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REVIEW

Clinical aspect and molecular mechanism of DNA aneuploidy in gastric cancers

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Abstract The biological characteristics of cancers depend mostly on genetic alterations in the cancer cells of individuals. Gastric cancers show a high frequency of DNA aneuploidy, a phenotype of chromosomal instability. Compared to diploid tumors, gastric carcinomas with aneuploidy have been shown to have high proliferative activity and high metastatic or invasive potential; these characteristics lead to a poor prognosis. It has been suggested that an abnormal spindle assembly checkpoint is involved in DNA aneuploidy, but the underlying mechanism is still unclear. This review, in order to determine whether gastric carcinomas that display aneuploidy are associated with a poorer prognosis than diploid tumors, and to discuss the biological mechanisms that induce aneuploidy, summarizes the results of studies on DNA ploidy in gastric cancer published in the English literature. Analysis of DNA ploidy in gastric cancer may provide clinically useful information from diagnostic, therapeutic, and prognostic standpoints. Further investigations may be needed to clarify the relationship between chromosome instability and DNA ploidy.

Keywords DNA aneuploidy · Flow cytometry · Loss of heterozygosity · Gastric cancer · Prognosis · BubR1 · Mad2

Introduction

Gastric cancer is one of the most common causes of death among patients with malignant diseases around the world. DNA aneuploidy is one of the most frequent genetic aberrations in gastric cancer. However, the molecular mechanisms and roles of DNA aneuploidy are controversial topics. Because fresh biopsy samples or resected specimens can be obtained from gastric cancers, various genetic analyses have been conducted on samples collected worldwide. Gastric cancer is a chronic proliferative disease with multiple genetic and epigenetic alterations [1, 2]. This review summarizes the genetic and chromosomal alterations that have been found in gastric cancer. We focus on aneuploidy in particular, give an outline of the mechanisms involved in the development of aneuploidy, and discuss the significance and future of research on DNA aneuploidy in gastric cancer.

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Genetic alterations in gastric cancer

Multiple genetic and epigenetic alterations in oncogenes, tumor suppressor genes, cell-cycle regulators, cell adhesion molecules, DNA repair genes, genetic instability factors, and telomerase activation are implicated in the multistep process of gastric carcinogenesis. The specific combination of alterations differs in the 2 histological types of gastric cancer, suggesting that intestinal-type and diffuse-type carcinomas have distinct carcinogenetic pathways. Chromosomal instability (CIN); in particular, loss of heterozygosity (LOH), genomic amplifications, and DNA aneuploidy, are frequently observed in intestinal-type gastric carcinoma [3, 4]. Numerical abnormalities in specific chromosomes have been reported for chromosomes 1, 7, 8, 9, 17, 20, X, and Y in gastric tumors [5]. Although the relationship between the numerical abnormality in each chromosome and gastric carcinogenesis has not been elucidated, several reports have demonstrated that aneuploidy is related to cancer progression. Alterations in

chromosome 17 have been shown to be related to tumor progression and malignant potential in primary gastric cancer [6], and numerical abnormalities in chromosomes 3, 10, 11, 12, 17, and Y have also been shown to cause dramatic differences in outcomes [7]. Wu et al. [8], in a comparative genomic hybridization analysis, reported that frequent abnormalities were found in advanced cancers, including gains on the 8q chromosomal arm and losses on the 16q chromosomal arm. Other combinations of gains and losses have also been reported [9]. LOH is the deletion of one allele among paired chromosomes. LOH on chromosome 6 could be associated with an unfavorable prognosis [10]. Simultaneous alteration showing LOH on chromosome 16q and gains on 8q was also reported to result in poor outcomes [11].

Tumor suppressor gene mutations or LOH have been reported in over half of all human cancers, and they appear to occur in the early stages of cancer, indicating the important role that such mutations may play in the carcinogenesis of various organs [12]. LOH of the gene encoding phosphate and tensin homolog (*PTEN*) was observed in 17.1% of patients diagnosed with gastric cancer in our previous analysis [13]. p53 mutations were also found in more than 20% of gastric cancer samples, accompanied by LOH [12]. Genetic instability has long been considered an integral component of human neoplasias. In a small fraction of tumors, mismatch repair (MMR) deficiency leads to microsatellite instability (MSI) at the nucleotide sequence level [14]. In other tumors, an abnormal chromosome number (aneuploidy) has suggested genomic instability, but the nature and magnitude of the postulated instability are still matters of conjecture [15]. MSI is manifested as length variation in microsatellite sequences caused by MMR gene deficiency; MSI is found in around 20% of gastric cancer patients [16–20]. Gastric cancer with high-frequency MSI (MSI-H) represents a well-defined subset of carcinomas showing distinctive clinicopathological features. In colon cancer, tumors with MSI-H are characteristic of hereditary nonpolyposis colorectal cancer syndrome, which, in the majority of cases, is associated with an early age of onset and is caused by germline mutations in one of the MMR genes [21–24]. In contrast, the MSI-H phenotype in gastric cancer is predominantly caused by epigenetic hypermethylation of the h*MLH1 MMR* gene rather than being caused by germline mutations in one of the MMR genes [14, 25–27]. Aneuploidy is known to be associated with non-MSI tumors in both colon cancer and gastric cancer [28–31].

