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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

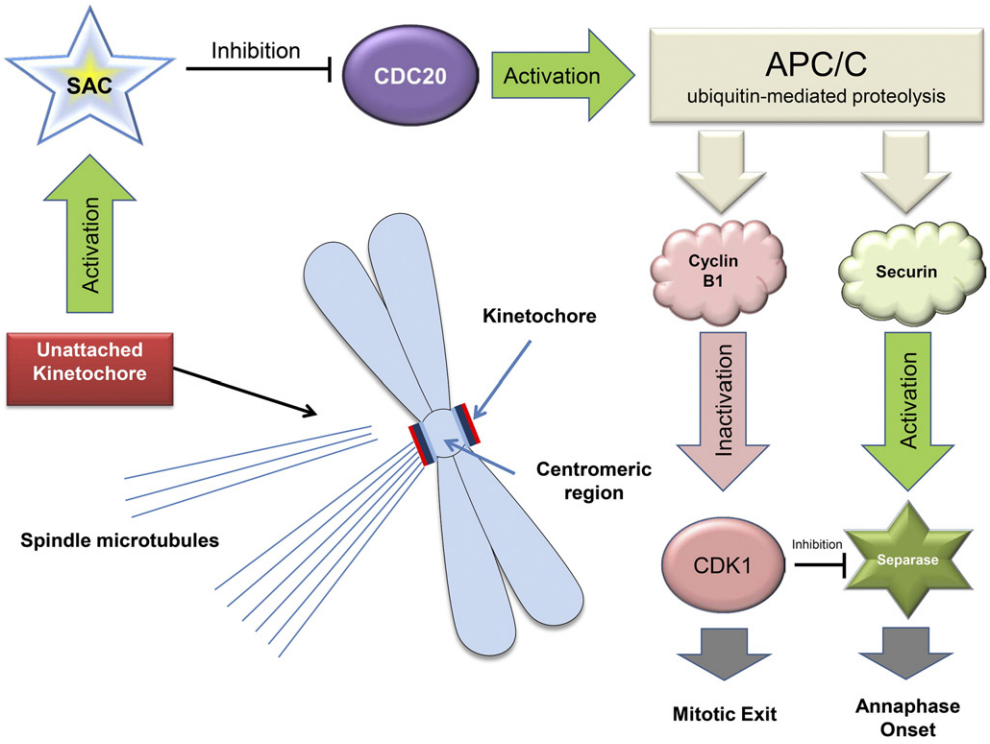

Figure 1. The mitotic checkpoint or SAC. SAC factors are activated when a signal is detected from unattached kinetochores to spindle microtubules. Active SAC inhibits CDC20. CDC20 is an essential regulator of cell division, which binds to and activates the APC/C. APC/C is a large complex of proteins with ubiquitin ligase activity. It triggers the transition from metaphase to anaphase by ubiquitylating cyclins (e.g., cyclin B1) and securin. Securin forms a complex with separase and thereby blocks separase activity. APC/C-mediated proteasomal degradation of securin liberates and activates separase. A second regulatory step of separase activity is its phosphorylation by CDK1. APC/C-mediated proteasomal degradation of cyclin B1 inactivates CDK1. Activated separase cleaves cohesin, resulting in the separation of sister chromatids and triggering the anaphase. Thus, inhibition of CDC20 and APC/C by SAC leads to a temporary pause of mitosis and continuation of the attachment of the sister chromatids (due to inactive separase enzyme) until all kinetochores are joined to the mitotic spindle [24].

centromeric region, where the kinetochore is formed. If there is an overexpression of the CENP-A, it can be incorporated in noncentromeric loci, and the kinetochore will be formed at an inappropriate chromosomal site. If the kinetochores form in other locations rather than on the centromeres or form at multiple regions on the chromosome, the attachment to spindles is inaccurate. This leads to a fragmentation of the chromosome due to spindle forces. If a chromosome does not have a site for kinetochore formation, it will not attach to the spindle and will not be segregated during mitosis [27].

