JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

The role of noncoding RNAs in chromosomal instability in cancer

Swati Mohapatra[1,2], Melanie Winkle[1], Anh N Ton[1,3], Dien Nguyen[4], George A. Calin[1,5*]

1 - Department of Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

2- The University of Texas MD Anderson Cancer Center UT Health Graduate School of Biomedical Sciences (GSBS), Houston, TX, USA

3 - Program in Molecular Genetic Technology, School of Health Professions, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

4 - Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

5 - Center for RNA Interference and Non-Coding RNAs, The University of Texas MD Anderson Cancer Center, Houston, TX, United States

**Correspondence: George A. Calin, M.D., Ph.D., 2130 W Holcombe Blvd, Houston, TX, 77030, gcalin@mdanderson.org*

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

Running title: Noncoding RNAs in chromosomal instability in cancer

Pages: 30

Figures: 1

Tables: 1

Total word count: 9226

Abstract: 200

Introduction: 412

Conclusion: 442

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Chromosomal instability (CIN) is characterized by an increased frequency of changes in chromosome structure or number and is regarded as a hallmark of cancer. CIN plays a prevalent role in tumorigenesis and cancer progression by assisting the cancer cells' phenotypic adaptation to stress, which has been tightly linked to therapy resistance and metastasis. Both, CIN-inducing and CIN-repressing agents are being clinically tested for the treatment of cancer to increase CIN levels to unsustainable levels leading to cell death, or to decrease CIN levels to limit the development of drug resistance, respectively. Noncoding RNAs (ncRNAs) including micro RNAs (miRNAs) and long noncoding RNAs (lncRNAs) have been fundamentally implicated in CIN. The miR-22, miR-26a, miR-28, miR-186 target important checkpoint proteins involved in mediating chromosomal stability and their expression modulation has been directly related to CIN occurrence. LncRNAs derived from telomeric, centrosomal and enhancer regions play an important role in mediating genome stability, while specific lncRNA transcripts including Ginir, GUARDIN, CCAT2, PCAT2, and NORAD have been shown to act within CIN-associated pathways. In this review, we discuss how these ncRNAs either maintain or disrupt the stability of chromosomes and how these mechanisms could be exploited for novel therapeutic approaches targeting CIN in cancer patients.

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

Significance Statement

Chromosomal instability increases tumor heterogeneity and thereby assists the phenotypic adaptation of cancer cells, causing therapy resistance and metastasis. Several microRNAs and long noncoding RNAs that have been causally linked to chromosomal instability could represent novel therapeutic targets. Understanding the role of noncoding RNAs in regulating different genes involved in driving chromosomal instability will give insights into how noncoding RNAs can be utilized towards modifying chemotherapeutic regimens in different cancers.

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

1. Introduction

Noncoding RNAs (ncRNAs) play diverse transcriptional and post-transcriptional regulatory roles across both homeostatic cellular functions and disease states (Cech and Steitz 2014, Slack and Chinnaiyan 2019). It is now known that approximately 70% of the human genome is transcribed across different cell types (Djebali, Davis et al. 2012) and current annotations describe almost 18,000 long noncoding RNA (lncRNA) loci that produce nearly 50,000 transcripts (Frankish, Diekhans et al. 2021). In addition, the latest miRbase v22 release describes 1,917 hairpin precursors producing over 2,500 mature human microRNAs (miRNAs) (Kozomara, Birgaoanu et al. 2019). While lncRNAs have been shown to perform diverse transcriptional as well as post-transcriptional gene regulatory functions (reviewed in (Kopp and Mendell 2018, Mohapatra, Pioppini et al. 2021, Statello, Guo et al. 2021) the function of miRNAs is more defined, specifically interacting with complementary messenger (m)RNAs to downregulate their expression via degradation or inhibition of translation (Lim, Lau et al. 2005). The prevalent involvement of both, lncRNAs and miRNAs in cancer cell biology has been established (Calin, Dumitru et al. 2002) and miRNA-based therapeutics are actively tested in phase I and II clinical trials (Winkle, El-Daly et al. 2021). In this review, we summarize current knowledge on the causative roles of lncRNAs and miRNAs in chromosomal instability (CIN) in cancer. Here, we specifically make a distinction between genomic instability, that is, the acquirement of genomic mutations during the replicative cell cycle, and chromosomal instability.

Chromosomal instability (CIN) is a sub-type of genomic instability characterized by changes in chromosome structure and/or count which arise due to chromosome segregation defects, replication stress, defects in the DNA damage

response or telomere dysfunction (Gollin 2004, Burrell, McClelland et al. 2013, Wilhelm, Said et al. 2020). A further subdivision is made according to the presence of structural (sCIN) or numerical (nCIN) changes (Wilhelm, Said et al. 2020). sCIN are pre-mitotic defects arising during interphase (e.g. due to replication stress, defective DNA damage response or telomere dysfunction) and are represented by partial deletions, translocations, rearrangements, amplifications and other aberrations (e.g. dicentric or ring chromosomes). nCIN arise due to chromosomal segregation defects during mitosis because of impairments in regulatory structural components (e.g. centromeres, kinetochores, microtubule, spindle assembly checkpoint) and result in variations of chromosome number (ploidy). There is further distinction to be made between nCIN and aneuploidy: while nCIN is an ongoing process stemming from defects in chromosome segregation that generally leads to aneuploidy, stable aneuploidy can also exist in the absence of nCIN (Schukken and Foijer 2018).

2. The role of CIN in cancer

The vast majority of human tumors show both, aneuploidy and chromosomal abnormalities indicative of CIN (Schukken and Foijer 2018). Aneuploidy in cancer cells is often associated with worse patient survival as the abnormal karyotypes are generally not random, but specifically result in gains of oncogenes and losses of tumor suppressor genes thus increasing cancer cell fitness (Nicholson and Cimini 2013). The role of CIN in cancer cells is more paradoxical, with detrimental effects on tumor fitness in some models (Funk, Zasadil et al. 2016, Zasadil, Britigan et al. 2016), while leading to tumorigenesis and correlating with drug-resistance and metastasis in other models (Schukken and Foijer 2018). The rate of CIN may play a

role conferring these differences as it has been described that a high degree of CIN can be detrimental to tumor cells and can potentially support chemotherapeutic treatment regimens (Funk, Zasadil et al. 2016). Conversely, persistent low-grade CIN contributes to tumorigenesis by increasing intra-tumoral heterogeneity, and the following clonal selection increases metastatic potential and drug resistance (Schukken and Foijer 2018). In addition, sCIN can produce translocations creating oncogenic fusion genes that may play a pivotal role in early tumorigenesis (Mitelman, Johansson et al. 2007). CIN has furthermore been directly linked to innate immune reactions through the release of double stranded DNA in the cytosol. While this mechanism was linked to increased tumor invasion and metastasis (Bakhoum, Ngo et al. 2018), defects in type I interferon and other immune pathways within tumor cells and/or their microenvironment are thought to lead to immune evasion of such CIN-introduced immunostimulatory effects (Bakhoum and Cantley 2018, Tijhuis, Johnson et al. 2019).

3. MicroRNAs implicated in CIN

Several miRNAs have been functionally implicated in CIN through their regulation of specific target genes (i.e. checkpoint proteins regulating DNA repair and mitosis) that have a direct link to CIN (**Figure 1**). Other miRNAs targeting important structural components such as the cohesin complex (involved in sister chromatid cohesion and the DNA damage response) (reviewed in (Kuru-Schors, Haemmerle et al. 2021, Yamada, Morooka et al. 2022), could also promote CIN although the direct causal relationship requires further study.

miR-22 directly bound and repressed the levels of MDC1 in three different cell lines (HEK293T, HeLa, U2OS) (Lee, Park et al. 2015). MDC1 (*Mediator Of DNA*

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

Damage Checkpoint 1) is an intra-S phase checkpoint protein that functions early in the DNA damage response, interacting with H2AX (*H2A Histone Family Member X*) at DNA double strand breaks and recruiting ATM (*Ataxia-telangiectasia Mutated*) kinase to mediate H2AX phosphorylation. Loss of MDC1 in mice confers chromosomal instability (i.e. chromosome and chromatid breaks, fragmentation, dicentric chromosomes) (Lou, Minter-Dykhouse et al. 2006) and the tumor suppressor protein is frequently mutated, lost or repressed in human cancers (Ruff, Logan et al. 2020). Multiple chromosomal abnormalities including recurring clonal amplifications and deletions were detected in miR-22 overexpressing GM00637 (fibroblast) cells. Furthermore, miR-22 overexpression increased the frequency of chromosome breaks in U2OS (osteosarcoma) cells and this effect was fully rescued by reconstitution of MDC1 containing a mutated miR-22 binding site. In addition, it was shown that overexpression of AKT1 (*AKT Serine/Threonine Kinase 1*), a positive upstream regulator of miR-22, reduced homologous recombination via miR-22 mediated suppression of MDC1 (Lee, Park et al. 2015). Suppression of homologous recombination is known to be causative for aneuploidy and CIN (Griffin 2002). Of note, miR-22 was shown to form a feed-forward loop with AKT1 via repression of PTEN (*Phosphatase and Tensin Homolog*), a negative regulator of AKT1 (Bar and Dikstein 2010). The miR-22-MDC1 regulatory axis and its effect on CIN was further confirmed in colorectal cancer cell lines. In this context, TFAP4 (*Transcription Factor AP-4*) was identified as a dual regulator of MDC1 expression, via direct transcriptional induction of MDC1 and via negative regulation of miR-22 which targets MDC1. TFAP4 deficient cells accrodingly showed a marked decrease in homologous recombination activity and increased micronuclei formation and gains in chromosome numbers, indicative of CIN. Treatment of TFAP4-deficient cells with

miR-22 antagomirs (or ectopic MDC1) could restore homologous recombination (HR)-mediated DNA damage repair, while TFAP-4 proficient cells treated with miR-22 mimics (or MDC1 siRNA) showed defective HR-mediated DNA damage repair (Chou, Kaller et al. 2022). These studies show that miR-22 directly regulates MDC1 in multiple cellular contexts and this majorly affects HR-mediated DNA repair and consequently causes CIN.