What is DNA aneuploidy?

DNA aneuploidy is a state in which cells have an abnormal number of chromosomes. Usually, CIN has been divided into numerical CIN and structural CIN forms. Numerical CIN includes DNA amplification and an abnormal number of chromosomes, and structural CIN includes DNA translocation and LOH. The term "DNA aneuploidy" means an abnormal number of chromosomes, but recently the term has been used to indicate both forms of CIN in a wide sense (Table 1). More than a century ago, David Paul Hansemann observed that cancer cells generally had an abnormal number of chromosomes [32]. In 1997, Lengauer et al. [15] reported that DNA aneuploidy was seen in 85% of colorectal cancers. This form of CIN is thought to reflect a continuing cellular defect that persists throughout the lifetime of the cancer cell and is independent of MSI, a recessive trait [15]. DNA aneuploidy is an important phenotypic characteristic of cancer cells; however, whether or not DNA aneuploidy may be a cause of carcinogenesis is still controversial. Recent evidence indicates that persistent missegregation of chromosomes results in gains and losses of chromosomes and may be an important cause of aneuploidy. This form of chromosome instability may contribute to tumor development and progression by facilitating LOH and the phenotypic expression of mutated tumor suppressor genes by favoring polysomy of chromosomes that harbor oncogenes [33]. Single nucleotide polymorphism array techniques can reveal all chromosomal alterations. DNA from aneuploid tumors shows alterations in almost all chromosomes, including DNA amplification and LOH (Fig. 1). Kawaguchi et al. [34] have reported that DNA aneuploidy is linked with gain of 8p23 and loss of 22q11 in gastric cancers. Gain or loss of specific chromosomal regions might have enough of an impact to generate the aneuploid phenotype. DNA aneuploidy is less frequent in early gastric cancer. Therefore, previous studies of early gastric cancers suggested that in pure diploid superficial carcinomas, genetic instability might lead to a cell clone that has undergone a ploidy shift, becoming more aggressive [35–37]. However, DNA ploidy in advanced gastric carcinoma is less heterogeneous than that in early gastric cancer. These observations suggest that gastric cancer tumor progression leads to the development of a dominant and more aggressive aneuploid cell clone [37]. Sasaki et al.

Table 1 Chromosomal instability

Numerical chromosomal instability
DNA amplification
Abnormal number of chromosome (loss)
Abnormal number of chromosome (gain); DNA aneuploidy
Structural chromosomal instability
Partial duplication
Partial deletion; Loss of heterozygosity
Inversion
DNA translocation

