Long noncoding RNAs at the crossroads of cell cycle and genome integrity

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Abstract

The cell cycle is controlled by guardian proteins that coordinate the process of cell growth and cell division. Alterations in these processes lead to genome instability, which has a causal link to many human diseases. Beyond the well-characterized role of protein-coding genes, an increasing body of evidence has revealed that long noncoding RNAs (lncRNAs) actively participate in the regulation of cell cycle and safeguarding of genome integrity. LncRNAs are versatile molecules that act via a wide array of mechanisms. In this review, we discuss how lncRNAs are implicated in the control of cell cycle and maintenance of genome stability and how changes in lncRNA-regulatory networks lead to proliferative diseases such as cancer.

Keywords: long noncoding RNAs, cell cycle, cell division, genome stability, cancer.
Emerging roles for lncRNAs in cell cycle control and genome stability

Multiple protein networks are involved in regulation of the cell cycle (Box 1), which is crucial to avoid the transmission of any errors during DNA replication and mitosis to the progeny. Alterations in any of these processes can lead to genome instability (see Glossary) which underlies many human diseases. One of the surprising discoveries of the postgenomic era revealed that only 2% of the mammalian genome codes for protein-coding genes, with long noncoding RNAs (lncRNAs) representing a significant portion of the human genome [1-3].

Despite more than 50,000 lncRNAs being annotated to date [2, 3], functional characterization has been performed only for a subset. However, evidence is accumulating that many lncRNAs play important roles in the regulation of cell cycle and genome stability [4, 5]. Understanding the role of lncRNAs in this context will not only provide new insights into regulation of fundamental cellular processes but also reveal novel pathogenetic mechanisms that contribute to human diseases such as cancer.

LncRNAs are a heterogenous class of RNA molecules that are transcribed by RNA polymerase II (Pol II), are longer than 200 nucleotides and have low coding potential [6]. LncRNAs exhibit cell-type and tissue-specific expression, not only in healthy conditions, but also in pathological states, and are subjected to rapid turnover during evolution [5, 7]. LncRNAs can regulate numerous cellular processes including cell cycle, proliferation, apoptosis and DNA damage response (DDR), all pathways whose dysregulation has been associated with cancer [4, 8, 9]. Mechanisms through which lncRNAs regulate these pathways include transcriptional and post-transcriptional gene regulation, chromatin organization, cell signalling and post-translational regulation of protein activity [5, 10]. LncRNAs are known to exhibit distinct subcellular localization [11], being found in the cytoplasm and/or nucleus where changes in lncRNA subcellular localization often have a profound effect on their function. Indeed, the biological significance of lncRNAs cellular distribution on the DDR is starting to emerge [12-16].
Expression of lncRNAs is deregulated in cancer and many cancer-associated lncRNAs are implicated in the cell cycle progression and maintenance of genome stability [2, 5, 17], whose misregulation is the hallmark of cancer [8]. In this review, we discuss recent findings that shed light on how lncRNAs control cell cycle and maintain genome stability. We highlight emerging lncRNA-mediated mechanisms in these processes (Fig. 1, Key Figure and Fig. 2) to better understand how alterations in lncRNA-regulatory networks contribute to human diseases such as cancer. For the purpose of this review we will categorize lncRNAs based on specific stages of the cell cycle where they have been implicated in, including quiescence (Box 2), DNA replication and cell division (Table 1), and those that directly or indirectly contribute to the DDR and maintenance of genome stability (Table 2).

**Box 1. An overview of the cell cycle regulation (see Figure for Box 1)**

The cell cycle is a tightly controlled process of cell growth and cell division that is required for normal development and tissue homeostasis, a process usually altered in cancer [18]. The mammalian cell cycle is divided into four main stages: G1 (gap 1), S-phase (DNA synthesis), G2 (gap 2) and mitosis (M, cell and nuclear division). Quiescence (G0), the fifth stage of the cell cycle, is a reversible cell cycle arrested state where cells cannot proliferate but still maintain their ability to re-enter the cell cycle and resume proliferation. The main players of the cell cycle are four different classes of cyclins (the A-, B-, D- and E-type), CDKs, CDKi, retinoblastoma protein (Rb)- and p53-related pathways. Different CDKs have distinct roles during cell cycle progression and their activities are regulated not only by cyclins, but also by phosphorylation or dephosphorylation. For example, CDK4 and 6 are active during G1 phase, CDK2 functions during both G1 and S phase while CDK1 kinase is active during G2 and mitosis. In turn, INK4 (p16INK4a, p15INK4b, p18INK4c and p19INK4d) and/or Cip/Kip (p21Cip1, p27Kip1 and p57Kip2) are families of CDKi, which can bind to cyclin-CDK complexes and inhibit their activities in specific stage of the cell cycle. While p21Cip1, p27Kip1 and p57Kip2 inhibit a broader spectrum of cyclin-CDK complexes, p15INK4b, p16INK4a, p18INK4C and p19INK4D specifically inhibit CDK4/6 activity.
In early G1-phase D-type cyclins (cyclin D1, D2 and D3) bind to CDK4/CDK6, which is essential for CDK activation and for cells to enter the G1-phase of the cell cycle. The Rb tumor suppressor protein, which binds and inhibits the E2F transcription factors, is a target of cyclin D-CDK4/6 and cyclin E-CDK2 complexes. Phosphorylation of Rb leads to dissociation of Rb-E2F complex and activation of the E2F-dependent transcription of cell cycle genes. As the cell cycle progresses, CDK2 can now bind to cyclin E to control entry to S-phase or cyclin A during S-phase. In the G2-phase of the cell cycle, cyclin A binds to CDK1 and promotes cell cycle progression during the G2-phase. Finally, for cells to enter mitosis cyclin A is replaced by cyclin B and cyclin B-CDK1 complex is responsible for triggering mitosis where replicated chromosomes are equally distributed to daughter cells. To exit mitosis, cyclin B is degraded leading to inactivation of the cyclin B-CDK1 complex.

