MINI-REVIEW

Microsatellite Instability of Nuclear and Mitochondrial DNAs in Gastric Carcinogenesis

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Abstract

Genetic instability contributes to the development and progression of gastric cancer, one of the leading causes of cancer death worldwide. Microsatellite instability (MSI) has been hypothesized to be involved in carcinogenesis, althgough its mechanisms and exact roles in gastric cancer remain largely unknown. Our aim was to identify associated clinicopathological characteristics and prognostic value of MSI in gastric cancer and precancerous lesions including gastritis, metaplasia, dysplasia, and adenoma. Because mitochondrial DNA has a different genetic system from nuclear DNA, the results of both nuclear MSI and mitochondrial MSI in gastric cancer were reviewed. This review provides evidence that genetic instability of nuclear and mitochondrial DNAs contributes to early stages of gastric carcinogenesis and suggests possible roles in predicting prognosis.

Keywords: Gastric cancer - dysplasia - metaplasia - microsatellite instability - mitochondrial microsatellite instability

Introduction

Gastric cancer (GC) is highly prevalent in Asia and the third most common cause of cancer death after lung cancer and liver cancer in Korea (Jung et al., 2012). The 5-year survival rate for gastric cancer is over 60%, however, the late onset of clinical symptoms occurred late diagnose at an advanced stage, which limits available therapeutic approaches in more than 50% of cases (Strong et al., 2010). A better prognostic result was obtained in Asian, possibly attributable to early detection and approaches.

According to clinicopathological studies, adenocarcinoma is the major histological type of gastric cancer, over 90% of all gastric malignancies, and it is divided into two distinct pathological entities, intestinal and diffuse types by Lauren classification. Intestinal type is associated with certain dietary factors, such as high intake of salt, smoked meats, and food preserved with nitrites or nitrates (Hamilton and Meltzer, 2006; Shah et al., 2011). It develops via an intestinal metaplasia-adenoma-carcinoma sequences by the accumulation of gene alterations. Histologically it is well differentiated and is seen more frequently in elder patients (Hamilton and Meltzer, 2006). In comparison to intestinal-type GC, diffuse type is less related to environmental influences and poorly differentiated with infiltrating, non cohesive cells (Correa et al., 1975; Shah et al., 2011). *Helicobacter pylori* infection plays a significant role in the development of intestinal GC through chronic inflammation, but without occurrence of gastric atrophy and intestinal metaplasia (Forman et al., 1991; Parsonnet et al., 1991).

Because GC is a complicated heterogeneous disease by genetic and environmental factors, various genetic pathways and genes involved in gastric carcinogenesis and progression have been studied by many authors (Tohdo et al., 1993; Wirtz et al., 1998; Fleisher et al., 2001; Wang et al., 2012; Najjar Sadeghi et al., 2013). The alterations in various genes regulate cell growth, apoptosis, DNA repair, etc. resulting from various underlying genetic and epigenetic changes. As a result, distinct GC clinicopathological profiles have been reviewed up to now, however, GC is still considered as the outcome of irregularities in complex biological processes (Yasui et al., 2005; Grabsch and Tan, 2013). Therefore, the clinicopathological characteristics of GCs vary from case to case, inducing the difficulty to choose their subtype and the optimal therapeutic approach.

In last few years, a large amount of studies about the genetic instability in GC have been accumulated. Recently, we and other groups investigated mitochondrial genetic instability in GC and precancerous legions (Maximo et al., 2001; Ling et al., 2004; Zhao et al., 2005; Lee et al., 2007; Jeong et al., 2010). These results show the change of mitochondrial DNA may be early event in gastric carcinogenesis and suggest its potential for progression of GC. Though nuclear microsatellite instability has been focused in GC by many authors, there was no study and overview on the importance of mitochondrial DNA (Nobili et al., 2011; Hudler, 2012; Shokal and Sharma, 2012). In this review, we intend to focus nuclear and mitochondrial microsatellite instability in GC and gastric carcinogenesis (precancerous lesions). Their clinical

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implications and novel knowledge about molecular pathway of carcinogenesis are presented.