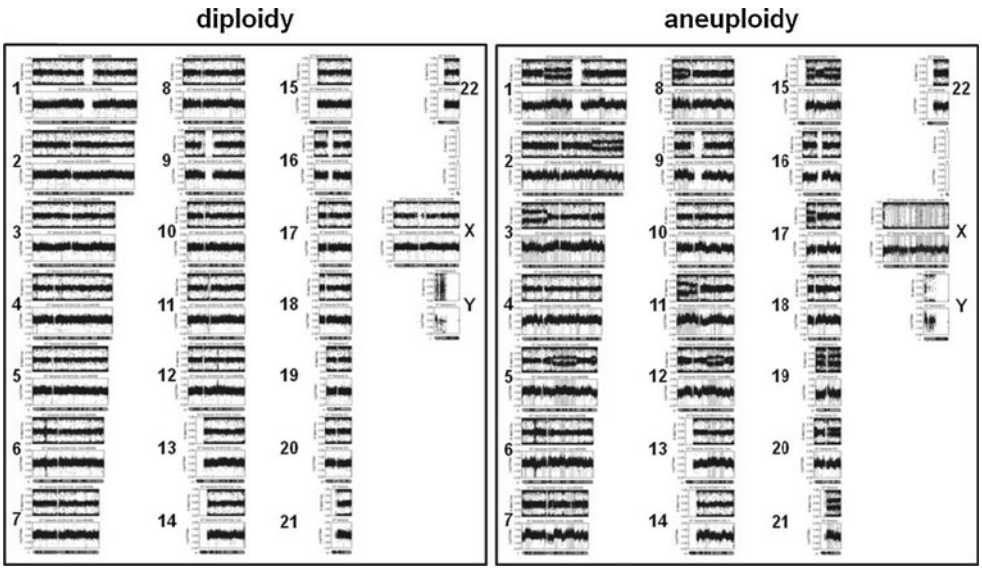

Fig. 1 Typical results of single nucleotide polymorphism arrays in gastric cancer. The *left panel* shows the result of single nucleotide polymorphism (SNP) arrays of DNA from diploid gastric cancers. The *right panel* shows the result of SNP arrays of DNA from aneuploid gastric cancers. There are numerous chromosomal changes in the *left panel*

[38] have shown that there is heterogeneity even in intramural gastric cancer. In addition, these results support the notion that aneuploid cells are generated from tumor stem cells, which selectively expand as aggressive tumors [39, 40].

Gastric cancer and DNA aneuploidy

DNA aneuploidy in gastric cancer has been reported since the 1980s [41]. Well-differentiated and moderately differentiated carcinomas display aneuploid patterns more frequently than poorly differentiated tumors [4, 42–45], though some reports have not agreed with that conclusion [36, 46].

Adenocarcinoma of the proximal portion of the stomach [gastroesophageal (GE) junction and cardia] is increasing in incidence. DNA aneuploidy is more common in GE-cardia tumors than in body-antrum tumors [47], and tumors displaying DNA aneuploidy have a greater proliferative activity, correlating with the Ki-67 index [44, 48]. Gastric adenoma, chronic gastritis, and intestinal metaplasia have also been investigated using flow cytometry [49], and these conditions show frequent chromosomal alterations [3], while none of the normal mucosae show aneuploidy [50]. DNA ploidy has also been reported in patients with primary gastric lymphoma. However, the impact of DNA ploidy on survival is still controversial [51, 52].

Is DNA aneuploidy a prognostic factor for gastric cancer?

Aneuploidy, as detected by flow cytometry, has been demonstrated as a useful prognostic marker during the progression of gastric carcinogenesis, due to the high proliferative activity associated with these tumors, leading to increased metastatic potential, poor prognosis, and shorter survival rates than rates in patients with diploid tumors [48, 53]. To date, numerous reports have been published that demonstrate the importance of DNA aneuploidy in gastric cancer (Table 2). Almost all of these reports have shown that DNA aneuploidy is associated with the prognosis of gastric cancer. However, the clinical impact of aneuploidy is debatable [54]. DNA aneuploidy is significantly correlated with lymph node metastasis, but not with tumor penetration [48, 55–57]. Although their case volume was low, Nesi et al. [58], in a prospective study, showed that aneuploidy was a prognostic factor in gastric cancer. Furthermore, even in multivariate analysis, DNA ploidy has been shown to be a prognostic factor [51, 59, 60]. However, some reports have demonstrated that DNA aneuploidy might be associated with a significantly shorter survival only in patients with intestinal-type tumors [61] or only in patients with diffuse-type cancer [62]. Taken together, this evidence strongly supports the hypothesis that aneuploidy is associated with the prognosis of gastric cancer.

Molecular mechanisms of DNA aneuploidy in gastric cancer

Defects in two distinct processes are considered to be the main causes of aneuploidy; namely, a failure in the centrosome-duplication cycle leading to multiple centrosomes, and a dysregulation of the cell division control machinery resulting in lagging chromosomes, mainly elicited by a weakened or an over-activated mitotic checkpoint also known as the spindle assembly checkpoint [63]. Figure 2

