, unstirred layer of the mucus prevents immediate mixing of the secreted HCO_3^- with the excess of acid in the lumen. **f.** The adherent mucus layer acts as a barrier to luminal pepsin, thereby protecting the underlying mucosa from proteolytic digestion. * Major normal gastric mucins.

and MUC6 are expressed in the embryonic stomach (Buisine et al., 2000). Normal adult gastric mucosa expresses MUC1 (Burchell et al., 1987; Girling et al., 1989; Ho et al., 1993; Walker et al., 1995; Reis et al., 1998) MUC5AC (de Bolos et al., 1995; Ho et al., 1995a; Byrd et al., 1997) and MUC6 (de Bolos et al., 1995; Ho et al., 1995b; Byrd et al., 1997; Pinto-de-Sousa et al., 2002). In the normal gastric mucosa, MUC1, MUC5AC, and MUC6 are expressed in a characteristic zonal pattern. MUC1 has an apical/membranous staining pattern observed in the surface and foveolar epithelium as well as the mucous neck zone cells (Burchell et al., 1987; Girling et al., 1989; Ho et al., 1993; Reis et al., 1998). Alternatively, in the oxyntic mucosa, chief and parietal cells show diffuse cytoplasmic staining and canalicular system staining for MUC1, respectively. In contrast, MUC5AC distribution is limited to the cytoplasm of the foveolar epithelium and mucous neck cells throughout the stomach. MUC6 is expressed in the cytoplasm of the antral pyloric glands but also in mucous neck cells and chief cells of the gastric body (Corfield et al., 2000; Jass, 2000). It has been shown that gastric mucins, particularly MUC5AC (Nordman et al., 1995) and MUC6 (Ho et al., 1995b) form a protective layer over the gastric epithelium surface and act as a selective diffusion barrier for HCl (Nordman et al., 1995). MUC2, MUC3, and MUC4 gene expression are generally absent in normal gastric specimens (Ho et al., 1995b). Our recent immuno-histochemical analysis (unpublished data) indicates a low level expression of MUC4 in the normal stomach. MUC13, a trans-membrane mucin that is normally expressed in hematopoietic precursors of bone marrow, is also present at moderate levels in the normal stomach. MUC13 protein has been demonstrated immunohistochemically in surface epithelium and deep glands of the stomach (Williams et al., 2001). MUC7, a secretory mucin, has been found in the salivary glands; however, published to date indicates that this mucin is not expressed in gastric epithelium (Bobek et al., 1993).

Mucins in non-neoplastic and preneoplastic conditions

Mucins in chronic H. pylori gastritis

H. pylori infection is implicated in the development of gastritis, glandular atrophy, intestinal metaplasia, and gastric malignancies (Zhang et al., 2005; Correa and Houghton, 2007). The organism is capable of exerting detrimental effects on the mucus layer, as well as the surface cells of the gastric epithelium. The result of *H. pylori* protease action is disintegration of the polymeric structure of mucin, whereas the elaborated lipases and phospholipase A2 result in mucus lipid degradation, and loss of mucosal surface hydrophobicity (Slomiany and Slomiany, 1992). The infection results in alteration of mucin glycosylation, which further facilitates bacterial attachment (Ota et al., 1998). The urease, produced by the bacteria, generates ammonia which is necessary for establishment of a neutral pH microenvironment and destabilization of the mucous layer and thereby helps in survival of the bacteria (Sidebotham et al., 1998; Andersen, 2007). These actions cause a collapse of the mucous barrier and favor bacterial binding to mucus, thereby facilitating the survival of *H. pylori* in the gastric environment. Cell culture experiments indicate that *H. pylori* alters the synthesis of the mucin core structures but does not influence the secretion of mucins. In *H. pylori* infection, MUC6 expression in surface mucous cells is increased whereas there is a decrease in the expression of MUC5AC and MUC1 (Byrd et al., 1997). This reversal of the normal gastric pattern is corrected on the elimination of infection (Byrd et al., 1997, 2000). Recent evidence suggests that gastric infection by *H. pylori* initially presents as a superficial gastritis. Later it may result in gastric atrophy with the development of intestinal metaplasia. Atrophic gastritis predisposes patients to gastric adenocarcinoma. Babu et al. showed an altered expression of MUC5AC and MUC6 in the surface columnar cells and a lack of MUC2 expression in atrophic gastritis similar to chronic gastritis (Babu et al., 2006). *H. pylori* is very closely associated with extra-cellular MUC5AC and epithelial cells that produce MUC5AC. This indicates that MUC5AC plays a role in the adhesion of *H. pylori* to the gastric mucosa (Van den Brink et al., 2000; Kocer et al., 2004). It is likely that following adherence to the surface epithelium, *H. pylori* down regulates the synthesis of the mucins. The alteration of the mucin expression profile in chronic gastritis does not correlate with the severity of bacterial infection.