Microsatellite Instability (MSI)

MSI

Genetic instability is referred to one of the hallmarks of cancer development and is believed to be one of the initial steps of gastric carcinogenesis (Lengauer et al., 1998; Belien et al., 2009). It is divided into two phenotypes: (1) chromosomal instability (CIN) which is characterized by gross chromosomal abnormalities, such as gain or loss of whole chromosomes (aneuploidy) and/or some part of chromosomes (loss of heterozygosity, amplifications, and translocations); (2) microsatellite instability (MSI) due to a defect in the DNA mismatch repair pathway. Microsatellites are short repetitive of 1-10 nucleotide long units in coding and non-coding DNA, as a valuable region both structurally and functionally. They are highly polymorphic in nature and show high rate of alteration in tumor samples. Their alterations are corrected by mismatch repair (MMR) and DNA exonuclease proof reading systems, reducing the error rate (Strand et al., 1993). However, patients with a deficiency or inactivation of one of the DNA mismatch repair proteins MLH1, MSH2, MSH6, or PMS2 in their cancer are unable to repair naturally occurring DNA replication errors, leading to the appearance of new alleles not present in the normal DNA (Buermeyer et al., 1999). These alterations like insertions, deletions, *ect.* are defined as microsatellite instability and may be a significant marker for prognosis and diagnosis in some cancers.

Since MSI has been focused on hereditary nonpolyposis colon cancer at first in 1993, and then, several studies suggested the clinical value of MSI in various genes (Aaltonen et al., 1993; Risinger et al., 1993). In 1997 meeting, standard markers for MSI test (Bethesda panel) were recommended by the National Cancer Institute as five microsatellite markers including two mononucleotide repeats (BAT26 and BAT25) and three dinucleotide repeats (D2S123, D5S346 and D17S250) (Boland et al., 1998). Based on the number of markers displaying instability per tumor, three groups of tumors are defined: those with ≥30-40% of the markers showing instability (MSI-H); those with ≤30-40% of the markers showing instability (MSI-L) and those showing no instability (MSS). For the detection of MSI-H cases, BAT26 and BAT25 are the most sensitive and commonly used markers and some studies suggested other markers of newly discovered efficiency, along with a few of the recommended markers, may serve as a better panel for MSI (Hoang et al., 1997; Suraweera et al., 2002; Buhard et al., 2006). As a result, pentaplex panel of five quasimonomorphic mononucleotide repeats (BAT25, BAT26, NR-21, NR-22 and NR-24) may be more sensitive for MSI-H tumors than other microsatellite markers (Umar et al., 2004). Also, Promega Corporation (Madison, USA) developed a fluorescent multiplex assay, called the MSI analysis system, which utilizes five mononucleotide microsatellite loci (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) and two penta-nucleotide repeat markers (Penta C and Penta D) (Bacher et al., 2004;

Murphy et al., 2006). Up to now, both Bethesda panel and revised panel have been used according to the preference of the investigators in many studies.

MSI in gastric cancer

Though Bethesda panel was proposed to apply only to colorectal cancer (Boland et al., 1998), MSI was investigated using this panel or other markers to identify its characteristics in GC. Previous studies showed that the frequency of MSI in GC varies from 10% to 50%, depending on the number of loci investigated (Hayden et al., 1998; Fleisher et al., 2001; Nobili et al., 2011; Hudler, 2012; Shokal and Sharma, 2012). When Bethesda or closely related criteria was used for MSI detection, its frequency was consistent from 9% to 23%. The frequency of MSI in GC was also different according to population and its incidence. In Asian, MSI and its cases were found higher than that in American and European due to environmental factors, diet, infection status, and et al. It was suggested that MSI may be associated with pathogenic agent like *Helicobacter pylori* and Epstein Barr virus (EBV) resulting in the impairment of MMR system (Chang et al., 2002; Chiaravalli et al., 2006; Machado et al., 2009; Ferrasi et al., 2010; Machado et al., 2010). However, other study showed similar frequency of MSI in GC with and without *H. pylori*, suggesting that *H. pylori* act in different pathogenesis of gastric carcinogenesis (Ohara et al., 2006). Though exact mechanism is not known, diet factor like red meat, nitrite and sodium intakes also may affect the MSI status in GC.