Another example is miR-26a which is ubiquitously expressed from three distinct genomic loci as miR-26a-1, miR-26a-2, and miR-26b from chromosome 3, 12 and 2 respectively. miR-26a has been described as both, oncomiR and tumor suppressor miRNA depending on the cancer type and target gene repertoire involved e.g. in proliferation, cell cycle regulation, apoptosis and metabolism (Rizzo, Berti et al. 2017, Li, Li et al. 2021). A study applying sustained miR-26a overexpression in breast cancer cells and mouse embryonic fibroblasts reported the occurrence of aneuploidy as well as centrosome defects such as fap1multipolar, monopolar, and defective bipolar cells (Castellano, Dabrowska et al. 2017). Multipolar spindles are strongly associated with chromosome mis-segregation (Silkworth, Nardi et al. 2009). Mechanistically, this effect is likely attributed to miR-26a directly targeting multiple genes involved in mitosis and cytokinesis (i.e. CHFR, LARP1, YWHAE). The restoration of CHFR expression rescued the occurrence of multipolar spindles in two miR-26a overexpressing breast cancer cell lines, while restoration of YWHAE (14-3-3 epsilon; *Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Epsilon*) showed this rescue effect in one of two breast cancer cell lines. CHFR (*Checkpoint With Forkhead And Ring Finger Domains*) is an antephase checkpoint protein that delays entry into mitosis (via inhibition of PLK1 (*Polo Like Kinase 1*) and AURKA (*Aurora Kinase A*) if mitotic

stress inhibits centrosome segregation (Scolnick and Halazonetis 2000, Sanbhnani and Yeong 2012). YWHAE is associated with the G2/M checkpoint (via interaction with CDC25C) (Telles, Hosing et al. 2009) during DNA replication and is thought to associate with centrosomes and/or microtubules during mitosis (Pietromonaco, Seluja et al. 1996, Abdrabou, Brandwein et al. 2020). Of note, miR-26a-1 is located within the 3p21.1 region that is frequently deleted in epithelial malignancies (Diederichs and Haber 2006), however if it affects the frequency of chromosomal instability has not been investigated.

miR-28 has been shown to potently regulate the levels of MAD2 protein via translational inhibition in various human and mouse cell types (i.e. HeLa embryonic kidney, HCT116 colon cancer, RPE-1 retinal pigment epithelial, and IMCD-3 murine kidney cells) (Hell, Thoma et al. 2014). MAD2 (*Mitotic Arrest Deficient 2*) is a mitotic spindle assembly checkpoint protein, deregulation of which is majorly involved in causing CIN through its regulatory roles in spindle assembly and kinetochore-microtubule attachments (Kabeche and Compton 2012, Schuyler, Wu et al. 2012). CIN can result from both up- and downregulation of MAD2 due to the requirement of a delicate balance between MAD1 and MAD2 for spindle checkpoint function (Schuyler, Wu et al. 2012). Overexpression of miR-28 in HeLa, RPE-1 and IMCD-3 cells abolished pro-metaphase arrest in the presence of the mitotic checkpoint activator ‘nocodazole’ and caused chromosome missegregation events (e.g. lagging chromosomes), mitotic slippage and leads to change in chromosome number. This effect was completely rescued upon expression of MAD2 lacking the 3’UTR required for regulation by miR-28. Furthermore, depletion or loss of the tumor suppressor gene VHL (*Von Hippel-Lindau Tumor Suppressor)* known to reduce MAD2 levels and introduce CIN) was shown to induce the expression of miR-28 at the

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

transcriptional level. Consequently, miR-28 inhibition could rescue the occurrence of CIN in pVHL-deficient cells, implying the suitability of anti-miR-28 therapy to target CIN in VHL-deficient cancers such as renal cell carcinomas (Hell, Thoma et al. 2014). The miR-28-MAD2 regulatory axis was independently confirmed in Burkitt lymphoma (P3HR and RAJI) cells, where overexpression of miR-28 caused G1 arrest and growth reductions (Schneider, Setty et al. 2014), but whether this effect was directly related to CIN remains to be elucidated. In this context, the proto-oncogene MYC was identified as a negtive regulator of miR-28 causing its reduced expression in Burkitt lymphomas. Restoration of miR-28 expression could prevent MYC-induced transformation of MCF10A breast epithelial cells, indicating that miR-28 reconstitution therapy could be suitable for the treatment of MYC-positive cancers (Schneider, Setty et al. 2014). However, the therapeutic targeting of the miR-28-MAD2 axis should be explored with caution due to the exact dosing of MAD2 required for correct function of the spindle assembly checkpoint (Schuyler, Wu et al. 2012).

The role of miR-186 overexpression in the presence and absence of arsenite has been investigated (Wu, Ferragut Cardoso et al. 2019). Arsenic exposure is known to introduce CIN via interference with DNA damage repair and disruption of mitotic progression (States 2015, Sage, Minatel et al. 2017). In two of three miR-186 overexpressing HaCaT (keratinocyte) clones, the occurrence of double minute, dicentric and ring chromosomes was increased. Exposure to arsenite further increased the number of double minute and dicentric chromosomes in miR-186 overexpressing clones. The authors suggest a functional link between miR-186 overexpression and BUB1 (*BUB1 Mitotic Checkpoint Serine/Threonine Kinase*) downregulation, however, direct regulation of BUB1 by miR-186 is not experimenally

supported (Wu, Ferragut Cardoso et al. 2019). Opposing this hypothesis, a recent report notes a link between BUB1 overexpression and mitotic segregation errors and CIN in multiple myeloma (Fujibayashi, Isa et al. 2020).

4. Long noncoding RNAs implicated in CIN

Multiple long noncoding RNAs have causal relationships for CIN through regulation of all CIN-related pathways, i.e. telomere integrity, chromosomal segregation and DNA damage response (Table 1). One of the mechanisms maintaining chromosomal integrity and stability during cell division is the telomere, a region of repetitive DNA complexed with specialized ribonucleoproteins (O'Sullivan and Karlseder 2010). Abnormal shortening of telomere length can cause CIN including abnormal structural alterations such as chromosome end-to-end fusions (Baird 2018, Turner, Vasu et al. 2019). Telomere length is regulated by telomerase, a telomeric-DNA-synthesizing ribonucleotide enzyme, the shelterin complex and various other proteins. In addition, telomeres are transcribed into lncRNAs of varying length termed telomeric repeat-containing RNAs (TERRA) (Azzalin, Reichenbach et al. 2007), which also regulate telomere length through various mechanisms. TERRA contain G-rich repetitive sequences complementary to the RNA component of telomerase (i.e. Terc), the interaction with which blocks the Terc template region and prevents telomere-telomerase interaction (Schoeftner and Blasco 2008). TERRA have furthermore been shown to bind to the telomerase reverse transcriptase (TERT) polypeptide (Redon, Reichenbach et al. 2010). Both of these TERRA functions, being a competitive inhibitor of Terc and an allosteric inhibitor of TERT, negatively regulate telomerase activity and block telomere extension (Schoeftner and Blasco 2008, Redon, Reichenbach et al. 2010). In yeast, it was furthermore

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

found that TERRA regulate the length of telomeres independently of telomerase through interaction with the Ku70/80 dimer. Ku70/80 binds to the telomere in order to protect the chromosome ends that have 3'-overhangs from degradation by EXO1 (*Exonuclease 1*). TERRA binding of Ku70/80 interfered with its ability to inhibit EXO1 (Pfeiffer and Lingner 2012). TERRA has been shown to interact with various other telomere-associated proteins including the shelterin complex component TRF2 (*Telomere Repeat Factor 2*) through its N-terminal Gly/Arg-rich (GAR) domain, which assisted the recruitment of ORC1 (*Origin Recognition Complex Subunit 1*). Depletion of TERRA caused decreased binding of the origin recognition complex at telomeres, loss of H3K9 (*Histone H3 Lysine 9*) trimethylation, thereby affected heterochromatin formation and caused telomere dysfunction (Deng, Norseen et al. 2009). A follow up study showed that the interaction between TRF2 and TERRA is mediated through G-quadruplex structures within the lncRNA. Interestingly, a G-quadruplex targeting compound, N-methyl mesoporphyrin IX, was found to specifically inhibit this interaction while also downregulating TERRA expression (Mei, Deng et al. 2021). In addition, the formation of DNA:RNA hybrid structures involving TERRA has been identified at telomeres. Such hybrids were enriched in ICF (Immunodeficiency, Centromeric instability and Facial anomalies) syndrome cells (that have short telomeres and increased TERRA) and were associated with increased levels of DNA damage at chromosome ends (Sagie, Toubiana et al. 2017). Involvement of TERRA in the regulation of telomere dynamics makes it an attractive therapeutic target in cancer, that may experience both shortened and elongated telomeres depending on cancer type and stage (Fernandes, Dsouza et al. 2020).