Some evolutionarily conserved proteins, i.e., MIS12, HEC1, and MCM21, are recruited to the outer plate of the kinetochore just before mitosis and form sites for microtubule attachment [24,27,28]. CENP-C/H/I/K/F, Ndc80, Zwint, and KNL1 (SPC105) are known to be other proteins important in kinetochore assembly and function. Ndc80 associated with MIS12 and KNL1 is essential for microtubule-kinetochore binding [27]. Dynein and kinesin (CENP-E) are important in kinetochore motility [27]. Six protein kinases regulate kinetochore assembly and ensure the formation of bipolar chromosome attachments to the spindle, i.e., Aurora B, Polo-like kinase-1 (PLK1), cyclin-dependent kinase-1 (CDK1), MPS1, BUB1, and BUBR1 (MAD3) [27]. Defects in any of these proteins can lead to aberrant chromatid segregation, aneuploidy, and hence CIN.

Merotellic Attachments. The attachment of the microtubules to the kinetochores is a stochastic process and error prone. Although the errors are usually detected by SAC proteins, merotellic attachments remain undetected by the checkpoint. Merotellic attachments occur when one or both kinetochores have an attachment to both spindle poles. This causes a hindrance in chromosome segregation due to its lagging and leads to aneuploidy in the daughter cells. This is a hallmark of CIN [26]. Aurora B has been indicated in correcting syntelic and merotelic chromosome attachments, and its inactivation can cause chromatid segregating defects (for a review, see [29]).

Faulty Sister Chromatid Cohesion and Separation. During the S phase of the cell cycle, cohesin, an evolutionarily conserved protein complex, forms between the sister chromatids and holds them together. During the transition of the metaphase into the anaphase, the activation of separase leads to a cleavage of cohesin and the separation of the sister chromatids. Separase cleaves Rad21, Mcd1, and SCC1 cohesins and if uncontrolled leads to premature separation of chromatids, lagging chromosomes, and anaphase bridges. Thus, the expression of separase (encoded by *ESPL1*) is important for the fate of chromosomes after mitosis [30]. Interestingly, abnormal sister chromatid cohesion due to somatic mutations can also lead to CIN [31]. *STAG2* encodes a cohesin complex subunit and is mutated in multiple CIN cancers [32].

Centrosome Amplification. Centrosomes are microtubule organizing centers that form bipolar spindles at the beginning of mitosis to ensure the equal distribution of the chromosomes. Each centrosome or microtubule organizing center contains two centrioles surrounded

by the pericentriolar matrix, which is the source of microtubule assembly. During the S phase of the cell cycle, the centrosome is duplicated. Daughter centrosomes split and move to opposite sites of the cell to form the mitotic spindle. After normal mitosis, each daughter cell receives a single centrosome [33].

If centrosome amplification is not regulated, it will lead to multiple centrosomes in the cell and multipolar spindles in mitosis, resulting in chromosome mis-segregation. The cyclin-dependent kinases (CDKs) and the Aurora I1l-like kinases are involved in the regulation of the centrosome duplication process [34,35]. Some studies provided evidence that *TP53* inactivation and STK15/AURKA overexpression lead to centrosome replication in a single cycle due to checkpoint alterations [36–38]. However, centrosome duplication may also occur in the absence of *TP53* mutations [39]. Aurora A and PLK1 are also known to be involved in centrosome duplication [40,41]. Cytokinesis failure, cell fusions, centrosome fragmentation, and cell cycle arrest in the S phase are additional events that lead to multicentrosome cells [33]. Extra centrosomes are known to promote chromosome mis-segregation and result in high merotelic attachments in CIN cancers [42]. More recent investigations indicated a role for Tpx2 in centrosome duplication regulation [43].

