Helicobacter pylori-Mediated Genetic Instability and Gastric Carcinogenesis

Takahiro Shimizu, Tsutomu Chiba and Hiroyuki Marusawa

Abstract *Helicobacter pylori* infection is the most important cause of human gastric cancer worldwide. Gastric cancer develops over a long time after *H. pylori* infection via stepwise accumulation of genetic alterations and positive selection of cells with growth advantages. *H. pylori* itself and the resultant chronic inflammation lead to the emergence of genetic alterations in gastric epithelial cells via increased susceptibility of these cells to DNA damage. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) in inflammatory and gastric epithelial cells, as well as the expression of cytidine deaminase in gastric epithelial cells, may link *H. pylori*-related inflammation and DNA damage. Recent comprehensive analyses of gastric cancer genomes provide clues for the possible molecular mechanisms of gastric carcinogenesis. In this chapter, we describe how genetic alterations emerge during gastric carcinogenesis related to *H. pylori* infection.

Contents

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T. Shimizu · T. Chiba (✉) · H. Marusawa
Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, 54 Kawara-Cho, Shogoin, Sakyo-Ku, Kyoto 606-8507, Japan
e-mail: chiba@kuhp.kyoto-u.ac.jp

T. Shimizu
e-mail: shimy@kuhp.kyoto-u.ac.jp

H. Marusawa
e-mail: maru@kuhp.kyoto-u.ac.jp

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1 Genetic Abnormalities in Gastric Cancer Tissues Related to *H. pylori* Infection

Gastric cancer is generally classified by histologic classification systems, including those of Lauren (1965) and the World Health Organization (WHO) (2010; www.who.int/en/). The Lauren classification includes two subtypes of gastric cancer, the intestinal type and the diffuse type, and the WHO classification has four subtypes, including papillary, tubular, mucinous and poorly cohesive types. Intestinal-type gastric cancers represent cohesive tumor cells with a glandular architecture. These cancer types are typically generated from *Helicobacter pylori*-infected gastric mucosa with chronic gastritis, atrophy and metaplastic changes (Correa 1988). While intestinal metaplasia has been focused as a precursor to gastric cancer, spasmolytic polypeptide-expressing metaplasia (SPEM) has also been highlighted as another metaplastic lesion (Goldenring et al. 2010). SPEM generates via the trans-differentiation of chief cells following parietal cell loss due to *H. pylori* infection (Nam et al. 2010). In addition, SPEM gives rise to intestinal metaplasia in animal models (Yoshizawa et al. 2007; Choi et al. 2016) and also progresses to further aberrant and invasive phenotypes in *H. pylori*-infected Mongolian gerbil models (Shimizu et al. 2016). Therefore, SPEM is thought to be the initial pre-neoplastic metaplasia predisposing to gastric cancer. Diffuse-type gastric cancer, by contrast, is composed of scattered, poorly cohesive cells with poor cellular differentiation. This type of cancer develops in *H. pylori*-infected mucosa with or without atrophic and metaplastic changes, as well as in the mucosa unaffected by *H. pylori* infection.

On the other hand, classification of gastric cancers based on comprehensive genome analyses has been recently proposed (Cancer Genome Atlas Research Network 2014; Cristescu et al. 2015). Cancer is a disease of genetic abnormalities (Stratton et al. 2009). Whole-genome sequencing and whole-exome sequencing which targets coding exons of genes using next generation technologies have been conducted on various cancer types and have identified numerous genetic alterations in cancerous tissues (Lawrence et al. 2013). The Cancer Genome Atlas (TCGA)