Similar to lncRNAs derived from telomeric sequences, the repetitive DNA regions of the centromere also produce RNA transcripts. The centromere is made up

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

of alpha satellite DNA, i.e. repeated ~171-bp monomer units, which associates with a complex of proteins during mitosis to form the kinetochore responsible for attaching spindle microtubules. The transcriptional activity of the centromere was first discovered in rice (Nagaki, Cheng et al. 2004) and later also identified in humans, producing centromeric RNAs (cenRNAs) (Wong, Brettingham-Moore et al. 2007, Chan, Marshall et al. 2012, McNulty, Sullivan et al. 2017, Ishikura, Nakabayashi et al. 2020). CenRNAs were shown to physically interact with centromere proteins, specifically CENP-A (*Centromere Protein A*) and its chaperone HJURP (*Holliday Junction Recognition Protein*) (Quenet and Dalal 2014). CENP-A is a histone H3 variant that epigenetically specifies centromere identity and function (Fachinetti, Folco et al. 2013) and depletion of cenRNAs lead to a loss of CENP-A and HJURP at centrosomes (Quenet and Dalal 2014). An independent study similarly identified loss of CENP-A loading upon cenRNAs depletion and additionally detected a loss of colocalization with CENP-C (*Centromere Protein C*), which was shown to form a stable complex together with cenRNA and CENP-A (McNulty, Sullivan et al. 2017). Other proteins associated with cenRNA are INCENP (*Inner Centromere Protein*) and AURKB (*Aurora Kinase B*), both subunits of chromosome passenger complex (CPC) that regulates the attachment of microtubules to the kinetochore (Wong, Brettingham-Moore et al. 2007, Ideue, Cho et al. 2014). CenRNAs depletion disrupted the localization of INCENP, its interactor survivin, and AURKB at kinetochores, resulting in abnormal chromosome segregation (Wong, Brettingham-Moore et al. 2007, Ideue, Cho et al. 2014). In addition, reduction in cenRNA transcription induced AURKB activation (Ideue, Cho et al. 2014), which is known to dysregulate microtubule attachment to the kinetochore (Murata-Hori and Wang 2002, Portella, Passaro et al. 2011). The overexpression of cenRNAs has

been detected in several human cancers, which lead to increased CIN, indicating cenRNA expression may be an early event in cancer development and could be a useful marker for neoplastic cells (Ting, Lipson et al. 2011, Chan, Moralli et al. 2017, Ichida, Suzuki et al. 2018). In tumor cells, CENP-A is often overexpressed and can mis-localize to regions outside centromeres altering the recruitment of centromere and kinetochore associated proteins and leading to CIN (Shrestha, Ahn et al. 2017). Ectopic accumulation of CENP-A is for example observed at chromosomal region 8q24, which harbors the frequently translocated oncogene c-Myc. Five lncRNAs are expressed from region 8q24: CCAT1 and 2 (*Colon Cancer-associated Transcript 1 and 2*), PCAT1 and 2 (*Prostate Cancer Associated Transcript 1 and 2*) and PVT1 (*Plasmacytoma Variant Translocation 1*). Interestingly, knockdown of several of these lncRNAs decreased the ectopic localization of CENP-A and co-localization of CENP-C, an effect that was most prominent upon knockdown of PCAT2. A direct interaction between PCAT2 and CENP-A that was dependent on transcriptionally coupled H3.3 chaperones HIRA (*Histone Cell Cycle Regulator*) and DAXX (*Death Domain Associated Protein*) caused this ectopic placement of CENP-A/C in colon cancer cells. Insertion of transgenic PCAT2 at a naïve chromosome locus (4q31) was sufficient to cause ectopic CENP-A/C localization (Arunkumar, Baek et al. 2022). This study thus shows that oncogenic lncRNAs may mimic cenRNAs to ectopically recruit centromere-associated proteins, thereby causing genomic fragility.

In B cells, tumorigenic genomic translocations are frequently associated with the off-targeting of AID (Activation-Induced cytidine Deaminase). AID mediates somatic hypermutation and class switch recombination, mechanisms designed to diversify B cell antigen receptors, by mediating deamination of cytosines, leading to mutations or double strand breaks within the immunoglobulin genes. AID is known to

have recurrent off-targets such as proto-oncogenes BCL6 (B-Cell Lymphoma 6) and MYC, causing their translocation and juxtaposition to potent Ig enhancers, which in turn results in their overexpression. AID off-targeting hotspots were shown to be characterized by convergent transcription stemming from antisense transcription of enhancer RNAs (eRNAs) within sense transcribed genes (Meng, Du et al. 2014, Qian, Wang et al. 2014). Such regions of convergent transcription were more prone to genomic instability upon depletion of the RNA exosome (region that regulates the ncRNA expression initiating from enhancers), which was shown to resolve RNA/DNA hybrid structures (R loops) stemming from regions of convergent transcription (Pefanis, Wang et al. 2015). Thus, the active transcription of eRNAs and their RNA exosome-mediated degradation play a major role in B cell specific chromosomal translocations arising from AID off-targeting.

The lncRNA Ginir (*Genomic Instability Inducing RNA*) and its antisense transcript (Ginir-as) display a tight and balanced spaciotemporal expression pattern during mouse embryonic development. Ginir expression was higher in proliferating cells during development, particularly in neuronal tissues, while Ginir-as expression was higher in non-proliferating cells of major organs in the adult mouse. The overexpression of Ginir (but not Ginir-as or the Ginir-Ginir-as combination) induced oncogenic transformation of NIH/3T3 (mouse fibroblast) cells *in vitro* and in murine xenograft models, inducing increased proliferation and invation potential. DNA double strand breaks, activation of the DNA damage response as well as mitotic defects were noted in Ginir-overexpressing cells, including multipolar spindles resulting in multinucleated cells. Mechanistically, this effect was mediated through interaction of Ginir with Cep112 (*Centrosomal Protein 112*) and Brca1 (*Breast Cancer Type 1 Susceptibility Protein*) (Panda, Setia et al. 2018). Brca1 is, in addition

to its prominent role in the DNA damage response, involved in centrosome regulation and its dysfunction causes increases in centrosome number (Yoshino, Fang et al. 2021). High levels of Ginir disrupted the interaction between Cep112 and Brca1 and downregulated their expression, consequently leading to centrosome amplification and this effect that was phenocopied by the individual knockdown of both, Cep112 and Brca1 (Panda, Setia et al. 2018). Ginir thus acted as an oncogene in adult murine cells by introduction of DNA damage and CIN.

LncRNA GUARDIN was discovered as a p53-responsive lncRNA, facilitating DNA damage response and modulating the p53 cytotoxic effect. Knockdown of GUARDIN reduced the proliferation and carcinogenic potential of HCT116 (colon cancer) cells. A dual role was defined for this lncRNA: On one hand it sequestered miR-23a (i.e. acted as an endogenous competing RNA), thereby affecting expression of the miR-23a target gene TRF2. TRF2 is a part of the shelterin complex, essential for telomere integrity and its levels are maintained through the GUARDIN-miR-23a-TRF2 axis. Consequently, GUARDIN depletion resulted in DNA damage at telomeres and end-to-end fusion of chromosomes. On the other hand, GUARDIN directly interacted with BRCA1 as well as BARD1 (*BRCA1 Associated RING Domain 1*) and depletion of GUARDIN resulted in downregulation of BRCA1 through proteosomal degradation. This affected the DNA damage response and markedly reduced the activity of homologous recombination and non-homologous end-joining pathways (Hu, Jin et al. 2018). The BRCA1-BARD1 interaction is also essential for centrosome regulation during mitosis (Yoshino, Fang et al. 2021), but whether GUARDIN affects centrosome number and function remains to be elucidated.

CCAT2 (*Colon Cancer-associated Transcript 2*) was first identified as a lncRNA arising from the cancer-associated 8q24 gene desert, and was selectively overexpressed in microsatellite-stable (MSS) colorectal cancer (CRC) patient samples (Ling, Spizzo et al. 2013), which generally have a worse prognosis compared to microsatellite-instable (MSI) CRCs (Boland and Goel 2010). *In vivo* xenograft models showed that CCAT2 overexpression promoted tumor growth and metastasis, and upregulated expression of the oncogene MYC. A strong link to the development of CIN was identified upon *in vitro* overexpression of CCAT2 in HCT116 cells, which caused the occurrence of aberrant metaphases resulting in both sCIN and nCIN and consequently, a dramatic increase in the amount of polyploid cells. CCAT2-overexpressing clones were marked by the presence of three or more centrosomes, causing faulty chromosome segregation (Ling, Spizzo et al. 2013). In a follow-up study, the precise functional role of CCAT2 in CIN was further unraveled, identifying the lncRNA as a positive regulator of BOP1 (*BOP1 Ribosomal Biogenesis Factor*), either via induction of MYC or directly. BOP1 overexpression in CRC cell lines phenocopied the effects of CCAT2, leading to abnormal spindles during metaphase as well as anaphase bridges, consequently causing chromosome fusions, breaks and fragmentation. In the functional model further established, BOP1 increased the active (i.e. phosphorylated) form of AURKB, while CCAT2 was found to form a complex with AURKB, possibly mediating the interaction between BOP1 and AURKB (Chen, Dragomir et al. 2020). AURKB is a part of the chromosomal passenger complex associated with centrosomes and controls spindle assembly and cytokinesis via different substrates (Hindriksen, Meppelink et al. 2015). Increased active AURKB disrupts chromosome-microtubule attachments and causes premature collapse of the mitotic spindle (Munoz-Barrera and Monje-Casas 2014).