LncRNAs regulating G1-phase of the cell cycle

The first study that identified cell cycle-regulated lncRNAs examined by custom microarrays noncoding RNAs (ncRNAs) transcribed from 56 cell cycle-linked loci, and identified lncRNAs periodically expressed during the cell cycle in human cells [19]. The expression of some of these lncRNAs was found to be responsive to DNA damage and deregulated in human cancers. The cell cycle-dependent expression of lncRNAs was also confirmed using RNA sequencing (RNA-seq) in human cervical cancer cell line (HeLa) [20]. The FANTOM 6 project, which used antisense LNA-modified GapmeR antisense oligonucleotide (ASOs) to deplete 285 lncRNAs in human primary fibroblasts, identified several lncRNAs associated with cell cycle defects further supporting the role of lncRNAs in cell cycle progression [21]. By analyzing cell cycle-staged single-cell RNA-seq data from mouse embryonic stem cells (ESCs), which have a shorter G1-phase, lncRNAs were shown to regulate cell cycle and contribute to cell type-specific adaptations of ubiquitous processes [22]. Another study performed deep RNA-seq on synchronized human cancer cells and identified >2000 lncRNAs that had cell cycle phase-specific expression [23]. In particular, 42% of genes that had elevated expression in
specific stage of the cell cycle were ncRNAs with the majority being IncRNA genes. Interestingly, most of these IncRNAs had higher expression in the G1-phase. Although differential expression alone does not prove a role in the cell cycle, it is reasonable to hypothesize that some of these cell cycle-regulated IncRNAs might play a role in G1/S transition.

Besides analyses of IncRNA expression during the cell cycle, the link between individual IncRNAs and G1/S transition has been specifically investigated. These G1-phase related IncRNAs control expression of cyclins, CDKs, CDKi and tumor suppressor genes by acting as protein scaffolds or as transcriptional, post-transcriptional or epigenetic regulators. For example, ncRNA_{CCND1} (ncRNA transcribed upstream of the CCND1 gene) negatively regulates CCND1 transcription in human cells through recruitment and activation of the RNA binding protein (RBP) TLS (Translocated in LipoSarcoma), leading to cell cycle arrest [24]. NcRNA_{CCND1}, which is transcribed from the promoter region of CCND1 is also the first example of IncRNA expressed upon DNA damage. LncRNA LAST (LncRNA-Assisted Stabilization of Transcripts), which cooperates with the single-stranded DNA/RNA-binding factor CNBP, also controls CCND1 function in human cells by regulating its mRNA stability [25]. Furthermore, overexpression of tumor suppressive IncRNA GAS5 (Growth Arrest Specific 5) leads to G1 cell cycle arrest and inhibition of cell proliferation in human cancer cells via negative and positive regulation of CDK6 [26] and P27^{kip1} levels [27], respectively. Additional examples of IncRNAs controlling G1-phase through post-transcriptional mechanisms are Gadd7 (growth-arrested DNA damage-inducible gene 7) [28, 29], CCAT1 (colon cancer-associated transcript-1) [30-32] and MIR100HG [33].

In addition to cyclins and CDKs, IncRNAs can also regulate CDKi levels. Together with ARF (alternate reading frame), p16^{INK4a} and p15^{INK4b} genes are located within the INK4/ARF locus, which is critical for cell cycle progression. Given the importance of the INK4/ARF locus in tumor suppression [34], its transcription must be tightly regulated. LncRNA ANRIL (antisense non-coding RNA in the INK4 locus, p15AS or CDKN2B-AS1) is involved in epigenetic repression of the INK4/ARF locus, and is also required for cell cycle progression in
human cells. Mechanistically, ANRIL recruits Polycomb Repressive Complex 1 and 2 (PRC1/PRC2) to the INK4/ARF locus resulting in a decrease of $p15^{ink4b}$ and $p16^{ink4a}$ levels [35, 36]. Another study showed that KNCQ1OT1, an imprinted IncRNA, regulates $p57^{kip2}$ by binding to PRC2 and G9a chromatin regulators, which through epigenetic modifications repress $p57^{kip2}$ transcription [37, 38].

Rb-mediated E2F regulation has a key role in controlling G1-phase. Many IncRNAs act downstream of E2F1 and contribute to E2F1-regulatory networks such as H19 [39], ANRIL [40], MA-linc1 (mitosis-Associated Long Intergenic Non-Coding RNA 1) [41] and ERIC (E2F1-Regulated Inhibitor of Cell death) [42]. For example H19 IncRNA, which acts as a primary miRNA precursor generating miRNA-675, downregulates Rb [43] and promotes breast cancer cell proliferation [39].

Some IncRNAs such as MALAT1 (Metastasis Associated Lung Adenocarcinoma Transcript 1) [44] and MA-linc1 [41] can affect more than one phase of the cell cycle. MALAT1 is an abundant IncRNA that functions in G1/S transition and mitosis, and is deregulated in cancer [45-47]. p53 is a downstream mediator in MALAT1 function and is required to induce G1- or S-phase arrest in MALAT1-depleted cells [44]. MALAT1’s role in G1/S transition is independent of its function in mitosis, which is consistent with MALAT1 expression being highest during the G1/S and M-phase. In addition to MALAT1, expression of LAST and MIR100HG is also peaking in the G1-phase indicating that expression of some IncRNAs is closely associated with its specific cell cycle stage function.

Since dysregulation of IncRNAs involved in G1/S transition have been observed in cancer, cardiovascular and overgrowth syndromes [26, 30-33, 38, 39, 41, 43, 45-48], these findings indicate that IncRNA-mediated cell cycle regulation has an important impact on physiological processes as well as pathogenesis of diseases.