MSI was associated with various clinicopathological features. MSI was generally associated with female and old age, however, its strong relation with GC in young female was also found (Sasao et al., 2006; Arai and Takubo, 2007). These paradoxical results suggested that various molecular pathways may be involved in gastric carcinogenesis. MSI was also associated with mucinous GC and mucosa-associated lymphoid tissue lymphomas. Importantly, most studies described a strong association of MSI in intestinal-type GC (Vauhkonen et al., 2005; Kim et al., 2011). And GC with MSI was more frequently Borr-mann type I or II and had a many tumor-infiltrating lymphocyte. The intestinal type is preceded from chronic gastritis followed by atrophic gastritis, intestinal metaplasia, dysplasia and cancer. MSI in these precancerous legions will be described further and this result supports that intestinal type of GC undergoes much genetic instability compared to diffuse type.

GC patients with MSI were usually diagnosed at early stage and showed good prognosis (Hayden et al., 1997; Schneider et al., 2000; Beghelli et al., 2006; Corso et al., 2009; Yashiro et al., 2009), though some studies have found no significant difference between MSI and MSS (Ottini et al., 1997; An et al., 2005; Oki et al., 2009; An et al., 2012). Recent summary about the correlation between MSI and survival status in GC showed a trend of better prognosis of patients with MSI tumors than that without MSI (Fang et al., 2012). Chemotherapy for GC is used selectively, therefore, there was two studies with large cases of GC investigating the effect of chemotherapy according to MSI status. Some study showed that both

survival results and response to 5-FU-based chemotherapy did not correlate with MSI status (Oki et al., 2009). However, other study described that MSI status had no prognostic value itself, but it can predict chemotherapeutic response in stage II and III gastric cancer (An et al., 2012).

MSI in gastric precancerous legions

As mentioned above, because MSI was deeply associated with intestinal-type GC, MSI in gastric metaplasia and dysplasia as its precursors were also focused. At first, high frequency of MSI in precancerous legions was found, suggesting its early role in gastric carcinogenesis (Semba et al., 1996; Hamamoto et al., 1997). To evaluate its role in gastric carcinogenesis, some studies discriminated between metaplasias that are adjacent to GC and those not coexisting with GC in evaluating legions for MSI (Semba et al., 1996; Hamamoto et al., 1997; Leung et al., 2000; Lee et al., 2002; Lee et al., 2004). Previous results of MSI in gastric precancerous legions according to the markers and samples were summarized in Table 1 (Semba et al., 1996; Hamamoto et al., 1997; Endoh et al., 2000; Kashiwagi et al., 2000; Kim et al., 2000; Kobayashi et al., 2000; Leung et al., 2000; Jin et al., 2001; Kim et al., 2002; Lee et al., 2002; Garay et al., 2004; Lee et al., 2004; Liu et al., 2005; Zaky et al.,

2008; Park et al., 2013). In gastric precancerous legions without GC, the frequency of MSI was low under 10% of cases except the studies by Semba et al. (1996) (33.3%, 4/12) and Zaky et al. (2008) (20.5%, 8/39). Gastric adenoma tends to have MSI more frequently than gastritis or metaplasia and MSI may be accumulated according to their progression.

In gastric precancerous legions adjacent to GC, the frequency of MSI was different according to the markers examined for MSI detection (Semba et al., 1996; Hamamoto et al., 1997; Kobayashi et al., 2000; Leung et al., 2000; Jin et al., 2001; Kim et al., 2002; Lee et al., 2002; Lee et al., 2004; Zaky et al., 2008). In data using the Bethesda markers or closely related criteria, MSI was found in about 10% of cases except the studies by Kim et al. (2002) (0%) and Zaky et al. (2008) (48.2%). However, the studies using other markers showed rare frequency of MSI in precancerous legions adjacent to GC (Semba et al., 1996; Hamamoto et al., 1997; Kobayashi et al., 2000; Jin et al., 2001). These results suggested that the pathway of gastric carcinogenesis may be different between precancerous legions adjacent to GC and that without GC. Kashiwagi et al. (2000) showed the gastritis with MSI developed into gastric adenoma or adenocarcinoma and suggested MSI as a possible marker for predicting