Telomere Dysfunction. Telomeres are structures at the endings of chromosomes, which prevent the formation of DNA rings and chromosome fusions. Each DNA replication round shortens the telomeres, and telomerase maintains a functional length of telomeres. In cells with defective telomerase function, chromosomes finally reach a critical shortening of the telomeres. The exposed chromosome ends activate the DNA damage response which connects the chromosome ends together, and causes flaws in chromosome segregation. A series of breakage fusion bridge cycles occurs in proliferating cells, giving rise to CIN (reviewed in [44]).

Cell Cycle Checkpoint Defects

The three cell cycle checkpoints, i.e., G1-S, S-G2, and G2-M, consist of mechanisms that control the cell cycle and detect errors in DNA repair, DNA synthesis, and chromosome segregation. Signals are then sent to the replication/segregation machinery to repair the damage [45]. Cdks drive cell division and regulate the different phases of the cell cycle. They consist of a catalytic subunit (cdk) and a regulatory subunit (cyclin). The Cdk1 also known as cell division cycle 2 (CDC2) is a mitotic kinase controlling the G2-M checkpoint or the "mitotic checkpoint" [46]. Cdk1along with cyclin A or B controls the start of mitosis and the centrosome cycle. When Cdk1 is activated, it phosphorylates more than 70 substrates to trigger centrosome separation and perform many other tasks [46]. Among other kinases, NEK, Aurora kinase, and Polo-like kinase are known regulators of this phase. Other regulators of this checkpoint, i.e., MPS1, BUB1, and BUBR1, have been described previously. Upregulation of CDK1 and its regulators, i.e., cyclin B1/B2, cyclin A, CDC25, CKS1, and CKS2, has been reported as a signature of CIN in malignant tumors [46]. Cdk2, Cdk4, and Cdk6 are kinases active in the interphase (G1-S checkpoint). Cdk4 and Cdk6 are activated by D-type cyclins, whereas Cdk2 is activated by E-type cyclins [46]. The "DNA damage checkpoint," which checks the DNA for replication accuracy, ceases G1/S and G2/M transitions in case of DNA damage by repression of CDK activity. Hyperactive CDKs, caused by mutations in genes active in the DNA damage response pathway, lead to the continuation of the cell cycle and proliferation of the cells containing damaged DNA. These series of events lead to an instability of the genome [46]. The G1-S checkpoint induces G1 arrest via a pathway including several molecules. The pathway starts when DNA damage is sensed by ATM/ATR protein. This protein phosphorylates p53 transcription factor and targets MDM2 protein, too. The CHK1/CHK2 also target p53 and MDM2 proteins. P53/MDM2 modifications lead to p53 accumulation and its higher activity. The transcriptional target of p53 is the $p21^{cip1}$/WAF1, an inhibitor of cyclin-dependent kinases. Activation of this pathway results in cyclin E/CDK2 kinase inhibition and thus G1 arrest [47]. Elimination of the G1-S checkpoint, for instance, by *TP53* mutation, results in gene amplifications, deletions, and high genomic rearrangement frequency [45]. Cyclin E upregulation induces CIN in tumors [46], and CDK4 amplification is also said to result in mitosis deregulation and CIN by duplicating centrosomes [48]. Replication fork integrity during S phase (replication phase) is controlled by the S-G2 checkpoint. In this case, stalled replication activates the checkpoint, leading to inhibition of DNA double-strand breaks [47].

The Oncogene-Induced Mitotic Stress Theory

The mechanisms described above all lead to incorrect chromosome segregation during mitosis. However, CIN necessitates additional major cellular changes. Chromosomal alterations are usually negatively selected, cannot be tolerated by cells because they are deleterious, and would induce apoptosis. This can be overcome by genetic alterations that inactivate apoptosis like *TP53* mutations [49]. Thus, the chromosomal segregation errors have to be accompanied by defects in tumor suppressor genes and the overexpression of oncogenes that simulate proliferation and inactivate apoptosis.