project revealed that the gastric cancer genome has, on average, 11.4 mutations per megabase (Cancer Genome Atlas Research Network 2014). Although most of these genetic alterations may be passenger mutations that do not contribute to carcinogenesis, 2–6 mutations on average per each cancer tissue could be oncogenic driver mutations (Kandoth et al. 2013). Based on the abundant information of genetic changes in tumors, TCGA research network demonstrated that gastric cancer is subdivided into four subtypes: tumors positive for Epstein-Barr virus (EBV), tumors with microsatellite instability (MSI), tumors with chromosomal instability (CIN) and genomically stable (GS) tumors. EBV-positive cancer shows extreme DNA hypermethylation, *PIK3CA* mutations and amplification of *JAK2*, *PD-L1*, and *PD-L2* genes. The characteristics of MSI tumors are epigenetic silencing of *MLH1*, one of the DNA mismatch repair genes, in the context of a CpG island methylator phenotype (CIMP). *MLH1* silencing can lead to subsequent genetic changes in hundreds to thousands of genes. The frequency of MSI is reportedly higher in intestinal-type gastric cancer, older females and distal gastric cancer (Kim et al. 2011). Early gastric cancer genomes with MSI show a level of mutations comparable to that of advanced MSI gastric cancer in terms of the number, sequence composition, and functional consequences of mutations (Kim et al. 2014). These findings suggest that genetic or epigenetic alterations characterized as MSI are already present in early gastric cancer genomes. CIN tumors account for 50% of gastric cancers, and most of them are histologically of the intestinal-type. This type of cancer typically has tumor protein p53 (*TP53*) mutations and chromosomal aberrations, including marked aneuploidy, and focal amplification, such as receptor tyrosine kinases. *TP53* mutations are frequently seen in non-cancerous gastritis mucosa with *H. pylori* infection (Shimizu et al. 2014), and various chromosomal aberrations are present in gastric adenoma (Uchida et al. 2010). These findings suggest that *TP53* mutations and various chromosomal alterations are early events during *H. pylori*-related gastric carcinogenesis with atrophy-metaplasia-dysplasia sequence. GS tumors that lack these specific features are predominantly of the diffuse histologic subtype, and half of them harbor mutations or fusion in E-cadherin (*CDH1*) or Rho GTPase family genes (Wang et al. 2014; Kakiuchi et al. 2014).

Combined histologic and genetic analyses are essential for understanding the process of gastric cancer development. Although each cancer has a very different profile, these analyses elucidate several possible processes from early genetic events to progression in *H. pylori*-related gastric carcinogenesis. In addition to these approaches for uncovering the process of gastric carcinogenesis, molecular mechanisms by which genetic alterations generate and accumulate during *H. pylori* infection are also important. Two main types of factors can influence the generation of genomic abnormalities: one that induces DNA damage and another that repairs damaged DNA (Fig. 1), which will be discussed in this chapter.

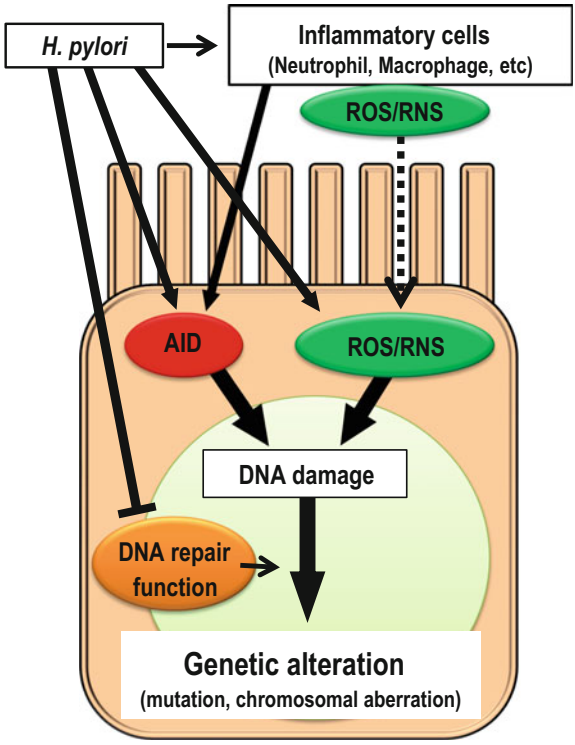

Fig. 1 The mechanisms how genetic alterations generate during *H. pylori*-related gastric carcinogenesis. *H. pylori* itself and the resultant chronic inflammation induce DNA damages in gastric epithelial cells via the expression of reactive oxygen species (ROS) and reactive nitrogen species (RNS) as well as activation-induced cytidine deaminase (AID). In addition to these genotoxic or genome editing agents, the alteration of DNA repair function induced by *H. pylori* infection also influences the generation of genetic alterations