Table 2 DNA aneuploidy and patient prognosis

Year	Method	Number of cases	Rate of aneuploidy (%)	Relationship with prognosis	References
1988	Microspectophotometry	254	24	Poor prognosis	[91, 92]
1989	Flow cytometry	70	61	Poor prognosis	[93]
1990	Flow cytometry	117	30.8	Poor prognosis	[55]
1990	Flow cytometry	493	53	Poor prognosis	[94]
1991	Flow cytometry	125	34	Poor prognosis	[95]
1991	Microspectophotometry	66	96	Poor prognosis	[96]
1993	Flow cytometry	74	43	Poor prognosis	[97]
1993	Flow cytometry	84[a]	39	ND	[35]
1994	Flow cytometry	104	36.5	Not prognostic	[57]
1995	Flow cytometry	63	44	Poor prognosis	[98]
1995	Flow cytometry	97	–	Poor prognosis	[99]
1995	Flow cytometry	270	62.9	Poor prognosis	[89]
1996	Flow cytometry	216	–	Poor prognosis	[56]
1996	Flow cytometry	52	42	Poor prognosis	[100]
1996	Flow cytometry	127	84.3	High rate in advanced cancer	[101]
1997	Flow cytometry	161	43.5	Poor prognosis	[102]
1997	Flow cytometry	130	–	Poor prognosis	[60]
1997	Flow cytometry	289	36	Poor prognosis only in diffuse-type cancer	[62]
1998	Flow cytometry	66	41	Poor prognosis	[103]
1998	Flow cytometry	76	62	Poor prognosis	[59]
2000	Laser scanning cytometry	183	56	High rate in advanced cancer	[104]
2001	Flow cytometry	270	35.9	Not prognostic	[105]

ND not determined

[a] Early gastric carcinomas (T1) were selected

shows the spindle checkpoint kinases and chromosomal separation. This checkpoint is the mechanism that delays the separation of sister chromatids until all the chromosome kinetochores are correctly attached to the spindle.

BUB1 is a human homolog of the yeast mitotic checkpoint gene that plays an important role in chromosome segregation. Mutations in *BUB1* have not been found in gastric cancer [64], but the protein encoded by *BUB1*, BUBR1, is overexpressed in gastric cancer [65–68]. We studied the expression of BUBR1 by immunohistochemistry in 181 gastric cancer samples. Ninety-one (50.3%) cases had high expression of BUBR1, and those cases were significantly correlated with the presence of DNA aneuploidy ($P < 0.05$). Also, high expression of BUBR1 was significantly correlated with deep invasion, lymph node metastasis, liver metastasis, and poor prognosis [65]. Transfection of gastric cancer cell lines with full-length BUBR1 resulted in changes to the ploidy pattern. BUBR1 forms a complex with Bub3, mitotic arrest deficient 2 (Mad2), and cell division cycle 20 (CDC20) at the spindle assembly checkpoint, thus inhibiting CDC20 activity [69]. In gastric cancer cells with high BUBR1 expression, the formation of this complex might be compromised, and the spindle assembly checkpoint may be overridden, resulting in DNA aneuploidy. In contrast, the overexpression of *MAD2* in gastric cancer is not associated with aneuploidy or with any of the disease's clinicopathological characteristics [70]. However, mutations in the *MAD2* gene were reported in gastric cancer, and overexpression of mutant *Mad2* in HeLa cells led to the appearance of aneuploid cells [66]. We investigated the relationship between *Mad2* expression and aneuploidy. Aneuploidy in gastric cancer was significantly correlated with high expression of Mad2 protein (unpublished data), and *Mad2* overexpression was found in gastric tumors harboring p53 mutations, indicating that p53 mutations may cause the upregulation of *Mad2* and result in the generation of aneuploid cells within the tumor. The tumor-amplified kinase BTAK was cloned from breast cancer cells and mapped on chromosome 20q13 as a target gene for this amplification in human breast cancers. Transfection of BTAK in near-diploid gastric cancers induced the formation of another aneuploid cell population as well [71].