Mucins and reactive gastropathy

Reactive gastropathy is a relatively common finding in gastric biopsies. Histologic hallmarks of this condition include foveolar hyperplasia of surface epithelium, presence of dilated capillaries and prominence of smooth muscle with minimal edema and inflammation of the lamina propria. In most instances, it is associated with either a reflux of duodenal contents or therapy with non-steroidal anti-inflammatory drugs. Alterations of the expression of membrane (MUC1) and secreted (MUC5AC and MUC6) mucins frequently observed in reactive gastropathy are different from those reported for *H. pylori* gastritis. This difference may be due to the involvement of different etiological factors in the pathogenesis of reactive gastropathy than *H. pylori* infection. Loss of apical MUC1, aberrant expression of MUC5AC, extending deep in pyloric glands, and aberrant expression of MUC6, extending to the upper foveolar glands, is seen (Mino-Kenudson et al., 2007). It is proposed that the alteration of MUC1, which is involved in cell adhesion and polarity, may play a role in the development of the serrated epithelial profile of reactive gastropathy. Still, mucin profile alteration and its implications in reactive gastropathy remains largely

to be elucidated.

Mucins in gastric intestinal metaplasia

Intestinal metaplasia (IM) is one of the lesions identified in the cascade of events that precede the development of gastric carcinoma. The mucin expression profile in different types of intestinal metaplasia allows for the identification of two main patterns of IM. The type I or complete intestinal metaplasia is characterized by the presence of absorptive cell, paneth cells, and goblet cells that secrete sialomucins. Incomplete intestinal metaplasia (type II and III) is characterized by the presence of columnar and goblet cells that secrete sialo and/or sulphomucins. Types II and III intestinal metaplasia are morphologically indistinguishable. Type I intestinal metaplasia displays little or no expression of MUC1, MUC5AC and MUC6. De novo expression of MUC2 and MUC4 is conspicuous in goblet cells (Reis et al., 1999; Corfield et al., 2000; Jass, 2000; Babu et al., 2006; Wang and Fang, 2006). High levels of MUC2 and MUC3 mucin mRNA and immunoreactive protein are found in specimens with intestinal metaplasia (Ho et al., 1995b). Types II and III intestinal metaplasia share an identical pattern of mucin expression. Gastric mucin core proteins MUC1 and MUC5AC are expressed in both goblet cells and columnar cells. MUC6 expression occurs in the lower crypt and glandular epithelium but not to the extent observed in normal gastric mucosa. MUC2 and MUC4 are expressed in goblet cells. Therefore, the mucin expression profile in the different types of intestinal metaplasia allows for the identification of two patterns. One pattern is defined by decreased levels of expression of "gastric" mucins (MUC1, MUC5AC, and MUC6) and the expression of MUC2 intestinal mucin, which corresponds to type I or complete intestinal metaplasia. The other is defined by the co-expression of "gastric mucins" (MUC1, MUC5AC, and MUC6) together with the MUC2 mucin, encompassing type II and III intestinal metaplasia. Type II intestinal metaplasia represents a transitional or transient form that subsequently transforms into complete IM (type I) or incomplete type III intestinal metaplasia by deregulation of mucin glycans processing with sulfation (Reis et al., 1999).