2 Genotoxic Mediators: ROS/RNS

2.1 ROS/RNS in H. pylori*-Infected Gastric Mucosa*

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated in human tissues are considered potential genotoxic factors (Hussain et al. 2003). The high expression levels of ROS and RNS in the gastric mucosa of *H. pylori*-infected patients correlate well with histologic mucosal damage (Suzuki et al. 1996; Davies et al. 1994). Sources of ROS and RNS are inflammatory cells such as neutrophils, as well as gastric epithelial cells (Hardbower et al. 2014). In general, the oxidative burst from phagocytes in neutrophils is the main innate mechanisms of immunity against pathogenic bacteria (Naito and Yoshikawa 2002). In neutrophils, ROS production is catalyzed by nicotinamide adenine dinucleotide phosphate oxidase on

the membrane (Handa et al. 2011). Upon recognition of pathogenic bacteria, neutrophils immediately engulf the bacteria, form phagosomes and kill the bacteria by ROS production. In addition, nitric oxide (NO) is produced by macrophages as a normal host immune response against *H. pylori*. In *H. pylori*-infected gastric mucosa, however, ROS and RNS cannot kill these bacteria because of bacterial defense mechanisms (Gobert and Wilson 2016). On the one hand, ROS and RNS derived from neutrophils and macrophages increase in *H. pylori*-infected gastric mucosa, and on the other hand, the bacterial cytotoxin-associated gene A(CagA) protein stimulates multiple responses in gastric epithelial cells including oxidative stress (Backert et al. 2015). In gastric epithelial cells, *H. pylori* CagA induces the expression of spermine oxidase (SMOX), an enzyme for the back-conversion of spermine to spermidine (Xu et al. 2004; Chaturvedi et al. 2011). This reaction leads to the production of H_2O_2 as a by-product. Increased H_2O_2, however, causes ROS accumulation via mitochondrial membrane depolarization and the activation of caspase-mediated apoptosis (Chaturvedi et al. 2004). In fact, gastric epithelial cells in individuals infected with CagA-positive *H. pylori* express high SMOX levels (Chaturvedi et al. 2011).

2.2 *DNA Damage Induced by ROS/RNS in* H. pylori*-Infected Gastric Mucosa*

ROS and RNS induce various types of DNA damage, including point mutations, DNA adducts and single- or double-strand DNA breaks (DSBs). Among these, 8-hydroxydeoxyguanosine (8-OHdG), which is the main oxidatively modified product of DNA, is significantly expressed in gastric cancer tissues as well as in adjacent tissues in humans (Lee et al. 1998). Also, NO produced in *H. pylori*-infected gastric mucosa is highly reactive and rapidly reacts with superoxide (O_2^-) to produce highly toxic peroxynitrite ($ONOO^-$), inducing nitrative and oxidative DNA damage, such as the formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine and 8-nitroguanine, which are used as biomarkers of oxidative or nitrative DNA damage (Handa et al. 2011; Borrego et al. 2013). These damaged guanines preferentially lead to G > T transversion mutations during the genome replication process, although repair systems are closely involved in this formation as described later (Bruner et al. 2000). ROS and RNS are considered to induce single- or double-strand DNA breaks. Indeed, *H. pylori* infection leads to significantly increased levels of phosphorylated histone H2A variant X (H2AX), a marker of DSBs in gastric epithelial cells (Toller et al. 2011). In addition to ROS or RNS, the involvement of some repair systems is needed for the formation of DSBs as well as resultant chromosomal aberrations.

Oxidative stress also induces apoptosis or autophagy in gastric epithelial cells (Cover and Blanke 2005; Tsugawa et al. 2012). Therefore, oxidative stress has a mutagenic role as well as a preventive role in carcinogenesis. Recent studies demonstrated that cancer stem cells possess enhanced mechanisms for protection against oxidative stress (Tsugawa et al. 2012). Expression of a variant 9 form of the

receptor CD44 (CD44v9), a possible cancer stem cell surface marker (Lau et al. 2014) contributes to ROS defense via up-regulation of the synthesis of reduced glutathione (GSH), the primary intracellular antioxidant. CD44v9 interacts with and stabilizes xCT, a subunit of the cysteine-glutamate transporter xc(-), and thereby promotes cysteine uptake for GSH synthesis (Ishimoto et al. 2011). Cancer stem cells able to defend against ROS due to CD44v9 expression are thus thought to drive tumor growth, chemoresistance and metastasis. In *H. pylori*-infected gastric mucosa, SPEM, which is considered a precancerous lesion, expresses high levels of CD44v9 (Wada et al. 2013). These findings suggest that the balance of the accumulation of genetic alterations by ROS and cell survival via protection against ROS is important for the generation of cancer cells in *H. pylori*-infected gastric mucosa.