**LncRNAs regulating S-phase of the cell cycle**

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The role of lncRNAs in S-phase progression is less characterized compared to lncRNAs involved in G1-phase. An elegant study by Marchese et al. showed that CONCR (COhesion regulator NonCoding RNA), a lncRNA with peak expression in mid-late G1-phase, controls DNA replication and cell cycle progression [49]. CONCR interacts with and regulates the activity of a DNA helicase involved in DNA replication, and its depletion leads to sister chromatid cohesion and S-phase progression defects in human cells. Moreover, CONCR is activated by transcription factor MYC and its upregulation is observed in several cancers [49]. Similarly, SUNO1 (S-phase-upregulated Non-coding-1) is a lncRNA whose levels are enriched during G1/S and early S-phase and SUNO1-depleted cells display S-phase progression defects in human cell lines [23]. Mechanistically, SUNO1 promotes transcription of genes involved in cell proliferation by stabilizing the interaction between the RNA helicase DDX5 and Pol II on chromatin. In keeping with its role in promoting cell survival, increased levels of SUNO1 have been associated with tumorigenicity in colorectal mucosa and poor colon cancer prognosis. Despite SUNO1-depleted cells being sensitive to DNA damaging agents, its function in the DDR remains to be identified. Since other lncRNAs were shown to bind DNA/RNA helicases [50, 51], it remains to be seen whether the interaction between lncRNAs and DNA/RNA helicases is a common mechanism for lncRNAs to regulate S-phase.

By using nascent RNA-seq in HeLa cells, Ali et al. identified S-phase specific lncRNAs with functions in cell cycle control and cancer [52]. LncRNA SCAT7 (S-phase Cancer-Associated Transcript) regulates the FGF/FGFR signalling and affects proliferation, invasion and migration. Depletion of SCAT7 led to reduced FGF/FGFR signalling, which in turn affected expression of cell cycle genes such as CCND1. This work also represents a valuable resource of lncRNA-based oncogenic drivers with potential prognostic value. Subsequently, a similar study identified S-phase enriched lncRNAs in the non-transformed diploid cell line hTERT-RPE1, and showed diverse regulatory potential of linc00704, MIAT and LUCAT1 lncRNAs in S-phase progression [53].

Taken together, these findings indicate that lncRNAs involved in S-phase progression act either as effectors of DNA/RNA helicases or as regulators of pivotal signaling pathways.
LncRNAs regulating G2/M transition of the cell cycle

Growing evidence suggests that RNA-based mechanisms contribute to the fidelity of cell division through the activity of RBPs and different ncRNAs present on the mitotic spindle, kinetochores and centrosomes, the major components of the mitotic apparatus [54]. Although centromeric lncRNAs have been linked to chromosome segregation and kinetochore formation in Drosophila [55], the majority of lncRNAs are not present on mitotic chromatin [11, 56]. A Mitocheck consortium used a genome-wide siRNA library targeting 21,000 protein-coding genes and identified new proteins in cell division [57]. Once RNAi, ASO and CRISPR/Cas9-based libraries became available for lncRNAs, similar approaches were employed to identify lncRNAs involved in different cellular processes including cell division (Box 3). By targeting 600 cancer-relevant lncRNAs and analyzing their impact on cell division in HeLa Kyoto cells, linc00152 was identified as regulator of mitosis whose function was linked to the microtubule cytoskeleton and ubiquitin pathway [58]. However, no mitotic defects were observed in other human cell lines after linc00152 depletion, possibly due to lncRNA cell type-specific functions [59, 60]. A second study performed a larger RNAi imaging screen by depleting 2231 lncRNAs in HeLa cells, and identified lncRNAs with key roles in mitotic progression, chromosome segregation and cytokinesis [56]. These include linc00899 and C1QTNF1-AS1 that regulate mitosis through diverse mechanisms and whose expression is also altered in cancer [61, 62]. For instance, nuclear linc00899 was shown to regulate mitotic progression in multiple cell lines by binding and suppressing the transcription of TPPP, a microtubule-binding protein. By identifying lncRNAs with a role in mitosis and cytokinesis, these screens represent the basis for future studies aimed to understand how lncRNAs govern the fidelity of cell division.

Besides regulating the mitotic microtubule network certain lncRNAs control cell division by mediating interactions between proteins involved in mitosis. For example, lncRNA GiniR (antisense transcripts Genomic Instability Inducing RNA) [15] interacts with centrosomal protein Cep112 and BRCA1, a protein involved in DNA repair [63] and centrosome biogenesis.
Overexpression of GiniR in mouse fibroblasts leads to mitotic defects and genome instability. Intriguingly, Ginir was found to be cytoplasmic in normal and nuclear in transformed cells indicating that nuclear localization of Ginir might contribute to its oncogenic nature [15].

In parallel, IncRNAs were shown to modulate activities of protein kinases by acting as chaperones, allosteric regulators or preventing their post-translational modifications [10]. Polo-Like Kinase 1 (PLK1) is a major regulator of mitosis, whose spatio-temporal activity is central to error-free chromosome segregation [65]. Several IncRNAs such as IncRNA-RI (IncRNA Radiation Induced) [66] and APAL (Aurora A/Polo-like-kinase 1 [PLK1]–associated IncRNA) [67] regulate PLK1 expression or activity. OIP5-AS1 IncRNA was shown to regulate stability of cyclin G Associated Kinase (GAK), which is important for mitotic progression [68]. Depletion of IncRNA-RI, APAL and OIP5-AS1 led to mitotic defects indicating that IncRNA-mediated regulation of mitotic kinases is essential for error-free cell division. By exploring the role of IncRNAs in skeletal myogenesis, IncRNA SAM (Sugt1 associated muscle) was shown to regulate proliferation of mouse muscle stem cells and myoblasts [69]. Since loss of SAM induced degradation of Sugt1, a protein involved in kinetochore assembly, these data suggest that SAM-Sugt1 interaction is required for fidelity of cell division.