Helicobacter pylori infection

H. pylori infections have been reported to be associated with changes in DNA content and cellular proliferative

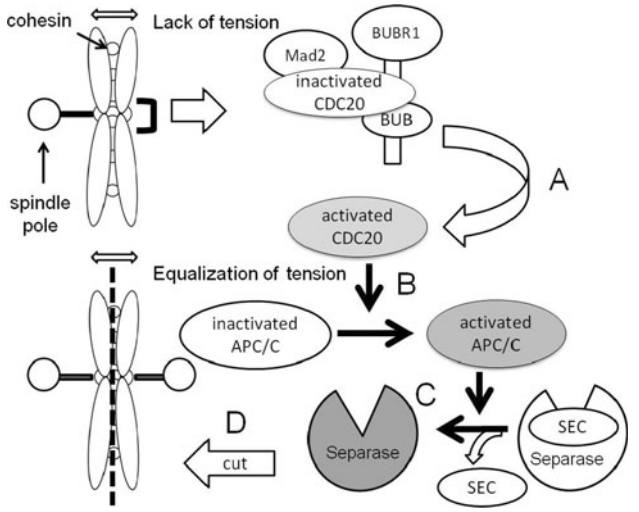

Fig. 2 Spindle checkpoint kinases and chromosomal separation. **a** In the mitotic stage, the spindle extends from both spindle poles to the kinetochore and adds tension to sister chromatids toward the 2 poles. If the tension applied to the sister chromatids is lacking in prometaphase, the mitotic checkpoint complex (MCC), formed by mitotic arrest deficient 2 (*Mad2*)-cell division cycle 20 (CDC20)-BUB-BUBR1, inactivates CDC20 and obstructs the separation of the chromosomes. **b** When there is equal tension, the MCC is removed, and CDC is activated. **c** Activated CDC20 stimulates the anaphase-promoting complex/cyclosome (*APC/C*), and activated APC/C poly-ubiquitylates and degrades securin, which inactivates separase. **d** Finally, separase cuts cohesin, which connects sister chromatids, and the chromosome is separated

activity [72, 73]. Chronic *H. pylori* infections were shown to be responsible for genomic instability in a subset of cases of *H. pylori*-positive chronic atrophic gastritis, and eradication of *H. pylori* infections might reverse genomic instability [74, 75]. Aneuploidy levels increased incrementally across the histological series from patients with gastritis to those with *H. pylori*-positive gastritis and those with atrophy/intestinal metaplasia (IM) [76, 77]. Methylation at E-cadherin was detected in patients with *H. pylori* infections, and *H. pylori* eradication therapy could reverse methylation in patients with chronic gastritis [78]. In diffuse gastric carcinoma, despite common E-cadherin gene (*CDH1*) mutations, *CDH1* LOH was absent from most tumors [79–81]. *CDH1* promoter methylation was found to be the second hit in more than half of the sporadic diffuse gastric carcinoma cases harboring *CDH1* mutations [79]. E-cadherin methylation is an early event in gastric carcinogenesis and can be initiated by *H. pylori* infection. *H. pylori* eradication therapy could reverse the methylation [78, 82]. The relationship between p16 methylation and *H. pylori* infections in precancerous gastric lesions was also investigated in a population-based study in China. The study showed that p16 methylation was significantly associated with *H. pylori* infections in precancerous gastric lesions [83].

Relationship between p53 mutations and DNA aneuploidy

Tumor-suppressor proteins such as p53, APC, and RB have been described to combine key regulatory functions of signaling pathways with protection from CIN [63]. Aneuploidy and inactivation of p53 frequently coincide in human cancers, but increasing evidence has shown that loss of p53 by itself is not the primary cause of aneuploidy [84]. The relationship between DNA ploidy and p53 mutations is still controversial [85]. 17p (p53) LOH and increased 4N or aneuploidy are closely associated with the early stages of gastric carcinogenesis [86]. A significant association was also found between increased 4N or aneuploidy and 17p (p53) LOH in all precancerous gastric lesions. However, no association between *H. pylori* infection and 17p (p53) LOH or increased 4N/aneuploidy in precancerous gastric lesions was reported. Recent analysis showed that LOH without copy number changes at the p53 locus was observed in p53 mutant esophageal squamous cell carcinomas. This copy-neutral LOH might be the major mechanism for inactivation of the intact allele in esophageal squamous cell carcinogenesis associated with p53 mutations [87]. Our data suggest that copy-neutral LOH, occurring because of CIN, might be the major mechanism for inactivation of the intact allele in esophageal squamous cell carcinogenesis associated with p53 mutations. Crypt isolation has enabled the separation of tumor tissues from stromal tissues, and thus, the DNA content in tumor cells can be accurately assessed [88, 89]. Furthermore, S-phase fractions were found to be more useful indicators than DNA aneuploidy if the crypt isolation method was used [88]. Another report clearly showed that diploid tumors generally did not display LOH or MSI, whereas, using the crypt isolation technique, it was found that aneuploid and multiploid tumors were associated with LOH and MSI [28].

Future perspectives

DNA aneuploidy is associated with the carcinogenesis and prognosis of gastric cancer. Therefore, there has been considerable interest in targeting cell-cycle checkpoints, particularly in emerging and alternative anticancer strategies [90]. Several molecules that inhibit cell-cycle kinases have been developed and clinically screened as potential anticancer agents, but none of these agents has been approved for commercial use [90]. The development of selection markers that lead to the choice of appropriate therapies for patients will be the primary focus of future research. Such development may lead to new treatments for gastric cancer in the future.

Conflict of interest None of the authors has anything to disclose.

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