Noncoding RNA Activated by DNA damage (NORAD) is a ubiquitously expressed, highly conserved lncRNA that is strongly induced by DNA damage. Knockout of NORAD was shown to result in a high frequency of mitotic errors (e.g. anaphase bridges, mitotic slippage) leading to nCIN (chromosome gains/losses, aneuploidy) as well as sCIN (rearrangements) in HCT116 (colorectal cancer) cells, and this phenotype could be rescued by NORAD restoration. Mechanistically, this effect was mediated by the sequestration of PUMILIO proteins (PUM2 and, to a lesser extent, PUM1) and 15 binding motifs (i.e. PUMILIO response elements, PREs) were identified within NORAD lncRNA (Lee, Kopp et al. 2016). An independent study suggested that the interaction between PUM2 and NORAD is mediated via KHDRBS1 (*KH RNA Binding Domain Containing, Signal Transduction Associated 1*; SAM68), which also has recurring binding sites within the lncRNA (Tichon, Perry et al. 2018). PUMILIO proteins post-transcriptionally regulate gene expression by binding to these specific response elements in the 3'UTRs of mRNAs (Wickens, Bernstein et al. 2002). Amongst PUMILIO target genes are key mitotic, DNA repair and DNA replication factors mediating genomic stability, which were repressed following the lack of PUM2/PUM1 sequestration in NORAD knockout cells (Lee, Kopp et al. 2016). A follow up study showed that the expression of a circular RNA containing four to eight PREs could rescue the chromosome segregation defects caused by CRISPR-mediated NORAD depletion, causing phase-seperation of PUM proteins into punctate cytoplasmic foci (i.e. NP bodies) (Elguindy and Mendell 2021).

5. Conclusions and Future perspectives

As CIN is an event occurring early in tumor development and has strong implications in tumor resistance to cytotoxic anticancer drugs (Pikor, Thu et al. 2013,

Vargas-Rondon, Villegas et al. 2017), its exploitation for therapeutic purposes requires fundamental understanding of the abnormal molecular pathways driving CIN. CIN may be exploited therapeutically in multiple ways: (1) by reducing CIN to hinder tumor adaptability and development of drug resistance, (2) by increasing CIN to produce unsustainable karyotypes leading to cell death, or (3) by targeting the CIN-tolerance mechanisms acquired by tumor cells (Thompson, Jeusset et al. 2017, Sansregret, Vanhaesebroeck et al. 2018). Multiple compounds such as APC/C (*Anaphase Promoting Complex/Cyclosome*) inhibitors (reduce CIN by prolonging metaphase/mitotic exit), SAC (*Spindle Assembly Checkpoint*) inhibitors (induce CIN by premature mitotic exit) or Aurora kinase inhibitors (induce CIN by inhibiting chromosome alignment and spindle assembly) show promising results in preclinical and/or early clinical studies (reviewed in (Thompson, Jeusset et al. 2017)). Multiple of the ncRNAs described here could similarly be targeted to increase or decrease the basal level of CIN in cancer cells. NcRNAs are furthermore involved in CIN-tolerance mechanisms. One such mechanism to compensate for the negative effects of aneuploidy is gene dosage compensation. For example, the OncomiR-1 cluster miRNAs miR-17, miR-19a and miR-20a have been found to be involved in the compensation for increased copy numbers of proto-oncogene MYC. Accordingly, the inhibition of these miRNAs caused cytotoxicity that was stronger in cells with higher copy numbers of MYC (Acon, Geiss et al. 2021). The oncomiR-1 cluster has furthermore been shown to form a feedback loop with STAT3 (*Signal Transducer And Activator Of Transcription 3*) (Jo, Kim et al. 2014, Acon, Geiss et al. 2021), indicating a broader role for this cluster in gene dosage compensation.

The different modes of targeting CIN in cancer further imply that good biomarkers for CIN are urgently needed for patient stratification. Monitoring of

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

ncRNAs such as cenRNAs, the levels of which are thought to increase prior to CIN occurrence (Ting, Lipson et al. 2011, Chan, Moralli et al. 2017, Ichida, Suzuki et al. 2018) could deliver such much needed markers in the near future.

Thus, the noncoding RNAs described here thus not only grant a better understanding of mechanisms leading to CIN, but also further expand the possibilities for its therapeutic targeting. Increasing use of large-scale sequencing have immensely helped in a better understanding of the chromosomal changes incorporated in different cancers. However, different emerging factors including non-coding RNAs have been reported in regulating chromosomal stability directly or indirectly, thereby fueling the tumor heterogeneity and propagating the karyotype diversity that is important to understand in order to find better treatment strategies.

7. Acknowledgements

Dr. Calin is the Felix L. Haas Endowed Professor in Basic Science. Work in Dr. Calin's laboratory is supported by NCI grants 1R01 CA182905-01 and 1R01CA222007-01A1, NIGMS grant 1R01GM122775-01, NIDCR grant 5 R01 DE032018-02, DoD Idea Award W81XWH-21-1-0030, a Team DOD grant in Gastric Cancer W81XWH-21-1-0715, a Chronic Lymphocytic Leukemia Moonshot Flagship project, a CLL Global Research Foundation 2019 grant, a CLL Global Research Foundation 2020 grant, The G. Harold & Leila Y. Mathers Foundation, two grants from Torrey Coast Foundation, an Institutional Research Grant , an Institutional Bridge Fund, and Development Grant associated with the Brain SPORE 2P50CA127001. SM is supported by the CPRIT Research Training Grant (RP210028). We created the figure illustrations using Biorender.com.

8. Declaration of Interests

Dr. Calin is the scientific founder of Ithax Pharmaceuticals.

9. **Authorship Contributions**

Wrote or contributed to the writing of the manuscript: SM, MW, ATN, DN, GAC

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

10. References

Abdrabou, A., D. Brandwein and Z. Wang (2020). "Differential Subcellular Distribution and Translocation of Seven 14-3-3 Isoforms in Response to EGF and During the Cell Cycle." Int J Mol Sci **21**(1).
Acon, M., C. Geiss, J. Torres-Calvo, D. Bravo-Estupinan, G. Oviedo, J. L. Arias-Arias, L. A. Rojas-Matey, B. Edwin, G. Vasquez-Vargas, Y. Oses-Vargas, J. Guevara-Coto, A. Segura-Castillo, F. Siles-Canales, S. Quiros-Barrantes, A. Regnier-Vigouroux, P. Mendes and R. Mora-Rodriguez (2021). "MYC dosage compensation is mediated by miRNA-transcription factor interactions in aneuploid cancer." iScience **24**(12): 103407.
Aguilera, A. and T. Garcia-Muse (2012). "R loops: from transcription byproducts to threats to genome stability." Mol Cell **46**(2): 115-124.
Arunkumar, G., S. Baek, D. Sturgill, M. Bui and Y. Dalal (2022). "Oncogenic lncRNAs alter epigenetic memory at a fragile chromosomal site in human cancer cells." Sci Adv **8**(9): eabl5621.
Azzalin, C. M., P. Reichenbach, L. Khoriauli, E. Giulotto and J. Lingner (2007). "Telomeric repeat containing RNA and RNA surveillance factors at mammalian chromosome ends." Science **318**(5851): 798-801.
Baird, D. M. (2018). "Telomeres and genomic evolution." Philos Trans R Soc Lond B Biol Sci **373**(1741).
Bakhoum, S. F. and L. C. Cantley (2018). "The Multifaceted Role of Chromosomal Instability in Cancer and Its Microenvironment." Cell **174**(6): 1347-1360.
Bakhoum, S. F., B. Ngo, A. M. Laughney, J. A. Cavallo, C. J. Murphy, P. Ly, P. Shah, R. K. Sriram, T. B. K. Watkins, N. K. Taunk, M. Duran, C. Pauli, C. Shaw, K. Chadalavada, V. K. Rajasekhar, G. Genovese, S. Venkatesan, N. J. Birkbak, N. McGranahan, M. Lundquist, Q. LaPlant, J. H. Healey, O. Elemento, C. H. Chung, N. Y. Lee, M. Imielenski, G. Nanjangud, D. Pe'er, D. W. Cleveland, S. N. Powell, J. Lammerding, C. Swanton and L. C. Cantley (2018). "Chromosomal instability drives metastasis through a cytosolic DNA response." Nature **553**(7689): 467-472.
Bar, N. and R. Dikstein (2010). "miR-22 forms a regulatory loop in PTEN/AKT pathway and modulates signaling kinetics." PLoS One **5**(5): e10859.
Boland, C. R. and A. Goel (2010). "Microsatellite instability in colorectal cancer." Gastroenterology **138**(6): 2073-2087 e2073.
Burrell, R. A., S. E. McClelland, D. Endesfelder, P. Groth, M. C. Weller, N. Shaikh, E. Domingo, N. Kanu, S. M. Dewhurst, E. Gronroos, S. K. Chew, A. J. Rowan, A. Schenk, M. Sheffer, M. Howell, M. Kschischo, A. Behrens, T. Helleday, J. Bartek, I. P. Tomlinson and C. Swanton (2013). "Replication stress links structural and numerical cancer chromosomal instability." Nature **494**(7438): 492-496.
Calin, G. A., C. D. Dumitru, M. Shimizu, R. Bichi, S. Zupo, E. Noch, H. Aldler, S. Rattan, M. Keating, K. Rai, L. Rassenti, T. Kipps, M. Negrini, F. Bullrich and C. M. Croce (2002). "Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia." Proc Natl Acad Sci U S A **99**(24): 15524-15529.
Castellano, L., A. Dabrowska, L. Pellegrino, S. Ottaviani, P. Cathcart, A. E. Frampton, J. Krell and J. Stebbing (2017). "Sustained expression of miR-26a promotes chromosomal instability and tumorigenesis through regulation of CHFR." Nucleic Acids Res **45**(8): 4401-4412.
Cech, T. R. and J. A. Steitz (2014). "The noncoding RNA revolution-trashing old rules to forge new ones." Cell **157**(1): 77-94.
Chan, D. Y. L., D. Moralli, S. Khoja and Z. L. Monaco (2017). "Noncoding Centromeric RNA Expression Impairs Chromosome Stability in Human and Murine Stem Cells." Dis Markers **2017**: 7506976.
Chan, F. L., O. J. Marshall, R. Saffery, B. W. Kim, E. Earle, K. H. Choo and L. H. Wong (2012). "Active transcription and essential role of RNA polymerase II at the centromere during mitosis." Proc Natl Acad Sci U S A **109**(6): 1979-1984.