Table 1. MSI in Gastric Precancerous Legion

MSI Markers	Samples	MSI-H	MSI-L	Reference
D2S110, D2S123, D2S136,	Metaplasia*	0% (0/9)	33.3% (3/9)	Semba et al. (1996)
D3S1067, D5S505, D7S486, D11S29, TP53, D17S855	Adenoma	33.3% (4/12)	8.3% (1/12)	
D1S191, D7S486, Dl 1S29,	Metaplasia*	0% (0/15)	26.7% (4/15)	Hamamoto et al. (1997)
TP53, D17S855,BAT-RII BAT25, BAT26, BAT402 D1S162, D1S237, D2S119, D2S104, D3S1274, D3S1300, D3S1766, D3S1216, D4S174, D5S299, D4S1652, APC2, D5S346, D5S409, D6S271, D6S105, D6S310, D7S507, D7S1805, D8S254, D8S555, D9S104, D9S103, D10S183, D10S109, D11S875 (MISC), D11S897, D12S95, D12S375, D13S120, D14S51, CYP19, D16S403, D16S413, TP53, D17S796, D17S786, D17S520, D17S791, D18S53, D18S34, D18S386, D19S177, D19S416, D20S66, D20S17, D21S411, D21S258, IL2RB, BAT25, BAT26	Adenoma*	20.6% (13/63)	N.S.	Kim et al. (2000)
Bethesda panel, BAT40, D13S170, TP53	Metaplasia*	13.3% (4/30)	40.0% (12/30)	Leung et al. (2000)
	Metaplasia	6.7% (3/45)	37.8% (17/45)	
D2S123, D3S1067, D5S409, D13S153, TP53	Chronic gastritis	2.0% (1/55)	0% (0/55)	Kashiwagi et al. (2000)
	Adenoma	8.3% (1/12)	8.3% (1/12)	
D1S103, D2S123, D3S1067, D5S107, D13S153, D17S261, D18S34, BAT25, BAT40	Metaplasia*	3.0% (1/33)	30.3% (10/33)	Kobayashi et al. (2000)
BAT26	Non-invasive	33.3% (8/24)	0% (0/24)	Endoh et al. (2000)
D2S115, D4S404, D5S178, IL-9, D6S265, D7S490, D11S900, MYH6, TP53, D17S1176, D18S46, D21S1407	Metaplasia*	0% (0/17)	0% (0/17)	Jin et al. (2001)
Bethesda panel	Dysplasia	2.2% (1/46)	2.2% (1/46)	Lee et al. (2002);
	Adenoma	5.7% (2/35)	14.3% (5/35)	Lee et al. (2004)
	Dysplasia*	11.8% (4/34)	5.9% (2/34)	
Bethesda panel, D13S170, TP53	Metaplasia*	0% (0/15)	0% (0/15)	Kim et al. (2002)
D3S1067, D3S1262, D5S346, D12S347, D12S351, BAT26	Metaplasia	0% (0/58)	0% (0/58)	Garay et al. (2004)
Bethesda panel	Metaplasia	7.3% (3/41)	19.5% (8/41)	Liu et al. (2005)
Bethesda panel	Metaplasia	20.5% (8/39)	N.S.	Zaky et al. (2008)
	Metaplasia*	48.2% (40/83)	N.S.	
Bethesda panel	L- dysplasia	3.2% (1/14)	0% (0/14)	Park et al. (2013)
	H- dysplasia	0% (0/17)	0% (0/17)	

*adjacent to GC; Bethesda panel, BAT25, BAT26, D5S346, D17S250, D2S123; N.S., not shown

high-risk legions that may develop into intestinal-type GC. However, it had no statistical significance because of small sample sizes and low frequency of MSI.

Mitochondrial microsatellite instability (mtMSI)

Mitochondrial DNA

Human cells have hundreds of mitochondria which are ubiquitous organelles with the primary function to generate ATP through oxidative phosphorylation and this process generates reactive oxygen species (ROS). Recent studies showed that mitochondria play an important role in cell apoptosis and calcium signaling. Therefore, their dysfunctions have been associated with various human disease including neurodegenerative disease, diabetic mellitus, cardiomyopathy, kidney failure, and tumors (Bates et al., 2012; Zsurka and Kunz, 2013). Mitochondrial DNA is a double stranded and circular molecule, which consists of 16,568 base pairs. The compact mtDNA molecule encodes 37 genes; 13 polypeptides of the oxidative phophorylation system (OXPHOS), 22 tRNAs and 2 rRNAs. A non-coding control region (16,024-576 nucleotide positions) contains three conserved sequence blocks and a displacement loop (D-loop) including origins of mtDNA replication and promoters and enhancers for mtDNA transcription. Mitochondrial DNA (mtDNA) has different genetic system from nuclear DNA, and multiple copies of mtDNA are present in each mitochondrion. Mutation rate of mtDNA is higher than that of nuclear DNA due to abundant reactive oxygen species in the mitochondrial inner membrane, less repair mechanisms, and no mtDNA-coating proteins, like the histones in the nucleus (Howell et al., 1996).