In addition to G1/S transition roles described above, MALAT1 [44] and MA-linc1 [41] can also regulate mitosis. For example, MALAT1 controls the expression of B-MYB, a transcription factor regulating expression of genes involved in mitosis. Notably MALAT1-depleted cells show mitotic defects and reduced levels of B-MYB due to its aberrant splicing. Differently from MALAT1 and other IncRNAs involved in G1/S progression whose levels are cell cycle regulated [23, 25, 33, 44, 49, 52, 53], the expression of IncRNAs involved in G2/M phase does not seem to follow the same pattern. The reason for this could be either poor quantification of mitosis-specific levels of IncRNAs due to the shorter duration of mitosis or the IncRNA concentration is less crucial in mechanisms controlling the G2/M transition.

In summary, IncRNAs control cell division by regulating gene expression or modulating activities and stability of mitotic proteins. Their expression is often altered in cancer [41, 45, 47, 58, 61, 62, 67, 70] similarly to IncRNAs involved in other cell cycle stages.
Box 2. LncRNAs involved in cell cycle quiescence (G0)

Quiescence is a state of reversible proliferative arrest where cells are not dividing but still have the ability to re-enter the cell cycle upon receiving an appropriate stimulus [71]. Several lncRNAs have been showed to act in the G0 maintenance or in G0/G1 transition. LncRNA PAPAS (promoter and pre-rRNA antisense) was found to be upregulated in quiescent mouse embryonic fibroblasts (MEFs) and human cells [72]. By interacting with a histone lysine methyltransferase, PAPAS facilitates chromatin compaction in growth-arrested cells at specific genomic loci, contributing to the maintenance of a G0-specific transcriptional program. Another example is a PDCD4-AS1 lncRNA, which is expressed in human quiescent cells together with its sense counterpart, the tumor suppressor PDCD4. PDCD4-AS1 increases the stability of PDCD4, which by negatively regulating CCND1 expression, promotes the maintenance of G0-phase [73]. Similarly to PDCD4, expression of PDCD4-AS1 correlates with overall survival of triple negative breast cancer (TNBC) patients, indicating a tumor suppressive role also for PDCD4-AS1.

Quiescence has also been associated with cancer stemness, which protects cancer stem cells (CSCs) from antiproliferating anticancer drugs leading to CSC-mediated resistance to conventional therapies [74]. Several lncRNAs such as GAS5 have been identified as key players in maintaining the “stemness” state of tumors [75]. In addition, GAS5 was found to be highly expressed in a small fraction of human pancreatic cancer cells and its depletion released these cells from the cell cycle arrest [76]. Another study found lncRNA DANCR (Differentiation Antagonizing Non-Protein Coding RNA) to be upregulated in human leukemia stem cells (LSCs), which have abnormal self-renewal capacity and increased chemotherapy resistance [77]. Depletion of DANCR in LSCs led to decreased stem cell renewal and quiescence by downregulation of WNT pathway. Serum stimulation can also promote cell cycle re-entry and induce expression of lncRNAs regulating G0/G1 transition. For example, increased level of MIR222HG in serum-stimulated human fibroblasts dictates the cell cycle re-entry post quiescence by binding to ILF3/ILF2 RBPs and promoting stability of its interacting mRNAs [78].
Given the growing evidence of quiescence-specific IncRNAs and their link to cancer stemness, future studies will be required to address the mechanism how IncRNAs control the cell cycle re-entry and identify the upstream signalling networks that regulate IncRNA expression at the quiescence and proliferation crossroad.

Box 3. High-throughput methods for functional profiling of IncRNAs

RNAi-, ASO- and CRISPR-based technologies have been used to perturb IncRNA expression and identified IncRNAs involved in pluripotency, differentiation, proliferation, growth, signaling, cell division and resistance to targeted therapeutics. One of the first loss-of-function (LOF) approaches was a high-throughput shRNA screen targeting 226 IncRNAs in mouse ESC [79] indicating the importance of IncRNAs in maintenance of the pluripotency. Subsequently, other shRNA screens identified IncRNAs involved in pluripotency [80] and mouse fibroblast proliferation [81]. To identify IncRNAs involved in cell division, two RNAi-based screens were coupled with immunostaining [56] or live-cell imaging in human cancer cells [58].

CRISPR-Cas9 technology has been a break-through for manipulating IncRNA expression. The CRISPR-deletion screen targeted 700 cancer-associated IncRNAs and identified 51 candidates involved in cell proliferation of liver cancer cells [82]. An alternative CRISPR screen exploited sgRNA libraries targeting IncRNA splice sites and led to identification of IncRNA-mediated phenotypes in different cellular models [83]. A large CRISPR interference (CRISPRi)-based screen targeted 16,041 IncRNAs in seven human cell lines and identified 499 IncRNAs affecting cell growth [59]. Interestingly, majority of IncRNAs had a phenotype only in one cell type indicating that IncRNAs can have cell-type specific functions regardless of their expression [59, 60]. A CRISPRi screen was also used to identify IncRNAs as new therapeutic targets in human glioblastoma [60]. Simultaneously, gain-of function (GOF) methods based on CRISPR-activation (CRISPRa) or CRISPR/Cas9 synergistic activation mediator (SAM) system have identified IncRNAs involved in AKT signaling [84] and drug resistance in melanoma [85] or acute myeloid leukemia (AML) [86].
using human breast, melanoma and AML cell lines, respectively. Since majority of CRISPR screens have been performed in cancer cell lines, it is possible that cell-type specific function of lncRNAs could be due to higher mutational load of cancer cell lines and their ongoing instability [87, 88].

However, CRISPR-based approaches have some limitations. For instance, CRISPR-screen targeting lncRNA splice sites can result in false positives [89], while CRISPRi can inadvertently affect expression of neighboring genes [90] or lncRNAs close to other transcriptional units [91]. Instead, the FANTOM 6 project used ASOs to deplete lncRNAs in human primary fibroblasts and analyzed the effect of lncRNA depletion on cell growth and morphology [21]. They found a concordance between RNAi and ASO-mediated phenotype only for 3 out 9 lncRNAs. Since each LOF method can give rise to method-specific off-target effects [92], multiple LOF and GOF methods should be used when analyzing the function of individual lncRNAs [6].