Chen, B., M. P. Dragomir, L. Fabris, R. Bayraktar, E. Knutsen, X. Liu, C. Tang, Y. Li, T. Shimura, T. C. Ivkovic, M. C. De Los Santos, S. Anfossi, M. Shimizu, M. Y. Shah, H. Ling, P. Shen, A. S. Multani, B. Pardini, J. K. Burks, H. Katayama, L. C. Reineke, L. Huo, M. Syed, S. Song, M. Ferracin, E. Oki, B. Fromm, C. Ivan, K. Bhuvaneshwar, Y. Gusev, K. Mimori, D. Menter, S. Sen, T. Matsuyama, H. Uetake, C. Vasilescu, S. Kopetz, J. Parker-Thornburg, A. Taguchi, S. M. Hanash, L. Girnita, O. Slaby, A. Goel, G. Varani, M. Gagea, C. Li, J. A. Ajani and G. A. Calin (2020). "The Long Noncoding RNA CCAT2 Induces Chromosomal Instability Through BOP1-AURKB Signaling." Gastroenterology **159**(6): 2146-2162 e2133.

Chou, J., M. Kaller, S. Jaeckel, M. Rokavec and H. Hermeking (2022). "AP4 suppresses DNA damage, chromosomal instability and senescence via inducing MDC1/Mediator of DNA damage Checkpoint 1 and repressing MIR22HG/miR-22-3p." Mol Cancer **21**(1): 120.

Deng, Z., J. Norseen, A. Wiedmer, H. Riethman and P. M. Lieberman (2009). "TERRA RNA binding to TRF2 facilitates heterochromatin formation and ORC recruitment at telomeres." Mol Cell **35**(4): 403-413.

Diederichs, S. and D. A. Haber (2006). "Sequence variations of microRNAs in human cancer: alterations in predicted secondary structure do not affect processing." Cancer Res **66**(12): 6097-6104.

Djebali, S., C. A. Davis, A. Merkel, A. Dobin, T. Lassmann, A. Mortazavi, A. Tanzer, J. Lagarde, W. Lin, F. Schlesinger, C. Xue, G. K. Marinov, J. Khatun, B. A. Williams, C. Zaleski, J. Rozowsky, M. Roder, F. Kokocinski, R. F. Abdelhamid, T. Alioto, I. Antoshechkin, M. T. Baer, N. S. Bar, P. Batut, K. Bell, I. Bell, S. Chakrabortty, X. Chen, J. Chrast, J. Curado, T. Derrien, J. Drenkow, E. Dumais, J. Dumais, R. Duttagupta, E. Falconnet, M. Fastuca, K. Fejes-Toth, P. Ferreira, S. Foissac, M. J. Fullwood, H. Gao, D. Gonzalez, A. Gordon, H. Gunawardena, C. Howald, S. Jha, R. Johnson, P. Kapranov, B. King, C. Kingswood, O. J. Luo, E. Park, K. Persaud, J. B. Preall, P. Ribeca, B. Risk, D. Robyr, M. Sammeth, L. Schaffer, L. H. See, A. Shahab, J. Skancke, A. M. Suzuki, H. Takahashi, H. Tilgner, D. Trout, N. Walters, H. Wang, J. Wrobel, Y. Yu, X. Ruan, Y. Hayashizaki, J. Harrow, M. Gerstein, T. Hubbard, A. Reymond, S. E. Antonarakis, G. Hannon, M. C. Giddings, Y. Ruan, B. Wold, P. Carninci, R. Guigo and T. R. Gingeras (2012). "Landscape of transcription in human cells." Nature **489**(7414): 101-108.

Elguindy, M. M. and J. T. Mendell (2021). "NORAD-induced Pumilio phase separation is required for genome stability." Nature **595**(7866): 303-308.

Fachinetti, D., H. D. Folco, Y. Nechemia-Arbely, L. P. Valente, K. Nguyen, A. J. Wong, Q. Zhu, A. J. Holland, A. Desai, L. E. Jansen and D. W. Cleveland (2013). "A two-step mechanism for epigenetic specification of centromere identity and function." Nat Cell Biol **15**(9): 1056-1066.

Fernandes, S. G., R. Dsouza, G. Pandya, A. Kirtonia, V. Tergaonkar, S. Y. Lee, M. Garg and E. Khattar (2020). "Role of Telomeres and Telomeric Proteins in Human Malignancies and Their Therapeutic Potential." Cancers (Basel) **12**(7).

Frankish, A., M. Diekhans, I. Jungreis, J. Lagarde, J. E. Loveland, J. M. Mudge, C. Sisu, J. C. Wright, J. Armstrong, I. Barnes, A. Berry, A. Bignell, C. Boix, S. Carbonell Sala, F. Cunningham, T. Di Domenico, S. Donaldson, I. T. Fiddes, C. Garcia Giron, J. M. Gonzalez, T. Grego, M. Hardy, T. Hourlier, K. L. Howe, T. Hunt, O. G. Izuogu, R. Johnson, F. J. Martin, L. Martinez, S. Mohanan, P. Muir, F. C. P. Navarro, A. Parker, B. Pei, F. Pozo, F. C. Riera, M. Ruffier, B. M. Schmitt, E. Stapleton, M. M. Suner, I. Sycheva, B. Uszczynska-Ratajczak, M. Y. Wolf, J. Xu, Y. T. Yang, A. Yates, D. Zerbino, Y. Zhang, J. S. Choudhary, M. Gerstein, R. Guigo, T. J. P. Hubbard, M. Kellis, B. Paten, M. L. Tress and P. Flicek (2021). "Gencode 2021." Nucleic Acids Res **49**(D1): D916-D923.

Fujibayashi, Y., R. Isa, D. Nishiyama, N. Sakamoto-Inada, N. Kawasumi, J. Yamaguchi, S. Kuwahara-Ota, Y. Matsumura-Kimoto, T. Tsukamoto, Y. Chinen, Y. Shimura, T. Kobayashi, S. Horiike, M. Taniwaki, H. Handa and J. Kuroda (2020). "Aberrant BUB1 Overexpression Promotes Mitotic Segregation Errors and Chromosomal Instability in Multiple Myeloma." Cancers (Basel) **12**(8).

Funk, L. C., L. M. Zasadil and B. A. Weaver (2016). "Living in CIN: Mitotic Infidelity and Its Consequences for Tumor Promotion and Suppression." Dev Cell **39**(6): 638-652.