Cells harbor multiple copies of mitochondrial genes and each mitochondrion 2 to 10 copies of mtDNA as opposed to only 2 copies of each nuclear gene. Mitochondrial genes have an exclusively maternal mode of inheritance in mammals, while nuclear DNA is inherited from both parents and rearranged by recombination (Giles et al., 1980). This identical status of mtDNAs was refered to "homoplasmy". Mutant mtDNA occurred by environmental and genotoxic damage can coexist with wild-type mtDNA in a state called heteroplasmy. Therefore, the biological impact of a given mutation could vary, depending on the proportion of mutant mtDNAs carried by each cell. In cancer, a certain level of heteroplasmy by mutant mtDNAs accelerates tumor progression due to ROS generation and genetic instability. As a result, mutant mtDNA may become predominant in cancers, which lead to homoplasmy (Dunbar et al., 1995; Coller et al., 2001; He et al., 2010). This mechanism supports the presence of homoplasmic mutation of mtDNA in malignant cancers and it could be important key to identify the carcinogenesis and to suggest new insight for cancer treatment.

mtMSI in gastric cancer

Mitochondrial DNA alteration has been focused in gastric cancer and various mitochondrial mutations were introduced. At first, Burgart et al. (1995) studied low frequency of 4977-bp mitochondrial deletion, between nucleotides 8,470 and 13,447 of the human mtDNA, in GC (5.2%, 4/77). Interestingly, only GC located in gastroesophageal junction (12.5%, 4/32) showed 4977-bp mitochondrial deletion and 45 distal GC had no mitochondrial change. However, 4977-bp mitochondrial deletion was found in about half of GC (Maximo et al., 2001; Wu et al., 2005), and they also showed this alteration in noncancerous tissues (53.1%, 17/32).

In mitochondrial genome, several repetitive elements are present and their alteration is defined as mitochondrial microsatellite instability (mtMSI). Of these, the poly C-tract D310 (between nucleotide 303 and 315) located in the hypervariable region II of D-loop is the most frequent site of mtMSI in various cancers (Richard et al., 2000; Bianchi et al., 2001; Wang et al., 2005; Ashtiani et al., 2012; Dai et al., 2013). In GC, high frequency of mtMSI was found by Alonso et al. (1997) (3/8, 37.5%), Habano et al. (2000) (16.1%,10/62), Maximo et al. (2001) (18.7%, 6/32), Sanchez-Cespedes et al. (2001) (62%,5/8), Ling et al. (2004) (38.2, 26/68), Wu et al. (2005) (46.9.0%, 15/32), Zhao et al. (2005) (20%, 4/20), Gargano et al. (2007) (11.0%, 11/100), and Jeong et al. (2010) (10.2%, 5/49). Habano et al. (2000) showed a deep association between MSI and mtMSI in GC, suggesting a common cause for instability of nuclear and cytoplasmic genomes Gargano et al. (2007) demonstrated that mtMSI had a positive-relationship with MSI and RUNX3 methylation, and higher frequency of mtMSI was found in stage I GC than other stages. No association with any of the clinicopathological characteristics are reported in above studies whereas mtMSI suggested an association between tumor-node-metastasis staging by Gargano et al. (2007) or intestinal type by Ling et al. (2004) and Jeong et al. (2010).

Although high frequency of mtMSI was found in GC, the causative role of mtMSI in D-loop is still unknown. Certainly, the D-Loop contains the major mtDNA transcription promoters, and therefore, mtMSI in D-loop might alter mtDNA transcription and lead to a respiratory chain alteration which is responsible for high reactive oxygen species levels release (Sbisa et al., 1997; Chinnery et al., 2002). Moreover, D-loop, especially D310 region, is more susceptible to oxidative and electrophilic damage *in vitro* (Mambo et al., 2003). It contributes to gastric carcinogenesis by occurring nuclear genome damage and the dysfunction in mitochondrial induced apoptosis.