**LncRNAs that safeguard genome integrity**

Maintenance of genome stability depends on DNA replication fidelity, DNA repair and accurate chromosome segregation throughout the cell cycle. Defects in any of these processes can lead to genome instability, a hallmark of cancer, aging and other diseases [8, 93]. Genome instability is known to activate the multifaceted DDR pathway, an integrated protein signaling network in charge of detecting DNA damage and triggering its repair (Box 4). Recent studies suggest that lncRNAs play an important role in this context by establishing multiple interactions with DNA, RNAs or proteins through which they can control different pathways impinging on genome stability [9].
Box 4. An overview of the multifaceted DNA damage response pathway (see Figure for Box 4)

DDR is a genome stability surveillance system consisting of DNA repair proteins, tumor suppressor genes, DNA damage-induced kinases, cell cycle checkpoints and apoptosis genes, which operate collectively to prevent the deleterious consequences of DNA damage [93]. DDR activation leads to cell cycle arrest to allow repair of the DNA damage or apoptosis to remove cells bearing severe levels of DNA damage. Deficiency in DDR is associated with increased mutational load, genome instability and a variety of human disease such as neurodegeneration, immunodeficiencies, cancer and aging. DDR pathway is often deregulated in cancer cells and leads to resistance to DNA damage inducing agents, presenting a hurdle to successful cancer treatment.

Treatment of cells with different DNA damaging agents such as ultraviolet (UV) and ionizing radiation (IR), topoisomerase poisons, alkylating and crosslinking agents activate specific DNA repair pathways to physically remove the damage. This includes base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination (HR) and non-homologous end joining (NHEJ). The main DNA damage sensors are ATM, ATR and DNA-PKcs kinases, which are activated by DNA damage and replication stress and initiate DNA damage signalling. A key factor in the DDR is also the tumor suppressor p53 whose function is conserved across all vertebrates [94]. p53 acts as a transcriptional regulator to control cell cycle arrest and apoptosis upon DNA damage.

Most studies have focused on proteins involved in DDR pathway but recent evidence indicate unforeseen and important functions of RNA-based mechanisms in different stages of the DDR. In particular, IncRNAs have emerged as key players in DNA damage signalling and DNA repair, specifically in cancer cells [9]. Many of the IncRNAs involved in cell cycle control are also involved in DDR and are often deregulated in cancer. Indeed, many IncRNAs have cancer type-specific aberrant expression and are involved in the onset and progression of cancer [17]. Expression of many IncRNAs is directly induced by DNA damage or alternatively damaging agents are used to activate p53, which then activates IncRNA expression. These
**lncRNAs are involved in cell cycle arrest or apoptosis indicating their key roles in the DDR pathway. Understanding the varied mechanisms by which lncRNAs control cell cycle, DDR and DNA repair should provide new insights into the origins of cancer as well as the molecular basis for chemo- or radio-resistance, which in the future may open new RNA-based avenues for anti-cancer interventions.**

Increasing evidence suggests that many lncRNAs have important roles in p53 signalling, where lncRNAs can control expression or activity of p53, or are regulated by p53 and mediate the p53 response [95]. The lncRNA **PANDA** (*p21 Associated NcRNA DNA damage Activated*) is transcribed antisense to *p21*<sup>Cip1</sup>, a p53 target and a key regulator of G1/S progression, and its levels are induced upon DNA damage in a p53-dependent manner [19]. **PANDA** interacts with transcription factor NF-YA and inhibits apoptosis in human fibroblasts. **DINO** (*Damage Induced Noncoding*) is another lncRNA induced upon DNA damage in human fibroblasts and human cancer cells [96]. **DINO** is required for p53-dependent gene expression, cell cycle arrest and apoptosis and its depletion abrogates DNA damage signalling. Similar to **PANDA** [97], **DINO** can bind to and promote p53 protein stabilisation. Thus, by binding to transcription factors, lncRNAs contribute to DDR by creating a feedback loop to amplify cellular signalling networks.

**LincRNA-p21** is another p53-regulated lncRNA, which triggers p53-dependent apoptosis upon DNA damage in human and mouse fibroblasts [98]. While nuclear **lincRNA-p21** regulates p53-dependent apoptosis and cell cycle arrest upon DNA damage by binding to RBP hnRNP-K [98, 99], cytoplasmic **lincRNA-p21** is involved in translation and hypoxia-enhanced glycolysis [100, 101]. **SNHG1** (*small nucleolar RNA host gene 1*) is another example how lncRNA subcellular distribution can affect p53 regulatory networks upon DNA damage. **SNHG1** is retained in the nucleus after DNA damage in human cancer cells to compete with p53 for binding to RBP hnRNPC [12] leading to higher p53 levels and apoptosis.

Since the majority of human cancers acquire mutations that abrogate p53 function, it is not surprising that expression of p53-regulated lncRNAs is also altered in cancer [102-105].
For example, lncRNA *LINC-PINT*, which is induced upon DNA damage [106] is downregulated in multiple types of cancer [107]. Furthermore, *LINC-PINT* has tumor suppressive function by inhibiting invasion and migration of human colon and lung cancer cells [107]. *NEAT1*, a nuclear IncRNA acting as an integral part of paraspeckles, is another p53-regulated IncRNA involved in a negative feedback loop that attenuates oncogene-dependent activation of p53 [108].

Depletion of *NEAT1* leads to replication stress and its targeting can increase the sensitivity of human breast cancer cells to genotoxic chemotherapeutics. Similarly, mouse *Neat1* was also shown to be a p53 target by suppressing transformation and cancer initiation [109]. In addition, *NEAT1* was also shown to be deregulated in various cancers and its dysregulation is linked to tumor progression [110, 111].