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

Gollin, S. M. (2004). "Chromosomal instability." Curr Opin Oncol **16**(1): 25-31.
Griffin, C. S. (2002). "Aneuploidy, centrosome activity and chromosome instability in cells deficient in homologous recombination repair." Mutat Res **504**(1-2): 149-155.
Hell, M. P., C. R. Thoma, N. Fankhauser, Y. Christinat, T. C. Weber and W. Krek (2014). "miR-28-5p promotes chromosomal instability in VHL-associated cancers by inhibiting Mad2 translation." Cancer Res **74**(9): 2432-2443.
Hindriksen, S., A. Meppelink and S. M. Lens (2015). "Functionality of the chromosomal passenger complex in cancer." Biochem Soc Trans **43**(1): 23-32.
Hu, W. L., L. Jin, A. Xu, Y. F. Wang, R. F. Thorne, X. D. Zhang and M. Wu (2018). "GUARDIN is a p53-responsive long non-coding RNA that is essential for genomic stability." Nat Cell Biol **20**(4): 492-502.
Ichida, K., K. Suzuki, T. Fukui, Y. Takayama, N. Kakizawa, F. Watanabe, H. Ishikawa, Y. Muto, T. Kato, M. Saito, K. Futsuhara, Y. Miyakura, H. Noda, T. Ohmori, F. Konishi and T. Rikiyama (2018). "Overexpression of satellite alpha transcripts leads to chromosomal instability via segregation errors at specific chromosomes." Int J Oncol **52**(5): 1685-1693.
Ideue, T., Y. Cho, K. Nishimura and T. Tani (2014). "Involvement of satellite I noncoding RNA in regulation of chromosome segregation." Genes Cells **19**(6): 528-538.
Ishikura, S., K. Nakabayashi, M. Nagai, T. Tsunoda and S. Shirasawa (2020). "ZFAT binds to centromeres to control noncoding RNA transcription through the KAT2B-H4K8ac-BRD4 axis." Nucleic Acids Res **48**(19): 10848-10866.
Jo, D. H., J. H. Kim, C. S. Cho, Y. L. Cho, H. O. Jun, Y. S. Yu, J. K. Min and J. H. Kim (2014). "STAT3 inhibition suppresses proliferation of retinoblastoma through down-regulation of positive feedback loop of STAT3/miR-17-92 clusters." Oncotarget **5**(22): 11513-11525.
Kabeche, L. and D. A. Compton (2012). "Checkpoint-independent stabilization of kinetochore-microtubule attachments by Mad2 in human cells." Curr Biol **22**(7): 638-644.
Kato, H., J. Jiang, B. R. Zhou, M. Rozendaal, H. Feng, R. Ghirlando, T. S. Xiao, A. F. Straight and Y. Bai (2013). "A conserved mechanism for centromeric nucleosome recognition by centromere protein CENP-C." Science **340**(6136): 1110-1113.
Kopp, F. and J. T. Mendell (2018). "Functional Classification and Experimental Dissection of Long Noncoding RNAs." Cell **172**(3): 393-407.
Kozomara, A., M. Birgaoanu and S. Griffiths-Jones (2019). "miRBase: from microRNA sequences to function." Nucleic Acids Res **47**(D1): D155-D162.
Kuru-Schors, M., M. Haemmerle and T. Gutschner (2021). "The Cohesin Complex and Its Interplay with Non-Coding RNAs." Noncoding RNA **7**(4).
Lee, J. H., S. J. Park, S. Y. Jeong, M. J. Kim, S. Jun, H. S. Lee, I. Y. Chang, S. C. Lim, S. P. Yoon, J. Yong and H. J. You (2015). "MicroRNA-22 Suppresses DNA Repair and Promotes Genomic Instability through Targeting of MDC1." Cancer Res **75**(7): 1298-1310.
Lee, S., F. Kopp, T. C. Chang, A. Sataluri, B. Chen, S. Sivakumar, H. Yu, Y. Xie and J. T. Mendell (2016). "Noncoding RNA NORAD Regulates Genomic Stability by Sequestering PUMILIO Proteins." Cell **164**(1-2): 69-80.
Li, C., Y. Li, Y. Lu, Z. Niu, H. Zhao, Y. Peng and M. Li (2021). "miR-26 family and its target genes in tumorigenesis and development." Crit Rev Oncol Hematol **157**: 103124.
Lim, L. P., N. C. Lau, P. Garrett-Engele, A. Grimson, J. M. Schelter, J. Castle, D. P. Bartel, P. S. Linsley and J. M. Johnson (2005). "Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs." Nature **433**(7027): 769-773.
Ling, H., R. Spizzo, Y. Atlasi, M. Nicoloso, M. Shimizu, R. S. Redis, N. Nishida, R. Gafa, J. Song, Z. Guo, C. Ivan, E. Barbarotto, I. De Vries, X. Zhang, M. Ferracin, M. Churchman, J. F. van Galen, B. H. Beverloo, M. Shariati, F. Haderk, M. R. Estecio, G. Garcia-Manero, G. A. Patijn, D. C. Gotley, V. Bhardwaj, I. Shureiqi, S. Sen, A. S. Multani, J. Welsh, K. Yamamoto, I. Taniguchi, M. A. Song, S. Gallinger, G. Casey, S. N. Thibodeau, L. Le Marchand, M. Tiirikainen, S. A. Mani, W. Zhang, R. V. Davuluri, K. Mimori, M. Mori, A. M. Sieuwerts, J. W. Martens, I. Tomlinson, M. Negrini, I. Berindan-Neagoe, J. A. Foekens, S. R. Hamilton, G. Lanza, S. Kopetz, R. Fodde and G. A. Calin (2013). "CCAT2, a novel noncoding RNA

mapping to 8q24, underlies metastatic progression and chromosomal instability in colon cancer." Genome Res **23**(9): 1446-1461.
Lou, Z., K. Minter-Dykhouse, S. Franco, M. Gostissa, M. A. Rivera, A. Celeste, J. P. Manis, J. van Deursen, A. Nussenzweig, T. T. Paull, F. W. Alt and J. Chen (2006). "MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals." Mol Cell **21**(2): 187-200.
McNulty, S. M., L. L. Sullivan and B. A. Sullivan (2017). "Human Centromeres Produce Chromosome-Specific and Array-Specific Alpha Satellite Transcripts that Are Complexed with CENP-A and CENP-C." Dev Cell **42**(3): 226-240 e226.
Mei, Y., Z. Deng, O. Vladimirova, N. Gulve, F. B. Johnson, W. C. Drosopoulos, C. L. Schildkraut and P. M. Lieberman (2021). "TERRA G-quadruplex RNA interaction with TRF2 GAR domain is required for telomere integrity." Sci Rep **11**(1): 3509.
Meng, F. L., Z. Du, A. Federation, J. Hu, Q. Wang, K. R. Kieffer-Kwon, R. M. Meyers, C. Amor, C. R. Wasserman, D. Neuberg, R. Casellas, M. C. Nussenzweig, J. E. Bradner, X. S. Liu and F. W. Alt (2014). "Convergent transcription at intragenic super-enhancers targets AID-initiated genomic instability." Cell **159**(7): 1538-1548.
Mitelman, F., B. Johansson and F. Mertens (2007). "The impact of translocations and gene fusions on cancer causation." Nat Rev Cancer **7**(4): 233-245.
Mohapatra, S., C. Pioppini, B. Ozpolat and G. A. Calin (2021). "Non-coding RNAs regulation of macrophage polarization in cancer." Mol Cancer **20**(1): 24.
Munoz-Barrera, M. and F. Monje-Casas (2014). "Increased Aurora B activity causes continuous disruption of kinetochore-microtubule attachments and spindle instability." Proc Natl Acad Sci U S A **111**(38): E3996-4005.
Murata-Hori, M. and Y. L. Wang (2002). "The kinase activity of aurora B is required for kinetochore-microtubule interactions during mitosis." Curr Biol **12**(11): 894-899.
Nagaki, K., Z. Cheng, S. Ouyang, P. B. Talbert, M. Kim, K. M. Jones, S. Henikoff, C. R. Buell and J. Jiang (2004). "Sequencing of a rice centromere uncovers active genes." Nat Genet **36**(2): 138-145.
Nicholson, J. M. and D. Cimini (2013). "Cancer karyotypes: survival of the fittest." Front Oncol **3**: 148.
O'Sullivan, R. J. and J. Karlseder (2010). "Telomeres: protecting chromosomes against genome instability." Nat Rev Mol Cell Biol **11**(3): 171-181.
Panda, S., M. Setia, N. Kaur, V. Shepal, V. Arora, D. K. Singh, A. Mondal, A. Teli, M. Tathode, R. Gajula, L. C. Padhy and A. Shiras (2018). "Noncoding RNA Ginir functions as an oncogene by associating with centrosomal proteins." PLoS Biol **16**(10): e2004204.
Pefanis, E., J. Wang, G. Rothschild, J. Lim, D. Kazadi, J. Sun, A. Federation, J. Chao, O. Elliott, Z. P. Liu, A. N. Economides, J. E. Bradner, R. Rabadan and U. Basu (2015). "RNA exosome-regulated long non-coding RNA transcription controls super-enhancer activity." Cell **161**(4): 774-789.
Pfeiffer, V. and J. Lingner (2012). "TERRA promotes telomere shortening through exonuclease 1-mediated resection of chromosome ends." PLoS Genet **8**(6): e1002747.
Pietromonaco, S. F., G. A. Seluja, A. Aitken and L. Elias (1996). "Association of 14-3-3 proteins with centrosomes." Blood Cells Mol Dis **22**(3): 225-237.
Pikor, L., K. Thu, E. Vucic and W. Lam (2013). "The detection and implication of genome instability in cancer." Cancer Metastasis Rev **32**(3-4): 341-352.
Portella, G., C. Passaro and P. Chieffi (2011). "Aurora B: a new prognostic marker and therapeutic target in cancer." Curr Med Chem **18**(4): 482-496.
Qian, J., Q. Wang, M. Dose, N. Pruett, K. R. Kieffer-Kwon, W. Resch, G. Liang, Z. Tang, E. Mathe, C. Benner, W. Dubois, S. Nelson, L. Vian, T. Y. Oliveira, M. Jankovic, O. Hakim, A. Gazumyan, R. Pavri, P. Awasthi, B. Song, G. Liu, L. Chen, S. Zhu, L. Feigenbaum, L. Staudt, C. Murre, Y. Ruan, D. F. Robbiani, Q. Pan-Hammarstrom, M. C. Nussenzweig and R. Casellas (2014). "B cell super-enhancers and regulatory clusters recruit AID tumorigenic activity." Cell **159**(7): 1524-1537.
Quenet, D. and Y. Dalal (2014). "A long non-coding RNA is required for targeting centromeric protein A to the human centromere." Elife **3**: e03254.

