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Chromosomal Instability in Gastric Cancer Biology

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Abstract

Gastric cancer (GC) is the fifth most common cancer in the world and accounts for 7% of the total cancer incidence. The prognosis of GC is dismal in Western countries due to late diagnosis: approximately 70% of the patients die within 5 years following initial diagnosis. Recently, integrative genomic analyses led to the proposal of a molecular classification of GC into four subtypes, i.e.,microsatellite-instable, Epstein-Barr virus–positive, chromosomal-instable (CIN), and genomically stable GCs. Molecular classification of GC advances our knowledge of the biology of GC and may have implications for diagnostics and patient treatment. Diagnosis of microsatellite-instable GC and Epstein-Barr virus–positive GC is more or less straightforward. Microsatellite instability can be tested by immunohistochemistry (MLH1, PMS2, MSH2, and MSH6) and/or molecular-biological analysis. Epstein-Barr virus–positive GC can be tested by *in situ* hybridization (Epstein-Barr virus encoded small RNA). However, with regard to CIN, testing may be more complicated and may require a more in-depth knowledge of the underlying mechanism leading to CIN. In addition, CIN GC may not constitute a distinct subgroup but may rather be a compilation of a more heterogeneous group of tumors. In this review, we aim to clarify the definition of CIN and to point out the molecular mechanisms leading to this molecular phenotype and the challenges faced in characterizing this type of cancer.

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Introduction

Worldwide, gastric cancer (GC) is the fifth most common cancer and accounts for 7% of the total cancer incidence. The prognosis of GC is dismal in Western countries due to late diagnosis: approximately 70% of the patients die within 5 years following initial diagnosis. GC may occur sporadically, as a familial disease, or as a hereditary disease. The vast majority of GCs occurs sporadically, and only 5% to10% of the cases are truly hereditary GCs caused by germline mutations, such as in *APC Promoter 1B, CDH1*, or *CTNNA1*. A model for the carcinogenesis of sporadic GC was described by Correa [1]: the colonization of the stomach mucosa by *Helicobacter pylori*, a diet rich in salt, and medication lead to chronic atrophic gastritis, intestinal and pseudopyloric metaplasia, dysplasia, and finally the occurrence of GC. The genomic alterations found in GC enclose a wide range of genetic changes including, e.g., point mutations (for instance, base substitutions, base deletions, or nucleotide insertions), changes on the chromosome level [such as chromosome fusions, chromosomal translocations, chromosomal segment duplication, segment insertions, segment deletions, and chromosomal number alterations (aneuploidy)], and gene amplifications [2]. They affect a diverse number of proto-oncogenes and tumor suppressor genes, and GC belongs to the group of cancers with a high frequency of somatic mutations as well as a substantial interindividual variability of mutational load [3]. Recently, an integrative genomic analysis [2] led to the proposal of a molecular classification of GC into four subtypes, i.e., microsatellite-instable (MSI), Epstein–Barr virus (EBV)–positive, chromosomal-instable (CIN), and genomically stable GCs [2,4].

A molecular classification of GC is urgently needed. It advances our knowledge of the biology of GC and may spur translational research aiming to improve diagnostics and treatment of GC toward precision medicine [5]. A sound categorization of GC based on molecular subtypes has implications for validation studies as well as

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clinical trials. Diagnosis of MSI-GC and EBV+ GC is more or less straightforward. MSI can be tested by immunohistochemistry (IHC) using antibodies directed against DNA-mismatch repair proteins (MLH1, PMS2 MSH2, MSH6) and/or molecular biological analysis of MSI using mononucleotide markers. EBV+ GC can be tested by Epstein-Barr virus encoded small RNA (EBER) *in situ* hybridization. However, with regard to chromosomal instability (CIN), testing may be more complicated and may require a more in-depth knowledge of the underlying mechanism leading to CIN. It also raises the question of whether CIN really defines a distinct subgroup or is a compilation of a more heterogeneous group of tumors.

Definition of Chromosomal Instability

Malignant tumors may be characterized by high levels of abnormal genomic alterations referred to as genomic instability [6]. However, genomic alterations are not equal to genomic instability. A tumor cell is classified as genomically instable when the number of genomic alterations accumulates rapidly in a short period of time, i.e., there is a high rate of accumulating mutations [7]. Genomic instability can be categorized into microsatellite instability (MSI) and chromosomal instability (CIN) [8]. Both instabilities indicate a mutator phenotype in cancer [9].

Mutations occurring at a high rate in microsatellite regions of the DNA sequence are the hallmark of MSI, which is caused by genetic and/or epigenetic alterations of genes coding for DNA mismatch repair proteins, such as *MSH2, MSH6, PMS2*, and *MLH1* [10–12]. If such genomic alterations occur on the chromosomal level, they are referred to as CIN.

