Noncoding RNAs in chromosomal instability in cancer

The role of noncoding RNAs in chromosomal instability in cancer

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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.
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.
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 acquisition 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
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\textit{Damage Checkpoint 1}) is an intra-S phase checkpoint protein that functions early in the DNA damage response, interacting with H2AX (\textit{H2A Histone Family Member X}) at DNA double strand breaks and recruiting ATM (\textit{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 (\textit{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 (\textit{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 (\textit{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 accordingly 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 antagonism (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 fis1multipolar, 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 Halazoneti 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
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 protooncogene MYC was identified as a negative 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 experimentally
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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
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
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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
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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 IncRNAs 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 IncRNAs 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 IncRNAs 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
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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 spatiotemporal 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 invasion 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 long noncoding RNA (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 chromosomal instability (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 IncRNA 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).
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Noncoding RNA Activated by DNA damage (NORAD) is a ubiquitously expressed, highly conserved IncRNA 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 IncRNA (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 IncRNA (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-separation 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
Noncoding RNAs 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

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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
10. References


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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.
Table 1 – lncRNAs causative for chromosomal instability

<table>
<thead>
<tr>
<th>Name</th>
<th>Target</th>
<th>Mechanism</th>
<th>Correlation to CIN</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>TERRA</td>
<td>Terc TERT</td>
<td>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)</td>
<td>Negative/Positive</td>
<td>(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)</td>
</tr>
<tr>
<td>Enhancer RNAs</td>
<td>AID</td>
<td>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.</td>
<td>Positive</td>
<td>(Meng, Du et al. 2014, Qian, Wang et al. 2014, Pefanis, Wang et al. 2015)</td>
</tr>
<tr>
<td>PCAT2</td>
<td>CENP-A, HIRA, DAXX</td>
<td>Transcribed from fragile 8q24 locus; causes local, ectopic recruitment of CENP-A and other centromere-associated proteins resulting in genome fragility</td>
<td>Positive</td>
<td>(Arunkumar, Baek et al. 2022)</td>
</tr>
<tr>
<td>Ginir and Giniras</td>
<td>Cep112 Brca1</td>
<td>Disruption of the Cep112-Brcal interaction and downregulation of Cep112 and Brcal causes centromere defects, chromosome missegregation and increased occurrence of DNA double strand breaks.</td>
<td>Positive</td>
<td>(Panda, Setia et al. 2018)</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>GUARDIN</th>
<th>miR-23a BRCA1-BARD1</th>
<th>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.</th>
<th>Negative</th>
<th>(Hu, Jin et al. 2018)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCAT2</td>
<td>BOP1 AURKB</td>
<td>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.</td>
<td>Positive</td>
<td>(Ling, Spizzo et al. 2013, Chen, Dragomir et al. 2020)</td>
</tr>
<tr>
<td>NORAD</td>
<td>PUMILIO (PUM1/PUM2) SAM68</td>
<td>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.</td>
<td>Negative</td>
<td>(Lee, Kopp et al. 2016, Tichon, Perry et al. 2018, Elguindy and Mendell 2021),</td>
</tr>
</tbody>
</table>

### 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
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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-as: Antisense Genomic Instability Inducing RNA

GUARDIN: P53-responsive LncRNA

H2AX: H2A Histone Family Member Xγ

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"

IncRNAs: 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
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PLK1: Polo Like Kinase 1
PREs: PUMILIO Response Elements
PTEN: Phosphatase And Tensin Homolog
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
Figure 1