3 Genome Editing Enzyme: Cytidine Deaminase

3.1 APOBEC Family

Human beings have several kinds of genome editing enzymes. Among them, the apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) family, represents cytidine deaminases that convert cytosine (C) to uracil (U) (Cascalho 2004). Most APOBEC family members act against foreign genomes, such as those of some intruding viruses. For example, APOBEC3G inactivates the viral function of human immunodeficiency virus (HIV) or hepatitis B virus (HBV) via editing their genomes (Harris et al. 2003; Noguchi et al. 2005). In contrast, activation-induced cytidine deaminase (AID), APOBEC3A, and APOBEC3B induce genetic alterations in human DNA sequences. AID is normally expressed in activated B cells and is a key molecule for generating immune diversity via inducing both somatic hypermutation, which occurs in variable regions of the immunoglobulin genes, and class-switch recombination, which occurs in switch regions of the immunoglobulin genes. AID appears to act on single-stranded DNA that is generated during the transcriptional stage (Matsumoto et al. 2015a, b) in the form of transcription bubbles, resulting in the conversion of C to U. The generated U:G mismatches can usually be repaired to C:G by the high-fidelity repair system (Liu and Schatz 2009). If the U:G mismatch is not repaired before the onset of DNA replication and is replicated, it gives rise to C:G to T:A transitions. Alternatively, the removal of the uracil by uracil-DNA glycosylase (UNG) or the recognition by mismatch repair proteins such as MSH2 and MSH6 and error-prone translesion polymerases can induce various types of mutations. Various mutations in variable regions of the immunoglobulin genes result in increased antigen-binding affinity. In addition, nicks in the near sites of both strand sequences of switch regions are generated by the repair process of AID-induced U:G mismatches, resulting in DNA DSB (Stavnezer 2011). Recombination of DSB by non-homologous end joining (NHEJ) contributes to the class-switch recombination. APOBEC3A and 3B also have the capacity to edit the human genome. Although their functions in normal

conditions are unknown, recent reports demonstrated that high expression of APOBEC3A and 3B is linked with the mutation signatures of several cancer types, including breast cancer and lung cancer (Burns et al. 2013a, b). In fact, in vitro experiments showed that these enzymes induce mutations in human genomes (Shinohara et al. 2012; Burns et al. 2013a, b). As with AID, deamination of these enzymes and subsequent repair processes induce various mutations. The target sequences of these enzymes differ: APOBEC3A and 3B favor C residues flanked by 5′-T, and AID exhibits a strong preference for deaminating C residues flanked by a 5′-purine (*G* or *A*) (Schmitz and Petersen-Mahrt 2012; Beale et al. 2004). Although recent detailed analyses of these mutation signatures identified more complicated target motifs (Chan et al. 2015), mutator enzymes as well as various repair processes are deeply related to mutation patterns.