Paradoxically, most cancers with CIN harbor only few or no mutations in chromosome segregating genes [50]. A recent hypothesis, denoted "oncogene induced mitotic stress," proposed that mitotic processes controlling chromosome segregation are affected indirectly by oncogenes and tumor suppressor genes (Figure 2). An example is the *RAS* oncogene. Its overexpression induces centrosome over-duplication, the production of anaphase bridges, and the formation of multipolar spindles [51].

The overexpression of *MET* (hepatocyte growth factor receptor) can induce the rise of multiple centrosomes and deregulation of centrosome duplication, which in turn leads to multipolar spindles and aneuploidy. *MET* is said to impose this affect via the PI3K-AKT pathway. *MET* expression can induce CIN in *TP53*-deficient cells, which is important in understanding its function [52]. Among different *Raf* oncogenes, *BRAF* mutation was shown to induce aberrant spindles, supernumerary centrosomes, chromosome mis-segregation, and aneuploidy in malignant melanoma [53].

RB and *TP53* inactivation in turn may lead to MAD2 (and BubR1) overexpression and hence CIN [54]. Thus, these two genes are other candidates in this regard. However, a number of cancers with mutant *TP53* are chromosomal stable and show MSI [55]. The effect of *TP53* inactivation depends on the type of *TP53* mutation: If it does not affect the function of $p21^{cip1}$, the tumors are stable. The unstable tumors have a defective $p21^{cip1}$ function. $P21^{cip1}$ defective mice overexpress Mad2, and because $p21^{cip1}$ is a CDK inhibitor, it is important in preventing CIN. P53 represses *MAD2* through $p21^{cip1}$-dependent inhibition of CDKs and activation of Rb. Thus, two major tumor suppressors, i.e., *TP53* and *RB*, are important in genomic integrity (reviewed in [54]). Teh et al. indicated that the upregulation of *FOXM1* in epidermal keratinocytes triggers DNA

damage response of *TP53* gene without leading to cell cycle arrest or apoptosis. High FOXM1 expression induced copy number variation, loss of heterozygosity (LOH), and whole chromosomal gain, which are characteristics of genomic instability [56].

PLKs, also known proto-oncogenes, contribute to the maintenance of genomic stability. The overexpression of PLK1 can result in multinucleation and skipping of the G1 arrest checkpoint [57]. PLK4 plays a central role in centrosome duplication and precise reproduction of centromeres [40]. PLK1 also inhibits proapoptotic function of p53 via its phosphorylation [57].

Cyclin D1 is able to induce centrosome amplification and produce chromosomal abnormalities after expression in normal cells. Cyclin D1 is an activator of cdk2 which controls centrosome duplication. $p21^{cip1}$, which is activated by p53, is an inhibitor of cdk2 and can in turn inhibit centrosome duplication [48].

Replication Stress

Recently, Burrell et al. claimed that CIN occurring in colorectal cancer (CRC) is mostly a result of premitotic errors and damaged replication fork progression causing replication stress, and not the reason of mitotic errors. Thus, replication stress leads to chromosomal mis-segregation. While searching for causative gene mutations leading to this event, only *TP53* mutation was detected. Further analyses identified also a loss of 18q, which contained three genes [*PIGN* (MCD4), *MEX3C* (RKHD2), and *ZNF516* (KIAA0222)].These were considered as new CIN suppressors in CRC [58]. It is important to mention that amounts of segregation errors can vary among different cancer types. Thus, observations made in CRC cannot be generalized to other types of cancers, including GC. Multiple observations suggest both premitotic and mitotic events to be important in CIN occurrence [59].