Several lncRNAs such as *lincRNA-RoR* (*Regulator of Reprogramming*) and *MEG3* (*human maternally expressed gene 3*), act upstream of p53 to fine-tune p53 levels following DNA damage. *LincRNA-RoR* negatively regulates p53-mediated apoptosis in human induced pluripotent stem cells [112], and suppresses p53 mRNA translation in human cancer cells [113]. High expression of *LincRNA-RoR* has been observed in human breast cancer where it correlates with worse survival, suggesting an oncogenic function for *lincRNA-RoR* [114]. On the other hand, the imprinted lncRNA *MEG3* binds and activates p53 targets to induce cell cycle arrest and apoptosis in human cancer cells [115, 116]. However, in cardiac fibroblasts *MEG3* does not affect the p53 response indicating that *MEG3* regulates p53 in a cell type-specific manner [117]. *MEG3* can also protect endothelial function by regulating DDR [118], and is downregulated in multiple cancers where *MEG3*’s tumor suppressive role was linked to its tertiary structural elements [119].

Several other lncRNAs can contribute to DDR independently of p53. One such lncRNA is *DDSR1* (*DNA damage-sensitive RNA1*), which is induced by DNA damage in an ATM-dependent manner [120]. *DDSR1* binds to BRCA1 and hnRNPUL1, a RBP involved in DNA repair [121] (Fig. 2a). Depletion of *DDSR1* impairs DDR by increasing the accumulation of BRCA1 at the laser-induced double-strand breaks (DSBs). Induction of *ANRIL* upon DNA damage in human fibroblasts and cancer cells is also ATM dependent and increased levels of
ANRIL were shown to suppress the expression of \textit{INK4B–ARF–INK4A}, forming a negative feedback loop to the DDR [40]. Recently \textit{HITT} (\textit{HIF-1\textalpha{} inhibitor at translation level}) was identified as the first IncRNA that binds and inhibits ATM activation in human cancer cells [122]. \textit{HITT} is downregulated in many cancers and can restrain HR activity, which might have a role in sensitising cancer cells to genotoxic treatments. The expression of some IncRNAs such as estrogen-regulated \textit{CUPID1} and \textit{CUPID2} (\textit{CCND1-upstream intergenic DNA repair 1 and 2}) can be affected by \textit{single-nucleotide polymorphisms} (SNPs) that contribute to the risk of developing breast cancer by modulating pathway choice of DSB repair [123].

In addition to \textit{DDSR1} and \textit{HITT}, other IncRNAs can also serve as a platform for DDR proteins and thus directly contribute to DNA repair. For instance, IncRNA \textit{PRLH1} (\textit{p53-regulated IncRNA for HR repair 1}) regulates HR-mediated DSB repair in human liver cancer cells through binding and stabilization of RNF169, an E3 ubiquitin ligase which acts as a negative regulator of the ubiquitin-dependent signaling at sites of DNA damage [124]. \textit{MALAT1} was shown to bind PARP1 and Ligase III [47], key proteins in \textit{alternative NHEJ} (alt-NHEJ) [125], and its depletion inhibits alt-NHEJ activity. Another example is \textit{LINP1} (\textit{IncRNA in nonhomologous end joining (NHEJ) pathway 1}) that can enhance DSB repair in human breast cancer cells by acting as a scaffold for DNA-PKcs and Ku70/80, major players of the NHEJ [126] (Fig. 2b). Indeed, \textit{LINP1} depletion leads to reduced level of DNA-PKcs after DNA damage and increased the sensitivity of human breast cancer cells to radiation treatment, indicating that \textit{LINP1} is a functional IncRNA regulating DDR in TNBC. Furthermore, \textit{LINP1} is overexpressed not only in TNBC but also other cancers [16, 127] suggesting that \textit{LINP1}-dependent repair mechanism is a broad mechanism present in multiple cancers. Interestingly, \textit{LINP1} is another IncRNA whose cellular distribution changes upon DNA damage, with \textit{LINP1} translocating from the cytosol to the nucleus upon IR treatment of HeLa cells [16]. Recently, \textit{LINP1} was shown to be a flexible RNA molecule that can self-assemble into phase-separated condensates through RNA-RNA interactions and interact with Ku70/80 [128]. Furthermore, \textit{LINP1} can substitute PAXX, a protein involved in NHEJ that binds Ku70/80 and is recruited to DNA damage sites [129]. This work [128] together with structural studies on \textit{MEG3} [119]
represents the starting platform for future studies to unravel how lncRNA structure-to-function relationships impact lncRNA function in the cell cycle and in genome stability.

Some lncRNAs can also be recruited to the site of DNA damage or are directly formed at the DNA lesion sites to contribute to the DDR. Within 5 minutes after generation of DSBs, BGL3 IncRNA localizes to the chromatin and accumulates at the site of DNA damage in human cancer cells [14] (Fig. 2c). BGL3 binds to PARP1 and BARD1, key regulators of DDR [130]. Accordingly, BGL3 depletion causes genome instability and also sensitises cells to DNA damaging agents. Thus, BGL3 is required for HR and acts as RNA scaffold to enhance interaction between BRCA1/BARD1 complex and other DDR proteins. GiniR is another lncRNA that binds BRCA1 and its high levels can impair the interaction between BRCA1 and Cep112 leading to mitotic defects and genome instability [15]. Intriguingly, these studies indicate that the BRCA1/BARD1 complex is a very common interactor of lncRNAs, which is consistent with the findings that many DNA repair proteins have RNA-binding capacities [131].

DSBs can also serve as a promoter for Pol II and induce the synthesis of damage-induced lncRNAs (dilncRNAs) in mouse and human cells [132], which contribute to efficient DNA repair indicating the importance of site-specific ncRNAs in DDR (Fig. 2d).