3.2 The Role of AID in Gastric Carcinogenesis

AID protein is aberrantly expressed in a substantial proportion of *H. pylori*-associated human gastric epithelium and gastric cancer tissues, although no AID expression is observed in normal gastric mucosa (Matsumoto et al. 2007). In particular, mononuclear cell infiltration and intestinal metaplasia correlate with AID expression (Nagata et al. 2014). After eradication of *H. pylori*, AID expression is significantly decreased but still higher than that in *H. pylori*-negative gastric mucosa. Intriguingly, infection with *cag*PAI-positive *H. pylori* ectopically induces high expression of AID in human gastric epithelial cell lines, but *cag*PAI-negative *H. pylori* has no effect on AID expression (Matsumoto et al. 2007). Also, inflammatory cytokines such as tumor necrosis factor (TNF)-α increase the expression of endogenous AID protein in gastric epithelial cells via the nuclear factor (NF)-κB pathway. Furthermore, aberrant AID expression in gastric epithelial cells induced by these stimuli causes a number of somatic mutations in tumor-related genes, including the tumor-suppressor gene *TP53*, and knockdown of endogenous AID significantly reduces the number of *TP53* mutations observed in *H. pylori*-infected cells. AID transgenic mice that have constitutive and ubiquitous AID expression develop malignant lymphoma as well as various epithelial tumors, including gastric cancer (Okazaki et al. 2003; Morisawa et al. 2008). These findings suggest that aberrant AID expression in gastric epithelial cells induces mutations via a genome editing function. In addition, AID expression in gastric epithelial cells causes chromosomal aberrations, mainly submicroscopic deletions, at various chromosomal loci (Matsumoto et al. 2010). Among these deleted loci, the recurrently deleted chromosomal regions harbor the tumor-suppressor cyclin-dependent kinase inhibitor genes *CDKN2A/CDKN2B*. In *H. pylori*-infected wild-type mice, the copy numbers of the *Cdkn2b-Cdkn2a* locus in the gastric mucosa are reduced, whereas no such changes are observed in the gastric mucosa of *H. pylori*-infected AID-deficient mice. These findings suggest that AID induces point mutations as well as chromosomal aberrations in gastric epithelial cells.

4 Guardian of DNA Damage: DNA Repair System

4.1 Overview of the DNA Repair System

In general, the high-fidelity DNA repair system in humans has an important role in preventing the generation of genetic abnormalities. The spontaneous mutation rate during DNA replication is very low, typically $<10^{-9}$ per base pair per cell division (Lange et al. 2011). Defects in DNA repair function, however, can induce many mutations that result in cancer initiation (Lange et al. 2011; Eso et al. 2015). In fact, individuals with mutations of the mismatch repair gene *MUTYH* develop familial adenomatous polyposis and multiple cancers in the gastrointestinal tract (Nielsen et al. 2007). Some types of cancer have mutations or methylated silencing of DNA repair genes such as *MLH1* and polymerase ε (*POLE*) (Imai and Yamamoto 2008; Rayner et al. 2016).

The DNA repair system generally involves the removal of damaged or incorrect bases and DNA re-synthesis by DNA polymerases (Lange et al. 2011). Briefly, base excision repair (BER) mediates the removal of a single base residue by a specific DNA glycosylase, the incision of the resultant abasic site by an apurinic/apyrimidinic (AP) endonuclease, and DNA re-synthesis by DNA polymerase β. Nucleotide excision repair (NER) can remove various helix-distorting adducts caused by ultraviolet (UV), cisplatin, and others, followed by the re-synthesis of the resulting 27–29 nucleotide gap by polymerase δ, ε or κ. Mismatch repair (MMR) is an excision repair process that removes mismatched bases. Some mismatch repair proteins such as MSH2 and MSH6 can recognize mismatch regions, and a segment of DNA is excised between the mismatch and a nearby nick, followed by filling of the resultant gap by DNA polymerase δ.

DNA DSBs are cytotoxic lesions that promote carcinogenesis or are lethal if they are left unrepaired or inappropriately repaired. The presence of DSBs is first recognized by the MRE11-RAD50-NBS1 (MRN) complex (Stracker and Petrini 2011), and this complex and activated ataxia telangiectasia mutated kinase (ATM) induce the activation of downstream DNA repair genes and a cell cycle checkpoint such as checkpoint kinase 2 (CHK2) (Shiloh and Ziv 2013; Bartek and Lukas 2003). DSBs are repaired by two major pathways: homologous recombination (HR) and NHEJ (van Gent et al. 2001). HR occurs between two homologous sequences, usually two sister chromosomes, after DNA replication, and the BRCA2-RAD51 complex has a central role in HR (Esashi et al. 2005). On the one hand, HR is relatively error-free, while on the other hand, NHEJ is the simple ligation between two DNA ends independently of the chromosome locus, and is therefore relatively error-prone and sometimes results in chromosomal aberrations. In fact, NHEJ contributes to class-switch recombination in the immunoglobulin gene loci of activated B cells. Therefore, in addition to genotoxic factors, the alteration of some repair functions can contribute to the induction of genetic aberrations or chromosomal aberrations during tumorigenesis.