DNA replication deficiency can result in a collapse of the replication forks. Putative causes are the activation of oncogenes (like *CCND1* and *KRAS*) and the inactivation of tumor suppressor genes (like *RB1* and *APC*). These two events accelerate cell proliferation and lead to a hyperreplicative phase with DNA double-strand breakage and fork collapse. Usually, after replication stress, apoptosis is induced via the p53 pathway. In *TP53*-deficient cells, this leads to tumorigenesis. Apart from structural chromosomal abnormalities caused by replication stress, it can also lead to chromosome mis-segregation due to loss of CIN suppressors or an instability of centromeres (reviewed in [49]).

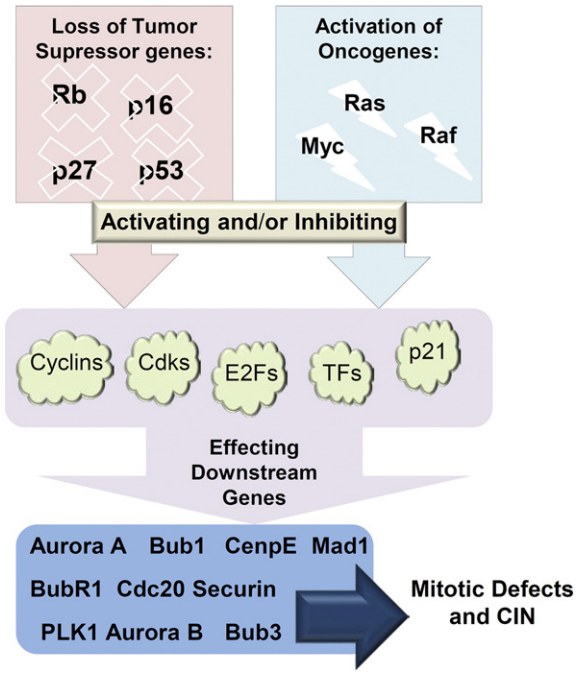

Figure 2. The "oncogene-induced mitotic stress" theory. Mutations on genes involved in accurate chromosome segregation are a rare event; nevertheles, CIN is prevalent in cancers. This theory proposes that the key oncogenes and/or tumor suppressor genes (top of diagram) have an indirect effect on mitosis genes (bottom of diagram), controlling chromosome segregation indirectly. Aberration in these main pathways results in mitotic stress and eventually CIN (simplified from [50]).

CIN Occurrence in Various Cancers

Defects in chromosome segregation are considered as a first step toward CIN, and the assessment of the expression of genes involved in chromosome segregation might give us insights into the biology of CIN cancers.

hBUB1, ATM, ATR, and *BRCA1/2* gene mutations have indicated CIN production in experimental models [13,60,61]. The *APC* (adenomatous polyposis coli) is a tumor suppressor gene coding for a regulator of the Wnt pathway and has many tasks like labeling β-catenin for degradation [62]. Loss of the C terminus of *APC* can induce CIN in CRC, making it a prominent factor involved in chromosomal stability [63]. Dunican et al. reported that *PLK, CCNA2*, and *RanBP2* are overexpressed in CIN-type CRC and claimed them to be a tool for separating CIN-type from MSI-type cancers [64]. Mutations in three gene classes, i.e., *MRE11* (double-strand break repair gene); *hZw10, hZwilch,* and *hRod* (chromosome segregation gene); and *Ding* were found in CIN CRC [65].

Yuan et al. analyzed sequences and mRNA expression profiles as well as protein expression patterns of SAC genes [*MAD1L1, MAD2L1, MAD2L2, BUB1, BUB1B, BUB3, CDC20*, and *TTK (MPS1)*] in breast cancer and found high expression of the genes in CIN type, especially for *BUB1B* gene, which could be a CIN breast cancer marker [66].

Carter et al. used gene expression data to infer chromosomal imbalance or functional aneuploidy in cancer. They assessed more than 10,000 gene expression data sets and found 70 genes to be highly associated with CIN in lymphoma, lung adenocarcinoma, glioma, medulloblastoma, mesothelioma, and breast cancers and called it the "CIN70" signature. Among the CIN70 genes, the genes with the highest CIN score were *TPX2, PRC1, CDC2, FOXM1*, and *KIF20A*. The top 70 genes included *AURKA/B, NEK2, H2AFX, CDC20, Zwint*, and *CCNB1/B2*, which are genes involved in chromosome segregation and cytokinesis [67].