Although CIN is a major characteristic in many types of cancers, it remains a dubious phenomenon with an inaccurate definition: some groups refer to it as aneuploidy or polyploidy, whereas others define CIN as multiple structural rearrangements or frequent changes in chromosome numbers [6,7,13,14]. According to Geigel et al., CIN refers to the rate at which whole or large segments of chromosomes are either gained or lost [8]. CIN is not equal to aneuploidy, but it can lead to aneuploidy. A tumor cell can be aneuploid but still have a stable karyotype [8,15]. Accelerated loss of heterozygosity in tumor suppressor genes or accelerated gain of oncogene copies due to chromosomal duplication is a result of CIN that leads to cancer [16]. Cancers with CIN reveal a very heterogeneous structure (karyotypically, molecularly, and histologically) in different parts of the tumor [17].

Although many studies have been carried out on CIN in cancers, the definite cause of its incidence still remains controversial. Several theories have been postulated with regard to causes of CIN. One theory states that CIN simply results from defects in oncogenes and tumor suppressor genes. Oncogenes like *RAS* can cause an increase in genomic aberrations [18,19], and tumor suppressor genes like *TP53* (p53) can make the CIN phenotype worse [20]. However, tumors with a stable karyotype may have mutations in the same genes making this theory unattractive. Another theory postulates that aneuploidy occurs when, by chance, an abnormal chromosome is present within cells that can cause abnormal cell division and instability of the cellular segregation machinery, therefore leading to karyotypically abnormal daughter cells. Preceding genetic alterations in, e.g., oncogenes or tumor suppressor genes, are not required to produce CIN in this scenario [21,22]. Another theory proposes that CIN cancers arise from early mutational events in a gene or genes responsible for the CIN phenotype, a mechanism similar to MSI [13]. To reveal the definite cause of CIN in cancers, the mechanisms and altered pathways causing CIN necessitate further studies.

CIN Mechanisms

The CIN phenotype can be induced by dysfunctions of different cellular processes, which can be categorized into 1) inaccurate chromosome segregation during mitosis, 2) cell cycle checkpoint defects, 3) oncogene induced mitotic stress, and 4) replication stress.

Inaccurate Chromosome Segregation During Mitosis

Sister chromatid segregation in mitosis is a regulated process, and many events can lead to faulty chromosome separation if not precisely controlled, i.e. mitotic checkpoint defects, kinetochore malfunctions, merotellic attachments, faulty sister chromatid cohesion and separation, centrosome amplification, and telomere dysfunction.

Mitotic Checkpoint Defects. Mitotic checkpoint, also known as the spindle assembly checkpoint (SAC), has many roles in the regulation of the mitosis [23]. Mitotic checkpoint dysfunction, which is due to mutations of the genes involved, can lead to the CIN phenotype [13]. SAC controls proper attachment of chromosomes at the centromeric regions (kinetochores) to microtubules of the mitotic spindles [23]. If the chromosomes are not properly attached to the microtubules, SAC is activated and delays the progress of mitosis [23]. SAC regulates this by a cascade of events even if one kinetochore of a chromosome is not attached [24]. SAC is able to inhibit CDC20 and thereby anaphase-promoting complex/cyclosome (APC/C). APC/C is a large complex of proteins with ubiquitin ligase activity. It triggers the transition from metaphase to anaphase by ubiquitylating cyclins (e.g., cyclin B1) and securin (Figure 1).

SAC components involved in APC/C inhibition are BUB1, BUB3, BUBR1, MAD1, MAD2, CMT2/p31, MPS1, CENP-E, ZW10, ZWILCH, and ROD [23,25]. Other components also known to be involved in SAC are Aurora B, MAPK, NEK2, PLK1, dynein, dynactin, CLIP170, and LIS1 [25]. If any of the SAC components is deregulated, the inhibition of APC/C will not take place, and cells containing unattached kinetochores will proceed with mitosis, leading to mis-segregation of chromosomes [23]. However, SAC only controls the interaction among the kinetochores and the spindle microtubules, and therefore, kinetochores are also an important determinant of chromosome segregation [25].

Kinetochore Malfunctions. Kinetochores are protein structures located at the centromeric regions of chromosomes. They form an interface between the chromosome and microtubules [26]. The kinetochore structure consists of three sections: the inner section (interacts with chromatin), the outer section (interacts with 15 to 20 of the spindle microtubules), and the central section [27]. The kinetochore functions involve regulation of proper attachment of microtubules to chromosomes, assistance of chromosome movement on spindles, and activation of a signaling pathway to stop cell cycle progression when defects are detected [26]. Determination of the place of kinetochore assembly and the kinetochore assembly itself are important for error-free chromosome segregation [27]. During mitosis, the kinetochore assembles on the surface of the peripheral centromeric regions [24]. The centromeric regions consist of tandemly repeated sequences called α-satellite regions and a CENP-B box, which is bound by the CENP-B protein. The centromere contains CENP-A protein, which is a histone variant (instead of H3 in nucleosomes) and some other additional proteins [24,28]. The CENP-A levels are important in determination of the