This result suggested the hypothesis that mtMSI in the D-loop may alter mitochondrial DNA copy number (mtCN) or gene expression, and mtCN was focused in various cancers (Lee et al., 2004; Yin et al., 2004; Dai et al., 2013; Guo et al., 2013). Though reduced mtCN in GC was found in some studies, there was little study about the correlation betwen mtCN and mtMSI in GC (Jun et al., 2012; Wen et al., 2013). One case-control study showed no difference of mtCN among cases and controls and no association between leukocyte mtCN and GC risk (Liao et al., 2011). Our recent result confirmed that no clinical and prognostic values of mtCN in GC and its precancerous legions (unpublished data). Therefore, further comprehensive approach is needed to identify the relationship between mtMSI and mtCN in GC.

mtMSI in gastric precancerous legions

Though high frequency of mtMSI was found in GC, its role in gastric carcinogenesis has not been focused. Sui et al. (2006) and Rigoli et al. (2008) showed high frequency of mitochondrial DNA alterations in GC, however, they did not suggest any role of mtMSI in gastric carcinogenesis. Ling et al. (2004) demonstrated a sequential accumulation of mtMSI during carcinogenesis of GC (12.5%, 5/40 in gastritis; 20.0%, 6/30 in metaplasia; 25.0%, 5/20 dysplasia; 38.2%, 26/68 in GC). However, Jeong et al. (2010) showed a similar frequency of mtMSI in gastric dysplasia (12.5%, 3/24) and GC (10.2%, 5/49), suggesting its early role in the progression of GC. And gastric dysplasia with mtMSI showed a poor prognosis statistically compared to mtMSI negative through progression to high-grade dysplasia or gastric cancer. Interestingly, these two studies confirmed deep association between mtMSI and intestinal type GC. Taken together, it is suggested that mtMSI is an early and important role in the sequence of gastric dysplasia-intestinal GC.

This hypothesis may be associated with *H. pylori* infection according to previous studies (Hiyama et al., 2003; Lee et al., 2007). A high frequency of mtMSI in precancerous legions with *H. pylori* suggested that *H. pylori* infection contributes to the accumulation of mtMSI at early steps of GC progression. *In vitro* and *in vivo* study introduced that *H. pylori* infection promotes gastric carcinogenesis by induction mitochondrial genetic instability like mtMSI (Machado et al., 2009). Moreover, recent study described that this instability may be caused by down-regulation of mitochondrial DNA repair pathways (Machado et al., 2013). Lower expression of APE-1 and YB-1 involved in mitochondrial base excision repair and mismatch repair and lower mtCN by *H. pylori* are able to impair mitochondrial function and these processes might trigger gastric carcinogenesis. Though it did not reach the statistical significance, Jeong et al. (2010) also showed the frequency of mtMSI was higher in gastric dysplasia with *H. pylori* infection (25.0% versus 6.2%). Therefore, further study should clarify genetic instability of both nuclear and mitochondrial DNA in precancerous legions with *H. pylori*.

Conclusion

Genetic instability has been suspected to play a significant role in the development of cancer. However, there was little study in GC and its role of mtMSI, especially, is still controversial. In the past several years, we studied a comprehensive investigation of mtMSI in various cancers and matched precancerous legions. Alterations of mitochondrial DNA were frequently found in precancerous legions, while these in nuclear DNA were rare. And generally, mtMSI may affect the carcinogenesis by interacting other molecules or environmental in various cancers. This review suggests that genetic instability of nuclear and mitochondrial DNAs induced by *H. pylori* is an early event for the progression to GC and provides an alternative angle to conquer cancer (Figure 1). Nevertheless, many unanswered questions remain. The relationship of genetic instability with clinical data and
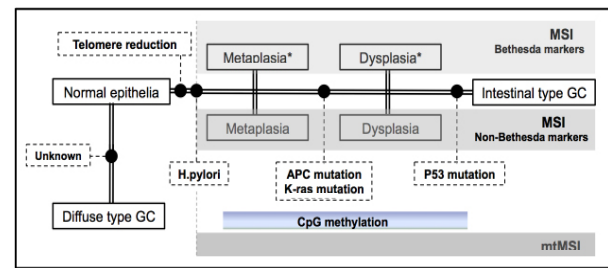
Figure 1. Events in the Development of Gastric Cancer.
*adjacent to GC

prognosis in GC should be studied more carefully. Further, it may construct the basis for identifying novel markers for cancer diagnosis and therapeutic approaches.

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