Redon, S., P. Reichenbach and J. Lingner (2010). "The non-coding RNA TERRA is a natural ligand and direct inhibitor of human telomerase." Nucleic Acids Res **38**(17): 5797-5806.
Rizzo, M., G. Berti, F. Russo, S. Fazio, M. Evangelista, R. D'Aurizio, M. Pellegrini and G. Rainaldi (2017). "Discovering the miR-26a-5p Targetome in Prostate Cancer Cells." J Cancer **8**(14): 2729-2739.
Ruff, S. E., S. K. Logan, M. J. Garabedian and T. T. Huang (2020). "Roles for MDC1 in cancer development and treatment." DNA Repair (Amst) **95**: 102948.
Sage, A. P., B. C. Minatel, K. W. Ng, G. L. Stewart, T. J. B. Dummer, W. L. Lam and V. D. Martinez (2017). "Oncogenomic disruptions in arsenic-induced carcinogenesis." Oncotarget **8**(15): 25736-25755.
Sagie, S., S. Toubiana, S. R. Hartono, H. Katzir, A. Tzur-Gilat, S. Havazelet, C. Francastel, G. Velasco, F. Chedin and S. Selig (2017). "Telomeres in ICF syndrome cells are vulnerable to DNA damage due to elevated DNA:RNA hybrids." Nat Commun **8**: 14015.
Sanbhnani, S. and F. M. Yeong (2012). "CHFR: a key checkpoint component implicated in a wide range of cancers." Cell Mol Life Sci **69**(10): 1669-1687.
Sansregret, L., B. Vanhaesebroeck and C. Swanton (2018). "Determinants and clinical implications of chromosomal instability in cancer." Nat Rev Clin Oncol **15**(3): 139-150.
Schneider, C., M. Setty, A. B. Holmes, R. L. Maute, C. S. Leslie, L. Mussolin, A. Rosolen, R. Dalla-Favera and K. Basso (2014). "MicroRNA 28 controls cell proliferation and is down-regulated in B-cell lymphomas." Proc Natl Acad Sci U S A **111**(22): 8185-8190.
Schoeftner, S. and M. A. Blasco (2008). "Developmentally regulated transcription of mammalian telomeres by DNA-dependent RNA polymerase II." Nat Cell Biol **10**(2): 228-236.
Schukken, K. M. and F. Foijer (2018). "CIN and Aneuploidy: Different Concepts, Different Consequences." Bioessays **40**(1).
Schuyler, S. C., Y. F. Wu and V. J. Kuan (2012). "The Mad1-Mad2 balancing act--a damaged spindle checkpoint in chromosome instability and cancer." J Cell Sci **125**(Pt 18): 4197-4206.
Scolnick, D. M. and T. D. Halazonetis (2000). "Chfr defines a mitotic stress checkpoint that delays entry into metaphase." Nature **406**(6794): 430-435.
Shrestha, R. L., G. S. Ahn, M. I. Staples, K. M. Sathyan, T. S. Karpova, D. R. Foltz and M. A. Basrai (2017). "Mislocalization of centromeric histone H3 variant CENP-A contributes to chromosomal instability (CIN) in human cells." Oncotarget **8**(29): 46781-46800.
Silkworth, W. T., I. K. Nardi, L. M. Scholl and D. Cimini (2009). "Multipolar spindle pole coalescence is a major source of kinetochore mis-attachment and chromosome mis-segregation in cancer cells." PLoS One **4**(8): e6564.
Slack, F. J. and A. M. Chinnaiyan (2019). "The Role of Non-coding RNAs in Oncology." Cell **179**(5): 1033-1055.
Statello, L., C. J. Guo, L. L. Chen and M. Huarte (2021). "Author Correction: Gene regulation by long non-coding RNAs and its biological functions." Nat Rev Mol Cell Biol **22**(2): 159.
States, J. C. (2015). "Disruption of Mitotic Progression by Arsenic." Biol Trace Elem Res **166**(1): 34-40.
Telles, E., A. S. Hosing, S. T. Kundu, P. Venkatraman and S. N. Dalal (2009). "A novel pocket in 14-3-3epsilon is required to mediate specific complex formation with cdc25C and to inhibit cell cycle progression upon activation of checkpoint pathways." Exp Cell Res **315**(8): 1448-1457.
Thompson, L. L., L. M. Jeusset, C. C. Lepage and K. J. McManus (2017). "Evolving Therapeutic Strategies to Exploit Chromosome Instability in Cancer." Cancers (Basel) **9**(11).
Tichon, A., R. B. Perry, L. Stojic and I. Ulitsky (2018). "SAM68 is required for regulation of Pumilio by the NORAD long noncoding RNA." Genes Dev **32**(1): 70-78.
Tijhuis, A. E., S. C. Johnson and S. E. McClelland (2019). "The emerging links between chromosomal instability (CIN), metastasis, inflammation and tumour immunity." Mol Cytogenet **12**: 17.
Ting, D. T., D. Lipson, S. Paul, B. W. Brannigan, S. Akhavanfard, E. J. Coffman, G. Contino, V. Deshpande, A. J. Iafrate, S. Letovsky, M. N. Rivera, N. Bardeesy, S. Maheswaran and D.

A. Haber (2011). "Aberrant overexpression of satellite repeats in pancreatic and other epithelial cancers." Science **331**(6017): 593-596.
Turner, K. J., V. Vasu and D. K. Griffin (2019). "Telomere Biology and Human Phenotype." Cells **8**(1).
Vargas-Rondon, N., V. E. Villegas and M. Rondon-Lagos (2017). "The Role of Chromosomal Instability in Cancer and Therapeutic Responses." Cancers (Basel) **10**(1).
Wickens, M., D. S. Bernstein, J. Kimble and R. Parker (2002). "A PUF family portrait: 3'UTR regulation as a way of life." Trends Genet **18**(3): 150-157.
Wilhelm, T., M. Said and V. Naim (2020). "DNA Replication Stress and Chromosomal Instability: Dangerous Liaisons." Genes (Basel) **11**(6).
Winkle, M., S. M. El-Daly, M. Fabbri and G. A. Calin (2021). "Noncoding RNA therapeutics - challenges and potential solutions." Nat Rev Drug Discov **20**(8): 629-651.
Wong, L. H., K. H. Brettingham-Moore, L. Chan, J. M. Quach, M. A. Anderson, E. L. Northrop, R. Hannan, R. Saffery, M. L. Shaw, E. Williams and K. H. Choo (2007). "Centromere RNA is a key component for the assembly of nucleoproteins at the nucleolus and centromere." Genome Res **17**(8): 1146-1160.
Wu, J., A. P. Ferragut Cardoso, V. A. R. States, L. Al-Eryani, M. Doll, S. S. Wise, S. N. Rai and J. C. States (2019). "Overexpression of hsa-miR-186 induces chromosomal instability in arsenic-exposed human keratinocytes." Toxicol Appl Pharmacol **378**: 114614.
Yamada, C., A. Morooka, S. Miyazaki, M. Nagai, S. Mase, K. Iemura, M. N. Tasnin, T. Takuma, S. Nakamura, S. Morshed, N. Koike, M. G. Mostofa, M. A. Rahman, T. Sharmin, H. Katsuta, K. Ohara, K. Tanaka and T. Ushimaru (2022). "TORC1 inactivation promotes APC/C-dependent mitotic slippage in yeast and human cells." iScience **25**(2): 103675.
Yoshino, Y., Z. Fang, H. Qi, A. Kobayashi and N. Chiba (2021). "Dysregulation of the centrosome induced by BRCA1 deficiency contributes to tissue-specific carcinogenesis." Cancer Sci **112**(5): 1679-1687.
Zasadil, L. M., E. M. Britigan, S. D. Ryan, C. Kaur, D. J. Guckenberger, D. J. Beebe, A. R. Moser and B. A. Weaver (2016). "High rates of chromosome missegregation suppress tumor progression but do not inhibit tumor initiation." Mol Biol Cell **27**(13): 1981-1989.

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

11. Footnotes

Funding Footnote: This work received no external funding.

Financial Disclosure: Authors declare no financial disclosures.

Figure Legend:

Figure 1: micro RNAs implicated in the generation of chromosomal instability. miR-22 directly regulates MDC1 through HR-mediated DNA repair and contributes to CIN. miR-26a, miR-28 and miR-186 regulates genes involved in cell cycle checkpoints and contributes to centrosome defect, aneuploidy, double minute, dicentric and ring chromosomes rendering CIN.

Conflict of interests: Authors declare no conflict of interests.