4.2 *Dysfunction of the DNA Repair System in* H. pylori*-Infected Gastric Epithelial Cells*

H. pylori infection has several effects on alterations of the DNA repair function. *H. pylori* infection in cultured gastric epithelial cells down-regulates the proteins involved in MMR and BER (Machado et al. 2009; Kim et al. 2002). In addition, down-regulation of MMR proteins occurs both in an *H. pylori*-infected mouse model and in human cases (Machado et al. 2009; Park et al. 2005). A combination of reduced expression of these repair genes with increased expression of genotoxic factors could enhance the accumulation of somatic mutations in gastric epithelial cells. A recent paper demonstrated that DSBs are introduced by NER, including endonucleases XPG and XPF, rather than BER (Hartung et al. 2015). Also, DSBs trigger a damage signaling and repair response involving ATM and its downstream target genes such as *53BP1* and *MDC1* (Hanada et al. 2014; Toller et al. 2011), but *H. pylori* infection induces the down-regulation of some components of several DNA repair pathways such as ATR, ATRIP, MRE11, and NBS1, which are involved in DSB repair (Koeppel et al. 2015). Interestingly, *H. pylori*-induced DSBs are repaired via NHEJ rather than HR, possibly due to the up-regulation of NHEJ-related genes and the down-regulation of HR-related genes (Hartung et al. 2015). These findings suggest that various alterations of DNA repair functions are closely linked with the formation of DSBs as well as chromosomal aberrations during the development of *H. pylori*-associated gastric cancers.

5 Molecular Mechanisms of DNA Aberrations

5.1 *Footprint of Carcinogenesis Process Determined by Comprehensive Genome Analyses*

Comprehensive cancer genome analyses reveal not only genetic abnormalities of the cancer genome, but also footprints of its carcinogenesis process. Each cancer type has its own specific dominant mutation signatures. Recent studies revealed that the mutation signature that accumulates in tumor tissues provides a clue to identifying the cause of genetic alterations during tumor development (Alexandrov et al. 2013; Helleday et al. 2014; Matsumoto et al. 2015a, b). Loss of function in DNA repair genes represents specific patterns of genomic alterations. As mentioned above, tumors with MSI in many cancer types have numerous substitutions, and small insertions and deletions due to defects of mismatch repair functions (Imai and Yamamoto 2008; Shah et al. 2010). Tumors with mutations in *POLE* or *POLQ*, which produce DNA polymerases with proofreading functions, have very large numbers of mutations (Heitzer and Tomlinson 2014). In tumors with inactivating mutations of HR-related genes *BRCA1* or *BRCA2*, such as some pancreatic cancers, substantial numbers of larger deletions (up to 50 bp) with overlapping

microhomologies at breakpoint junctions are found (Alexandrov et al. 2013). By contrast, some extrinsic mutagens have specific mutation signatures. UV light, a well-known extrinsic mutagen, induces mainly C:G > T:A transitions in the dipyrimidines. This mutation pattern is predominantly observed in melanoma and basal cell carcinoma, providing evidence that UV light plays a role as a causative factor in the development of these tumors (Krauthammer et al. 2012; Jayaraman et al. 2014). Benzo[a]pyrene, one of the convincingly established carcinogens contained in tobacco, typically causes C:G > A:T transversions. This mutation pattern is dominantly observed in lung cancers, especially those associated with smoking (Pfeifer et al. 2002; Alexandrov et al. 2013). As described above, intrinsic mutagens such as oxidative factors and APOBEC family members also have specific mutation signatures. Oxidative stress generally causes C:G > A:T transversions (Bruner et al. 2000). APOBEC3A, APOBEC3B, and AID are probably related to the development of various cancer types based on their expression levels and mutation signatures (Burns et al. 2013a, b; Schmitz and Petersen-Mahrt 2012). Thus, comprehensive cancer genome analyses can reveal the actual mechanisms of carcinogenesis in human tissues, which cannot be identified in cultured cells or animal models. Several mutation patterns, however, currently have unknown origins and may eventually elucidate novel mechanisms of carcinogenesis (Alexandrov et al. 2013).