Mucins in gastric adenocarcinoma

Different studies have supported the fact that alteration in the expression pattern of different mucins may contribute to alternation in epithelial cell growth, immune reaction, cellular adhesion and interaction with the extra-cellular matrix, which in turn may influence the tumorigenicity and metastatic properties of cancer cells (Hollingsworth and Swanson, 2004). The mucin expression pattern of gastric carcinoma is heterogeneous (Table 2). The process of neoplastic transformation in the stomach is associated with a decreased expression of mucins that are normally expressed in the gastric mucosa, including MUC5AC (Carrato et al., 1994; Ho et al., 1995b; Reis et al., 1997), MUC1 and MUC6 (Ho et al., 1995b), and the de novo expression of MUC2, MUC3 and MUC4 mucins that are normally expressed in other organs (Ho et al., 1995b). Characteristic patterns for MUC2, MUC3, MUC4, MUC5AC, and MUC6 are found in gastric cancers with increasing heterogeneity in advanced cancer stages (Ho et al., 1995b).

Table 2. Mucins with an altered expression pattern in gastric adenocarcinomas.

Mucin	Expression pattern	Method	Antibody used	References
MUC1	Over-expressed	IHC	NCL-MUC-1-CORE*, DF3*,MY.1E12*, and dHMFG-1*	Utsunomiya et al., 1998
			BC1*	Wang and Fang ,2006
	Down-regulation	IHC	139H2*	Ho et al., 1995b
MUC5AC	Over-expressed	IHC	CLH2*	Leteurtre et al., 2006
	Down-regulated	IHC	45M1*	Wang and Fang ,2006
	Down-regulated	IHC	CLH2*	Babu et al., 2006
	Down-regulated	IHC	CLH2	Reis et al., 1997
MUC5B	Over-expressed	IHC	Lum5B-2**	Leteurtre et al., 2006
			EU-MUC5Ba	Pinto-de-Sousa et al., 2002
MUC6	Over expressed	IHC	CLH5*	Leteurtre et al., 2006
	Down-regulated	IHC	Rabbit anti-MUC6**	Wang and Fang ,2006
	Down-regulated	IHC	CLH5*	Babu et al., 2006
MUC2	Over expressed	IHC	LUM2-3**	Leteurtre et al., 2006
	Over expressed	IHC	CCP58*	Wang and Fang, 2006
MUC3	Over expressed	IHC	M3P*	Ho et al., 1995b
MUC13	Over expressed	IHC	ppz0020*	Shimamura et al., 2005
MUC4	Over expressed	IHC	8G7*	our unpublished data

* Monoclonal antibody, ** Polyclonal antibody

Gastric carcinomas have been divided histologically into intestinal and diffuse types based on the histological classification described by Lauren (Lauren, 1965). These two types correspond to the differentiated and undifferentiated types described by Nakamura et al (Nakamura et al., 1968). Recently, a new classification of gastric carcinomas based on mucin expression has been proposed. Gastric carcinomas are classified as the gastric or intestinal phenotype on the basis of mucin expression by surface mucous cells, glandular mucous cells, and intestinal goblet cells (Fiocca et al., 1987; Machado et al., 2000; Sepulveda et al., 2000; Pinto-de-Sousa et al., 2002). MUC2 is employed as a marker of intestinal goblet cell differentiation and MUC5AC and MUC6 as a marker of the gastric phenotype to further understand the histogenesis and differentiation of gastric carcinoma. The differentiated-type tumor, which accounts for the majority of gastric carcinomas, is thought to display a predominantly intestinal phenotype (MUC2 positive) because it is preceded by a precancerous stage that is characterized by the sequential steps of atrophic gastritis, intestinal metaplasia, dysplasia, and intramucosal carcinoma. The diffuse type of gastric carcinoma shows the gastric phenotype (MUC5AC and MUC6 positive) and in general, lacks MUC2 expression. However, MUC2 expression is known to be lost in many colorectal cancers and a lack of MUC2 expression in the so called "diffuse type", therefore, does not exclude a background origin within intestinalized mucosa. Furthermore, researchers have concluded that the gastric-type differentiation or phenotype is retained in the majority of gastric carcinomas irrespective of the phenotype, being more prominent in diffuse and early than in intestinal and advanced carcinomas (Kabashima et al., 2000; Machado et al., 2000).