The 12-gene genomic instability signature assessed by Habermann et al. revealed overexpression of *CDKN2A, SCY18* and *STK15 (AURKA), CCNA2, CCNE1*, and *BIRC5* genes in genomically instable breast cancers compared with stable cohorts [68].

Beroukhim et al. analyzed a large data set of cancer specimens for somatic copy number aberrations. It was shown that most genes amplified were oncogenes (*MYC, CND1, ERBB2, CDK4, NKX2-1, MDM2, EGFR, FGFR1, KRAS*), BCL2 family genes (apoptosis regulators), and the NF-kB pathway–related genes [69].

Birkbak et al. applied the CIN70 signature to 2125 breast tumor expression profiles along with 3 ovarian cancers, 2 squamous cell lung

cancers, and a GC cohort. The results indicated the highest CIN70 score with estrogen receptor–negative and basal-like breast cancers. Structural complexity of chromosomes and CIN was also highly correlated with the CIN70 signature [70].

Watanabe et al. divided CRCs into two groups of CIN high and CIN low types based on LOH rates and found a set of differentially expressed genes characterizing the two groups. However, the genes were responsible for cell growth, cell communication, host pathogen interaction, and others, which are not attractive candidates to define CIN [71].

Szász et al. gathered expression profiles for the CIN70 genes in 10 publicly available breast cancer data sets. The highest correlated genes with tumor CIN score (Aurora A, FOXM1, TOP2A, and TPX2) were chosen as CIN4. These four genes indicated to be a good indicator of CIN and ploidy status in breast cancer. Thus, they claimed that the CIN4 expression status may serve as a prognostic marker in breast cancer [72].

Recently, How et al. examined CIN70 in cervical cancer to assess its relevance with clinical outcome prediction, which revealed high correlation [73].

CIN in Gastric Cancer

Many genes until now have been implicated in GC carcinogenesis. These include *MET*, *MYC*, *HST1/INT2*, and *ERBB2* amplification, some of which are associated with a poor prognosis [74]. However, genes involved in CIN type have not been precisely defined, and yet there is a need for identification of a marker or a set of markers to define CIN GC.

AURKA (BTAK) is a gene located on 20q13 and encodes a serine threonine kinase, which is regulated via the cell cycle [75]. *AURKA* plays a role in centrosome integrity and correct cytokinesis [76]. However, its overexpression leads to centrosome duplication and aneuploidy [38]. *AURKA* is amplified in GC, and its overexpression is possibly the reason for aneuploidy. *AURKA* can be overexpressed without gene amplification possibly due to accelerated transcriptional activation [77]. This gene interacts with *GSK-3b* and is important in β-catenin regulation in GC [78]. It is also involved in *TP53* regulation [79].

The *APC* gene regulates β-catenin [78] and chromosome segregation. *APC* mutations or LOH leads to structural chromosomal changes and aneuploidy. An *APC* mutation occurs in approximately 10% of GCs [80].

The The Cancer Genome Atlas network reported that the CIN GCs usually show an intestinal phenotype and are *TP53* mutated (71% of cases). They are enriched in amplifications of genes encoding receptor tyrosine kinases (*EGFR, ERBB2*, *ERBB3, FGFR2*, and *MET*). Other genes were also amplified in CIN GCs, i.e., *MYC, GATA4, GATA6*, and *ZNF217*, *VEGFA*, *KRAS, NRAS*, and cell cycle–related genes *(CCNE1, CCND1, CDK6)*[81].