Compelling evidence of how lncRNAs contribute to DDR comes from a study on IncRNA NORAD (noncoding RNA activated by DNA damage), which act as a decoy for RBPs to maintain genome stability. Inactivation of NORAD leads to increased rate of chromosome segregation errors resulting in aneuploidy and chromosomal instability (CIN) in human normal and cancer cells [133, 134] (Fig. 2e). NORAD is a conserved cytoplasmic IncRNA that maintains genome stability by sequestering PUMILIO proteins, RBPs that repress expression of genes involved in mitosis, DNA replication and repair [133-135]. Interestingly, deletion of Norad in mice leads to premature aging, aneuploidy, and mitochondrial defects [136]. Since genome instability is a hallmark of cancer and aging, the Norad-Pumilio regulatory axis could have an important role in the maintenance of genome stability for normal physiology and aging. Other NORAD-mediated mechanisms in genome stability have also been described. These include the formation of the high-order ribonucleoprotein complex with NORAD binding
to proteins involved in DNA replication and repair [13]. Although the origin of chromosome segregation errors in NORAD-depleted cells has not been determined, cells without NORAD exhibit reduced replication speed suggesting that these errors could be a consequence of DNA replication defects [13]. Notably, NORAD can change its localization to the nucleus upon DNA damage in human colon cancer cells [13], although another study did not observe a similar re-distribution [135]. In addition to NORAD, CCAT2 (Colon cancer associated transcript 2) is another lncRNA that contributes to CIN in human colon cancer cells [137, 138] and promotes colon cancer progression [32].

Some lncRNAs can have pleiotropic roles in genome stability, as observed with some lncRNAs involved in cell cycle regulation [41, 44]. One of them is lncRNA GUARDIN that regulates genome stability through two mechanisms [139] (Fig. 2f). Cytoplasmic GUARDIN acts as a miRNA sponge and indirectly contribute to DNA repair by stabilizing the expression of TRF2, an essential factor for telomerase protection. Simultaneously, GUARDIN promotes DNA repair by promoting the formation of the BRCA1/BARD1 complex, which regulates HR [63]. Accordingly, GUARDIN depletion leads to enhanced cytotoxicity to genotoxic drugs suggesting that GUARDIN may represent a new target for cancer treatment.

In summary, lncRNAs have a multi-faceted role in the DDR by regulating p53 signalling, directly binding DNA repair proteins at the site of DNA lesions or acting indirectly in transcriptional and translational regulation of genes involved in genome stability and DNA repair. Most studies on lncRNAs involved in the DDR employed DNA damaging agents that induce DSBs, which might have introduced a publication bias towards these agents. This could also explain why lncRNAs were frequently observed to bind to proteins involved in HR and NHEJ. By using various damaging agents to trigger other repair pathways, further work will reveal if lncRNAs can also bind other DNA repair proteins in order to maintain genome integrity.
Concluding Remarks

The main challenges in deciphering the mechanisms whereby lncRNAs regulate cell cycle progression and safeguard genome integrity relate to the diversity of the lncRNA modus operandi. Since lncRNA genes can function through their RNA transcripts, by the action of their transcription or via regulatory DNA elements, multiple LOF and GOF methods are required to establish whether the function of lncRNAs in cell cycle progression and genome stability is RNA-dependent.

The cell cycle and DDR are evolutionary conserved processes but the mechanisms contributing to their control might not be similarly conserved. In this context, lncRNAs contribute to diversity and precise control of the cell cycle progression and represent an additional regulatory layer in the maintenance of genome stability in conjunction with the well-characterized protein-based mechanisms. We noted that the majority of lncRNAs with a function in the cell cycle act during the G1-phase, which correlates with their higher expression in the G1/S phase and the larger number of core protein-coding genes controlling G1-phase compared to other cell cycle stages [23]. Although we categorize lncRNAs according to their roles in the cell cycle, future studies should dissect lncRNA-mediated mechanisms affecting cell proliferation without directly interfering with the cell-cycle control machinery. Further, lncRNA structure-to-function and sequence-to-function studies will be crucial to determine their role in cell cycle and genome stability. Since CRISPR-Cas13 system has been used to detect lncRNAs in live cell, the field could exploit this powerful system to investigate lncRNAs dynamics during the cell cycle and after DNA damage [140].

Continued efforts of the scientific community and constant technological improvement will help provide further insight into the RNA-based regulatory networks and uncover new mechanisms of how lncRNAs control cell cycle to safeguard genome integrity (see Outstanding Questions).
Glossary

- **Genome instability**: chromosomal, microsatellite and nucleotide-associated instability are three types of genome instability occurring from defects in cell division, replication stress, defects in DDR and telomere maintenance.

- **Long noncoding RNAs (IncRNAs)**: heterogeneous class of ncRNAs that are longer than 200 nucleotides.

- **DNA damage response (DDR)**: a network of multiple DNA repair pathways, damage tolerance processes and cell-cycle checkpoints that sense, detect and repair DNA lesions.

- **Noncoding RNAs (ncRNAs)**: RNA molecules transcribed from mammalian genome but are not translated into proteins.

- **Functional ANnoTation Of the Mammalian genome (FANTOM)**: international research consortium focused on functional annotation of the mammalian genome and characterization of transcriptional regulatory networks.

- **Antisense oligonucleotide (ASO)**: single stranded DNA or RNA molecule that targets RNAs to induce its silencing or degradation via the RNase H endonuclease.

- **RNA binding proteins (RBPs)**: proteins that directly bind to single or double stranded RNA molecules through RNA binding domains.

- **Imprinted IncRNA**: IncRNA transcribed from a gene that is regulated in a parent-of-origin specific manner.

- **DNA damaging agents**: reactive molecules that damage DNA and are widely used to treat cancer.

- **Mitocheck consortium**: a study providing in depth analysis of cell division phenotypes after RNAi-mediated depletion of ~21,000 proteins (https://www.mitocheck.org/).

- **RNA interference (RNAi)**: process of post-transcriptional gene silencing involving RNA-dependent cleavage of RNA molecules.
- **CRISPR/Cas9**: genome editing method involving the RNA-guided Cas9 nuclease which introduces DNA double strand breaks at specific genomic loci.