5.2 *Exploring the Molecular Mechanisms of Gastric Carcinogenesis According to Mutation Signature (Fig. 2)*

In gastric cancers, the most common mutation pattern is the C:G > T:A transition, more than half of which occurs in XpCpG trinucleotides (Wang et al. 2011; Zang et al. 2012; Nagarajan et al. 2012; Cancer Genome Atlas Research Network 2014; Shimizu et al. 2014). This pattern is the prominent mutation signature in many cancer types and is probably related to the spontaneous deamination of 5-methylcytosine (Alexandrov et al. 2013; Pfeifer 2006). In particular, this mutation signature is prominently observed in gastrointestinal cancers and therefore seems to be linked with inflammation-associated carcinogenesis (Burns et al. 2013a, b). In addition, gastric cancers have numerous C:G > T:A transitions at non-CpG sites that occur preferentially at GpCpX or ApCpX sequences (Nagarajan et al. 2012; Shimizu et al. 2014). This mutation pattern corresponds well with the mutation signature induced by AID activity (Olivier et al. 2014), suggesting the involvement of AID-mediated cytidine deamination in the induction of somatic mutations during gastric carcinogenesis. As mentioned above, DNA repair systems are deeply involved in the formation of mutations after DNA damage by AID activity, suggesting that MSI status could contribute to preserve the mutation signature induced by AID activity. Consistently, C:G > T:A transitions are more prominently

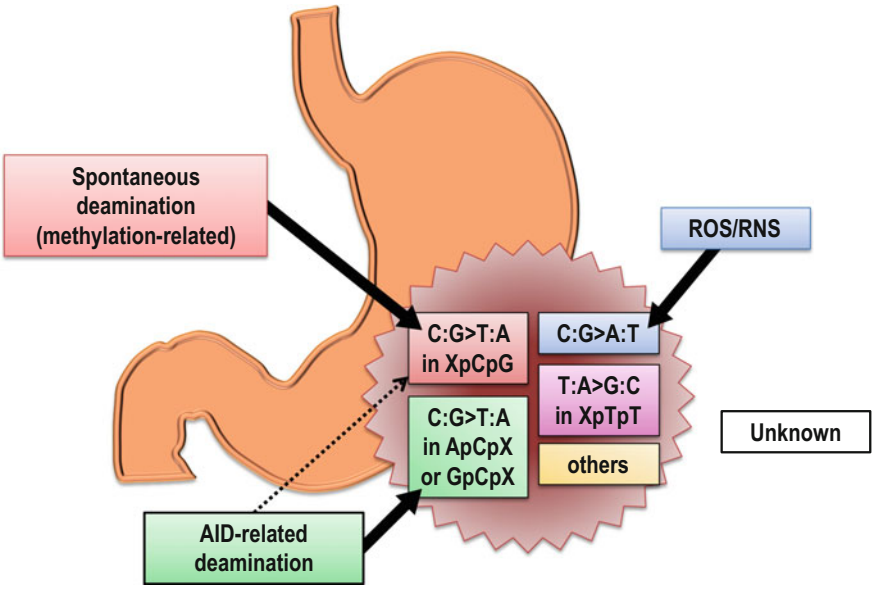

Fig. 2 Gastric carcinogenesis process according to mutation signatures of gastric cancer genome. Mutation signatures of gastric cancer genome represent the footprint of carcinogenesis. C:G > T:A transitions in XpCpG are most common pattern, indicating methylation-related spontaneous deamination. C:G > T:A transitions in ApCpX or GpCpX and C:G > A:T transversions suggest the involvement of AID-related deamination and ROS/RNS, respectively. Some patterns, including T:A > G:C transversions in XpTpT, suggest that unknown mechanisms still remain