Mucin expression is shown to be correlated with gastric tumor location. MUC5AC is associated with antral carcinoma and MUC2 with cardiac carcinomas (Pinto-de-Sousa et al., 2002). Tanaka et al. have shown the correlation between MUC1 and MUC2 expression patterns and the clinical-pathological features. They showed that tumor size, depth of invasion, presence of lymph node metastasis, and clinical stage positively correlates with MUC1 expression. MUC1 expressed in tumor functions as an anti-adhesion molecule that inhibits cell-cell adhesion thereby promoting tumor metastasis (Makiguchi et al., 1996). Immunohistological detection of MUC1 in gastric cancers correlates with gastric cancer development and progression, cell invasiveness, and poor prognosis (Utsunomiya et al., 1998; Reis et al., 1998; Nakagawa et al., 1999; Tanaka et al., 2003; Steininger et al., 2005). MUC2 is not normally found in the healthy gastric mucosa but is present in gastric mucosa with intestinal metaplasia and gastric carcinomas. It has been suggested that MUC2 can be considered as an intestinal metaplasia marker and may also be used for early detection in *H. pylori* infected pre-neoplastic gastric epithelium (Babu et al., 2006). MUC2 antigen expression is a prognostic factor associated with a favorable outcome in patients. Combined evaluation of the MUC1 and MUC2 mucin staining is clinically useful to predict the outcome in patients with gastric cancer (Utsunomiya et al., 1998). MUC5AC is expressed in the surface foveolar cells of normal gastric mucosa as shown by Northern blotting, in situ hybridization and immunohistochemical studies. Early gastric cancers, both diffuse and intestinal types, show MUC5AC expression, while advanced carcinomas lose reactivity (Reis et al., 1997). The expression of MUC5AC in early gastric carcinomas suggests that gastric carcinomas retain some normal gastric phenotype during the first step of neoplastic development.

In summary, normal human gastric epithelium has a unique organ- and cell-specific mucin gene pattern characterized by high levels of MUC1, MUC5, and MUC6. The process of neoplastic transformation in the stomach is associated with a decrease in these mucins and the additional expression of mucin MUC2, MUC3, and MUC4 genes which are normally expressed by the intestine. Advanced stage gastric cancers and poorly differentiated cancers are significantly more likely to express multiple mucin core peptides compared with early stage and well differentiated cancers. The organ microenvironment, etiologic agents and tumor-host interaction influence mucin production and the morphology of gastric carcinoma. Molecular mechanisms contributing to mucin expression still remain unknown. Examination of mucin gene expression may provide useful evidence for further classification and prognostic prediction in gastric carcinomas.

Combined evaluation of gastric mucins with other molecules

Although several studies have shown an altered mucin expression profile in gastric cancer, the use of the mucin expression profile as a marker of gastric cancer differentiation and prognosis is still contradictory. (Correa and Shiao, 1994; Byrd et al., 1997; Reis et al., 1998). For greater accuracy, a combined expression pattern of mucins with other molecules have been evaluated. The expression of several non-mucin molecular factors has been studied in gastric cancer (Table 3). Tanaka et al. 2003 have analyzed 209 histological samples of gastric carcinoma (141 early, 68 advanced) for a combined evaluation of MUC1 and E-cadherin expression. The absence of MUC1 and normal E-cadherin expression was proposed as a favorable prognostic marker (Tanaka et al., 2003). E-cadherin plays a crucial role in epithelial cell–cell interaction and in the maintenance of the normal architecture of epithelial tissues. The loss of E-cadherin function has been correlated with the aggressive biological behavior of gastric cancer. Ohno et al. (2006) have shown that the loss of E-cadherin and the expression of MUC1 in

advanced gastric cancer is an unfavorable prognostic marker (Ohno et al., 2006). An over expression of MUC1 reduces the E-cadherin mediated cell-cell adhesion and promotes cancer progression. Recently, a similar type of mechanism was proposed for MUC4-mediated cell's invasive property. Also, for the first time, our lab has shown that MUC4 is significantly over expressed in gastric adenocarcinoma (unpublished data).

CDX2, a caudal-related homeobox gene, is a regulatory factor for intestinal development and differentiation of goblet cells. Increased CDX2 expression is seen in intestinal-type cancers of Lauren and may play a role in gastric carcinogenesis (Bai et al., 2002). Recent studies have revealed that the MUC2 mucin gene is transcriptionally regulated by CDX2 (Mesquita et al., 2003; Yamamoto et al., 2003). CDX2 is a more specific marker for intestinal differentiation than MUC2 (Steininger et al., 2005).