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1 – lncRNAs causative for chromosomal instability

Name	Target	Mechanism	Correlation to CIN	Refs
TERRA	Terc TERT Ku70/80 TRF1/2 Telomere	Transcribed from telomeres; Regulate telomere length via inhibition of telomerase (Terc, TERT), exonuclease degradation of chromosome ends (Ku70/80-EXO1), formation of telomeric heterochromatin (TRF2-ORC1) and protection of chromosome ends from DNA damage (DNA:RNA hybrids)	Negative/ Positive	(Schoeftner and Blasco 2008, Deng, Norseen et al. 2009, Redon, Reichenbach et al. 2010, Aguilera and Garcia-Muse 2012, Pfeiffer and Lingner 2012, Sagie, Toubiana et al. 2017, Mei, Deng et al. 2021)
cenRNA	CENP-A HJURP CENP-C INCENP Aurora B	Transcribed from centrosomal DNA; Assists kinetochore assembly via interaction with various centromere-associated proteins and the chromosome passenger complex (CPC)	Positive	(Murata-Hori and Wang 2002, Wong, Brettingham-Moore et al. 2007, Portella, Passaro et al. 2011, Kato, Jiang et al. 2013, Ideue, Cho et al. 2014, Quenet and Dalal 2014, McNulty, Sullivan et al. 2017)
Enhancer RNAs	AID	In B cells, AID off-targeting to regions other than the immunoglobulin loci has been related to convergent transcription of enhancer RNAs. Convergent transcription leads to formation of R loops, which impose genomic fragility if not resolved by the RNA exosome, leading to translocations between proto-oncogenes and the potent immunoglobulin enhancers.	Positive	(Meng, Du et al. 2014, Qian, Wang et al. 2014, Pefanis, Wang et al. 2015)
PCAT2	CENP-A, HIRA, DAXX	Transcribed from fragile 8q24 locus; causes local, ectopic recruitment of CENP-A and other centromere-associated proteins resulting in genome fragility	Positive	(Arunkumar, Baek et al. 2022)
Ginir and Giniras	Cep112 Brca1	Disruption of the Cep112-Brca1 interaction and downregulation of Cep112 and Brca1 causes centromere defects, chromosome missegregation and increased occurrence of DNA double strand breaks.	Positive	(Panda, Setia et al. 2018)

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

GUARDIN	miR-23a BRCA1-BARD1	Maintains TFR2 (shelterin complex component) expression via sponging of miR-23a to prevent telomere dysfunction. Acts as an RNA scaffold for BRCA1-BARD1, forming a ribonucleoprotein complex that influences double strand break repair.	Negative	(Hu, Jin et al. 2018)
CCAT2	BOP1 AURKB	Positively regulates BOP1 expression which in turn upregulates phosphorylation and activation of AURKB. Possibly mediates BOP1-AURKB interaction by scaffolding. Increased pAURKB disrupts chromosome-microtubule attachments and chromosome missegregation.	Positive	(Ling, Spizzo et al. 2013, Chen, Dragomir et al. 2020)
NORAD	PUMILIO (PUM1/PUM 2) SAM68	Mediates phase-separation of PUM1 and PUM2 proteins, which bind to NORAD via PUMILIO response elements and/or via SAM68. This in turn inhibits the repressive effect of PUMILIO proteins on mRNA targets involved in DNA damage repair and mitosis regulation.	Negative	(Lee, Kopp et al. 2016, Tichon, Perry et al. 2018, Elguindy and Mendell 2021),

Abbreviations

AKT1: AKT Serine/Threonine Kinase 1

APC/C: Anaphase Promoting Complex/Cyclosome

ATM: Ataxia-telangiectasia Mutated

AURKA: Aurora Kinase A

AURKB: Aurora Kinase B

BARD1: BRCA1 Associated RING Domain 1

BOP1: BOP1 Ribosomal Biogenesis Factor

Brca1: Breast Cancer Type 1 Susceptibility Protein

BUB1: BUB1 Mitotic Checkpoint Serine/Threonine Kinase

CCAT2: Colon Cancer-associated Transcript 2

CENP-A: Centromere Protein A

CENP-C: Centromere Protein C

cenRNAs: centromeric RNAs

Cep112: Centrosomal Protein 112

CHFR: Checkpoint With Forkhead And Ring Finger Domains

CIN: Chromosomal Instability

CPC: Chromosome Passenger Complex

CRC: Colorectal Cancer

EXO1: Exonuclease 1

GAR: Gly/Arg-Rich Domain

Ginir: Genomic Instability Inducing RNA

Ginir-asAntisense Genomic Instability Inducing RNA

GUARDIN: P53-responsive LncRNA

H2AX: H2A Histone Family Member Xÿ

H3K9: Histone H3 Lysine 9

HJURP: Holliday Junction Recognition Protein

ICF: "Immunodeficiency, Centromeric Instability And Facial Anomalies Syndrome"

INCENP: Inner Centromere Protein

KHDRBS1: KH RNA Binding Domain Containing, Signal Transduction Associated 1"

lncRNAs: Long Noncoding RNAs

MAD1: Mitotic Arrest Deficient 1

MAD2: Mitotic Arrest Deficient 2

MDC1: Mediator of DNA Damage Checkpoint 1

miRNAs: MicroRNAs

MSI: Microsatellite-instable

MSS: Microsatellite-stable

MYC: Proto-oncogene MYC

nCIN: Numerical Chromosomal Instability

ncRNAs: Noncoding RNAs

NORAD: Noncoding RNA Activated by DNA Damage

ORC1: Origin Recognition Complex Subunit 1

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

PLK1: Polo Like Kinase 1

PREs: PUMILIO Response Elements

PTEN: Phosphatase And Tensin Homologÿ

PUM1: PUMILIO Homolog 1

PUM2: PUMILIO Homolog 2

SAC: Spindle Assembly Checkpoint

sCIN: Structural Chromosomal Instability

Terc: Telomerase RNA Component

TERRA: Telomeric Repeat-containing RNAs

TERT: Telomerase Reverse Transcriptase

TRF2: Telomere Repeat Factor 2

VHL: Von Hippel-Lindau Tumor Suppressor

YWHAE: Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Epsilon

JPET Fast Forward. Published on September 27, 2022 as DOI: 10.1124/jpet.122.001357
This article has not been copyedited and formatted. The final version may differ from this version.

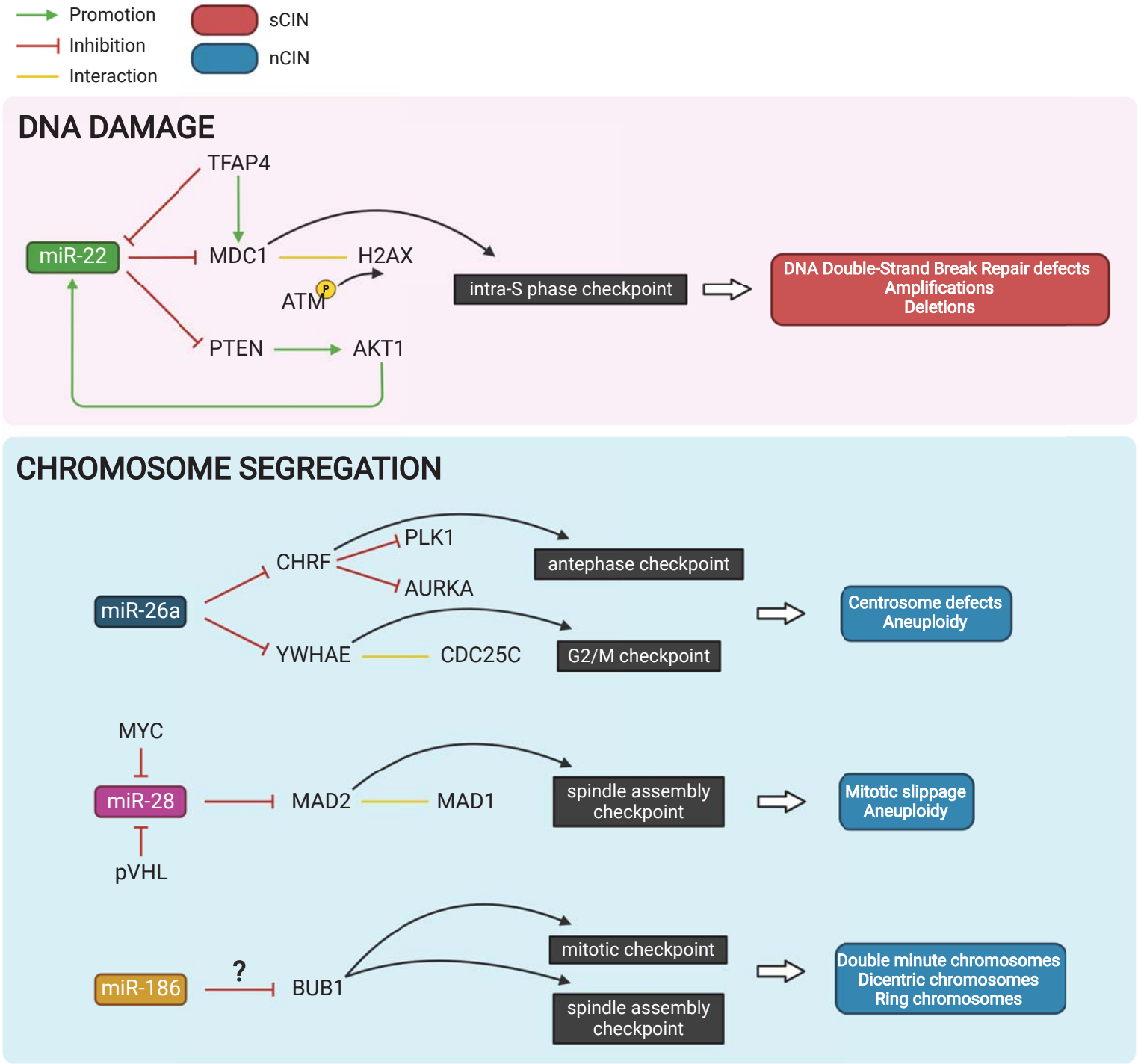

Figure 1