The expression of *EGFR, AKT*, and *HER2* was assessed in GC, and it was shown that *AKT* overexpression was related to *EGFR* and *HER2* expression. *EGFR/HER2* overexpression was highly correlated with aneuploidy in GC [82].

Table 1 summarizes the most important and frequent factors involved in producing the CIN phenotype in cancers that were mentioned previously.

GC Classification

The World Health Organization published a new classification system for malignant tumors of the gastrointestinal tract in 2010 [83]. GC is now subclassified into tubular, papillary, mucinous, poorly cohesive (including the signet ring cell variant), and mixed type. The carcinoma with lymphoid stroma (medullary carcinoma) and the hepatoid adenocarcinoma were added. However, the histological classification system described by Laurén is still widely used in many clinical trials and GC research publications [84]. Recently, several attempts had been undertaken to also classify GC molecularly. A morphomolecular classification of GC might be more attractive to better understand GC biology and to improve attempts for targeted therapy. It may spur the development of novel prognostic and predictive biomarkers for patient management [85].

Lei et al. [86] were among the first who categorized GC into three subtypes, i.e., the proliferative (with high mutation rates on *TP53*; high genomic instability; and high activity in E2F, MYC, and RAS oncogenic pathways), the metabolic (with low *TP53* mutation rates and high sensitivity to 5-fluorouracil), and the mesenchymal type (with low *CDH1* expression, and most sensitive to phosphatidyl inositol 3 kinase–AKT–mTOR inhibitors and expression of stem cell markers).

In 2014, The Cancer Genome Atlas published an integrative genomic analysis of 295 GCs including array-based somatic copy number analysis, whole-exome sequencing, array-based DNA methylation profiling, messenger RNA sequencing, microRNA sequencing, and reverse-phase protein array analysis. Finally, four

Table 1. List of the Most Common Gene Modifications Related to CIN

Chromosome Segregation Genes and Cell Cycle Genes Involved in CIN Cancers	Tumor Suppressor Genes or Oncogenes Involved in CIN Cancers	Gene Changes in CIN GC
PLK1 overexpression (proto-oncogene)	*TP53* mutation (tumor suppressor)	*AURKA (BTAK)* overexpression (chromosome segregation regulation)
AURKA (BTAK) and *AURKB* overexpression	MET overexpression (proto-oncogene)	*APC* mutation (tumor suppressor)
BUB1/BUB3/BUB1B overexpression	*RB1* mutation (tumor suppressor)	*EGFR/HER2/ERBB2* overexpression (proto-oncogene)
MAD1/MAD2/MAD3 (BUBR1) overexpression	*RAS (KRAS)* overexpression (proto-oncogene)	*RAS* (*KRAS* and *NRAS*) overexpression (proto-oncogene)
ZWINT overexpression	*ERBB2(HER2/neu/EGFR)* overexpression (proto-oncogene)	*MYC* overexpression (proto-oncogene)
hZw10, hZwilch, and *hRod* mutations	*FOXM1* overexpression (proto-oncogene)	*CCNE1, CCND1, CDK6* overexpression (cell cycle–related genes)
NEK2 and *MPS1 (TTK)* overexpression	*MYC* overexpression (proto-oncogene)	*MET* overexpression (proto-oncogene)
CCNA2 (cyclin A2)/CCNB1 (cyclin-B1)/*CCNB2/CCNE1/CCND1* overexpression	*MDM2* overexpression (proto-oncogene)	TP53 mutation (tumor suppressor)
CDK1(*CDC2*)/*CDK4* overexpression	*BIRC5* (survivin) overexpression (proto-oncogene)	*FGFR2* overexpression
CDC20 overexpression		*VEGFA* overexpression

The table includes gene modifications in various cancers and the gene modifications detected in CIN GC up to present.

molecular subtypes were proposed: EBV+ GCs with *CDKN2A* hypermethylation and frequent *PIK3A/PLL1* mutations, MSI-GCs (having hypermethylation and MLH1 silencing), genomic stable GCs (GS; with frequent *CDH1/RHOA* mutations), and CIN GC (frequently harboring *TP53* mutations) [81]. Based on a proposed algorithm, GC can be first recognized by EBV status and subsequent MSI analysis. The remaining cases can be divided into genomically stable or chromosomal-instable tumors [85].