observed in MSI gastric cancers and occur preferentially at XpCpG as well as at GpCpX or ApCpX sequences that are target sequences preferred by AID (Cancer Genome Atlas Research Network 2014, Shimizu et al. 2014). Recent reports demonstrated that the AID/APOBEC family also deaminates 5-methylcytosine or 5-hydoxymethylcytosine during the process of DNA demethylation (Bhutani et al. 2010; Popp et al. 2010). The deamination of 5-methylcytosine can induce C:G > T:A transitions in XpCpG sequences if the subsequent repair system does not work. Therefore, overexpression of AID also seems to be related to the emergence of C:G > T:A transitions in XpCpG sequences. More recently, deep sequencing of selected cancer-related genes, such as *TP53* in non-tumorous gastritis mucosa, revealed that C:G > T:A transitions at GpCpX sequences were strongly preferred, like those in gastric cancer tissues (Shimizu et al. 2014). Interestingly, human *TP53* knock-in (Hupki) mice with AID overexpression also had *TP53* mutations that led to C:G > T:A transitions at GpCpX sequences (Shimizu et al. 2014). Taken together, these findings indicate that AID is deeply involved in the emergence of mutations during gastric cancer development.

C:G > A:T transversion is also a mutation pattern frequently seen in gastric cancer genomes. This is the typical pattern induced by oxidative stress (Bruner et al. 2000). Considering the experimental data just discussed, oxidative stress is also an

important factor for inducing DNA damage during gastric carcinogenesis. In addition, T:A > G:C transversions at XpTpT sequences are unique patterns of gastric cancer genomes (Cancer Genome Atlas Research Network 2014). Esophageal adenocarcinoma has the same mutation patterns (Agrawal et al. 2012; Dulak et al. 2013; Nones et al. 2014), but another type of esophageal cancer, squamous cell carcinoma, for which the risk factors are tobacco and alcohol, does not have this mutation pattern (Song et al. 2014; Lin et al. 2014; Gao et al. 2014). Because esophageal adenocarcinoma is caused by duodeno-gastro-esophageal reflux, T:A > G:C transversions at XpTpT sequences may be linked with a currently unknown mediator of mutation induction.

Chromosomal aberrations are very important genetic alterations in gastric cancer, particularly CIN-type gastric cancer. As mentioned above, ROS and AID are possible inducers of DSBs during gastric carcinogenesis. In addition, the DNA repair system is deeply involved in the emergence of DSBs as well as in the repair of DSBs. Interestingly, CIN-type gastric cancers often have *TP53* mutations in addition to various chromosomal aberrations (Cancer Genome Atlas Research Network 2014). Many reports indicate that the functions of TP53 include the regulation of HR, repair genes, cell cycles, and others (Nicolai et al. 2015; Wang et al. 2015). Therefore, loss-of-function mutations of *TP53* could accelerate the emergence of DSBs as well as chromosomal aberrations.

6 Concluding Remarks

To fully understand the process of gastric carcinogenesis, precise molecular mechanisms of malignant transformation from gastric epithelial cells to cancer cells should be elucidated. Thus, we must know “what cells” are the origin of cancer, “what mutations” must occur, and “how” these cells can obtain these mutations. Recent comprehensive genome analyses revealed “what mutations” the gastric cancer genome possesses; however, it remains unknown “what cells” acquire “what mutations” for malignant transformation. Some reports demonstrated that normal stem cells can become cancer-initiating cells, while other reports indicated that differentiated cells may be better candidates for the origin of cancer cells (Beck and Blanpain 2013; Rycaj and Tang 2015; Brungs et al. 2016). The dynamic changes in the gastric glands during long-term *H. pylori* infection complicate the understanding of this process. Another difficulty is the lack of animal models that mimic human gastric carcinogenesis, although *H. pylori*- or *H. felis*-infected animal models are well established for the study of gastritis (Hayakawa et al. 2013). Mutation signatures in gastric cancer tissues can also provide information to help uncover actual mechanisms, showing “how” gastric epithelial cells acquire mutations. Some mutators, such as cytidine deaminase and oxidative stress, have been well investigated as key molecules involved in gastric carcinogenesis, but it is clear that unknown mechanisms still remain. For example, why are C:G > T:A transitions at CpG sites frequently observed? What induces T:A > G:C transversions at

XpTpT sites? How does the DNA repair system influence on the acquisition of mutations? Epigenetic alterations in gastric epithelial cells could also be involved in the induction of mutations. Further investigations will provide new insights toward understanding the whole process of gastric carcinogenesis.

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