Trefoil peptides (TFF peptides) constitute a group of small secretory peptides bearing one or more trefoil structural motifs (P-domains). In normal gastric mucosa, TFF1 (pS2) co-localizes with MUC5AC in the surface/foveolar epithelium and TFF2 (hSP) is expressed together with MUC6 in the mucous cells of the neck zone of the oxyntic mucosa and in the antral glands (Nogueira et al., 1999). Machado et al. characterized trefoil peptides and mucins in 96 gastric carcinomas and found that gastric carcinomas show a co-expression of TFF peptides, MUC5AC and MUC6 (Machado et al., 2000). TFF peptides and mucins may act in a synergistic manner in the pathogenesis of gastric cancer. Another non-mucin marker, Li-cadherin, a member of the cadherin family of cell adhesion molecules is specifically expressed in intestinal epithelium. Li-cadherin has been evaluated in correlation with the mucin phenotypes of gastric cancer. Li-cadherin expression is significantly associated with the MUC2-expressing, intestinal-type of gastric cancer (Motoshita et al., 2006). Other markers, such as concanavalin A (gastric pyloric glands) and CD10 (intestinal phenotypic cell markers), have been studied in association with mucins (Kabashima et al., 2000; Kawachi et al., 2003). Further study of the combined expression pattern of mucins and various non-mucin molecular markers in gastric cancer may be helpful in a better characterization of gastric cancer.

Table 3. Non-mucin molecular factors with an altered expression pattern in gastric cancer patients.

Molecular factors	Expression	Method	Reference
MSI/MMR	Over-expressed	IHC/PCR	Rhyu et al., 1994
VEGFR	Over-expressed	IHC	Zhang et al., 2002
TS	Over-expressed	IHC/PCR	Suda et al., 1999
EGFR	Over-expressed	IHC	Ross and McKenna, 2001
MMP	Over-expressed	IHC	Li et al., 2002
C-erb B-2	Over-expressed	IHC/FISH	Xue et al., 2003
P^{53}	Over-expressed	IHC/PCR	Feng et al., 2002
E-cadherin	Down-regulated	IHC/PCR	Mingchao et al., 2001

TS: Thymidilate synthase.

Conclusion and perspectives

Numerous alterations of gastric mucins have been described in pre-malignant and malignant diseases of the stomach. For gastric cancer diagnosis and prognosis purposes, intracellular and membrane-bound mucins have been studied by means of histochemical and immunohistochemical techniques. These techniques present the advantage of being carried out on bioptic specimens obtained during endoscopy; however, there is a drawback of finding a quantitative rather than a topographic-qualitative evaluation. Immunochemical data obtained by using antibodies which detect carbohydrate epitopes of mucins may be misleading because the staining intensity may be altered by a change in mucin protein expression or by alteration in glycosylation. This discussion suggests that the expression pattern of specific glycosyl transferase and mucins may be correlated in cell- and tissue-specific manners. Studies by Paulson et al. 1989 have shown that sialyltransferase are expressed in a tissue-specific manner (Paulson et al., 1989). It is known that the protein sequence of mucin may provide a recognition sequence for O or N-linked glycosylation (Brockhausen et al., 1990). Therefore, additional experiments are needed to correlate the specific mucin protein, carbohydrate antigen, and glycosyltransferase in gastric mucosa. Many findings have shown that the mucin antigens, like SIMA, LIMA (Filipe et al., 1988), BD-5 (Fiocca et al., 1988) and M3 (Nardelli et al., 1984) antigens are expressed in gastric cancer, normal small intestine and the colon, but they are absent in the normal gastric epithelium. So, these antigens may be present in those mucins that are over expressed in gastric cancer. Further comparative studies are needed. Since carbohydrate antigens are not limited to mucins alone and are found on other cellular glycoproteins and glycolipids, caution is required in the interpretation of histological analysis where no direct identification of mucin protein is made. Therefore, an analysis including both immunochemistry and in situ hybridization will be more accurate.

The organ microenvironment, etiologic agents and tumor-host interaction influence mucin production and the morphology of gastric carcinoma. Molecular mechanisms contributing to mucin expression are complex and still remain unknown. Examination of mucin gene expression may provide useful evidence for further classification and prognostic prediction in gastric carcinomas. A better understanding of their expression pattern during gastric cancer progression may allow them to be used as therapeutic targets.

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