The Asian Cancer Research Group classified GCs based on sequencing and gene expression data into four similar groups, i.e. MSI, MSS/EMT (corresponding to GS), MSS/TP53+ (corresponding to EBV+), and MSS/TP53– (corresponding to CIN). However, they noted the presence of some overlap in different molecular targets among the groups [87].

Recently, Gonzalez et al. classified GCs into four similar subtypes using IHC for p53 and MLH1 detection and fluorescent *in situ* hybridization for EBER detection in tissue sections. They classified GCs into EBER+ (EBV+), MLH1$^-$ (MSI), aberrant p53 (CIN), and the remaining sections into a fourth group (GS). However, they claimed difficulty in the detection of CIN cancers with wild-type *TP53*. This causes a problem in distinction of the CIN and GS types, and somatic copy number aberrations have to be defined molecularly rather than by IHC [62].

Setia et al. aimed at classifying GCs based on protein and mRNA expression of a set of biomarkers [EBER, p53, mismatch repair proteins (MLH1, PMS2, MSH2, MSH6), E-cadherin (CDH1), PD-L1, MUC2, MUC6, MUC5AC, CDX2, CD10, and HER2]. They classified GCs into five groups, i.e., EBER-positive GC, mismatch repair-deficient GC, E-cadherin aberrant GC, p53 aberrant GC, and GC with normal p53 expression. They further classified the p53 aberrant group into four subtypes (intestinal, gastric, mixed, and null) based on expression of MUC2, MUC6, MUC5AC, and CD10. It was suggested that p53 aberrant GCs incline toward higher HER2/neu expression, and the potential targeting molecules can be HER2, EGFR, VEGFR, MET, FGFR2, and cell cycle mediators (CCNE1, CCND1, and CDK6), which were all overexpressed. Oppositely, the p53 normal group had a high MUC6 expression and had been defined as the subtype with expression of normal gastric mucosa genes [63].

Based on all these findings, three groups can be characterized based on defined molecular markers: EBV+ GC, MSI-GC, and genomically stable GCs (*CDH1*-mutated and diffuse-type GC). The CIN GC is still vaguely characterized and ultimately may also be a diagnosis of exclusion. CIN GC more commonly shows an intestinal phenotype, is characterized by amplification of genes coding for receptor tyrosine kinases, and shows cell cycle–related gene overexpression. Thus, utilizing inhibitors targeting molecules involved (ERBB2, EGFR, MET, FGFR2, CDK2, KRAS) can be considered as a treatment option [88], but still less attention has been paid to the mechanism causing CIN.

CIN Characterization in GC

Despite the diversity of pathways leading to CIN, various CIN cancers share similar gene modifications, which lead to chromosomal rearrangements, aneuploidy, LOH, and complete disorder of the genome. However, molecularly and histologically, CIN GC still comprises a very heterogeneous group of tumors. A valid and "easy-to-use" CIN marker is still lacking and may be difficult to find. Several methods were utilized for the determination and quantification of CIN, including fluorescent *in situ* hybridization, LOH analysis, karyotyping, cytometry, single nucleotide polymorphism array, micronuclei counting, and comparative genomic hybridization [8,89]. None of these methods is optimal as a diagnostic tool in a more clinical setting. For CIN diagnosis in a cancer cohort, not only should the assay measure chromosomal imbalances (as a whole or in part), but it should also measure the rate of the chromosomal modifications by a simple, efficient, and rapid means, Thus, diagnostics of CIN GC have not reached the "bedside" [8], and further "benchwork" is needed to provide more insights into this phenomenon and develop reliable diagnostic tools.

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