- **CRISPR interference (CRISPRi)**: genome editing method involving Cas9 protein without its endonuclease activity (dCas9) fused to a Krüppel-associated box (KRAB) repression domain that can be directed to a specific genomic locus to prevent transcription.

- **CRISPR activation (CRISPRa)**: genome editing method involving dCas9 fused to active domains such as VP64 to enhance gene expression.

- **CRISPR/Cas9 synergistic activation mediator (CRISPR SAM)**: engineered protein complex activating gene expression composed of dCas9 fused to an active VP64 domain.

- **Base excision repair (BER)**: DNA repair pathway that deals with single-base modifications where the damaged base is recognized and removed.

- **Nucleotide excision repair (NER)**: DNA repair pathway that corrects bulkier and/or helix distorted lesions often induced by UV irradiation.

- **Mismatch repair (MMR)**: DNA repair pathway that deals with base-base mismatches and insertion/deletions mispairs generated during DNA replication and recombination.

- **Homologous recombination (HR)**: DNA repair pathway repairing DSBs. The HR process requires a homologous template to direct DNA repair and is generally recognised as a high-fidelity pathway. HR functions on stalled or collapsed replication forks in S phase and also contributes to DSB repair during G2 phase.

- **Nonhomologous end joining (NHEJ)**: second major pathway to correct DSBs, operating predominately during G1 phase.

- **Paraspeckles**: nuclear membranelles bodies that regulate gene expression through the retention of RNAs and proteins.

- **Replication stress**: a major source of genome instability arising from the impairment of DNA replication.
• **Single-nucleotide polymorphisms (SNPs):** a genetic variation of a single base pair present in a large fraction of the population.

• **Alternative-NHEJ:** a highly error prone DNA repair pathway that repair DSBs in less-efficient backup reactions.

• **CRISPR-Cas13:** CRISPR-Cas system involving Cas13 ribonuclease for targeted RNA knockdown.

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**Declaration of Interests**

The authors declare no competing interests.
Table 1. LncRNAs involved in cell cycle regulation

<table>
<thead>
<tr>
<th>Cell cycle phase</th>
<th>LncRNA</th>
<th>Mechanism</th>
<th>Regulation</th>
<th>Link to disease</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ncRNA\textsubscript{CCND1}</td>
<td>Suppression of \textit{CCND1} transcription by binding to TLS</td>
<td>Induced upon IR</td>
<td>Unknown</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>\textit{LAST}</td>
<td>Stabilization of \textit{CCND1} mRNA by binding to CNBP</td>
<td>c-MYC regulated; elevated levels during G1-phase</td>
<td>Potential oncogene</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>\textit{GAS5}</td>
<td>Negative regulation of \textit{CDK6} mRNA. Enhancement of E2F1 binding to the p27\textsuperscript{Kip1} promoter increasing p27\textsuperscript{Kip1} levels.</td>
<td>Unknown</td>
<td>Downregulated in multiple cancers</td>
<td>[26, 27]</td>
</tr>
<tr>
<td></td>
<td>\textit{Gadd7}</td>
<td>Negative regulation of \textit{CDK6} mRNA by binding to TDP43</td>
<td>Induced upon UV, cisplatin, alkylating agents and growth arrest signals</td>
<td>Unknown</td>
<td>[28, 29]</td>
</tr>
<tr>
<td></td>
<td>\textit{CCAT1}</td>
<td>Downregulation of \textit{CCND1} and \textit{CDK4}</td>
<td>c-MYC regulated</td>
<td>Upregulated in multiple cancers</td>
<td>[30-32]</td>
</tr>
<tr>
<td>LncRNA</td>
<td>Function</td>
<td>Regulation/Expression</td>
<td>References</td>
<td></td>
<td></td>
</tr>
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<td></td>
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</tr>
<tr>
<td><strong>MIR100HG</strong></td>
<td>Binding to HuR to promote its association with target mRNAs</td>
<td>c-MYC regulated; elevated levels during G1-phase</td>
<td>[33]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ANRIL</strong></td>
<td>Epigenetic silencing of the INK4/ARF locus by binding to PRC1/PRC2</td>
<td>ATM/E2F1-regulated</td>
<td>[35, 36, 40, 48]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>KNCQ1OT1</strong></td>
<td>Suppression of p57&lt;sup&gt;kip2&lt;/sup&gt; transcription through recruitment of PRC2 and G9a</td>
<td>Paternal expression</td>
<td>[37, 38]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>H19</strong></td>
<td>Downregulation of Rb mRNA through miRNA-675</td>
<td>E2F1- and cell cycle regulated</td>
<td>[39, 43]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MA-linc1</strong></td>
<td>Unknown</td>
<td>E2F1-regulated</td>
<td>[41]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ERIC</strong></td>
<td>Inhibition of E2F1-induced apoptosis</td>
<td>E2F1-regulated; induced upon etoposide</td>
<td>[42]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>Function</td>
<td>Stage(s) and Mechanism</td>
<td>Expression Status</td>
<td>References</td>
<td></td>
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</tr>
<tr>
<td><strong>MALAT1</strong></td>
<td>Positive regulation of genes involved in G1/S transition</td>
<td>Elevated levels during G1/S-phase</td>
<td>Deregulated in multiple cancers</td>
<td>[44-46]</td>
<td></td>
</tr>
<tr>
<td><strong>CONCR</strong></td>
<td>Binding to DDX11 helicase and modulation of its activity</td>
<td>Mid/late G1-phase expression; induced upon 5-FU and doxorubicin</td>
<td>Upregulated in multiple cancers</td>
<td>[49]</td>
<td></td>
</tr>
<tr>
<td><strong>SUNO1</strong></td>
<td>Interaction with DDX5 to promote transcription of genes involved in proliferation</td>
<td>S-phase specific expression</td>
<td>High expression of SUNO1 correlates with poor prognosis of colon cancer patients</td>
<td>[23]</td>
<td></td>
</tr>
<tr>
<td><strong>SCAT7</strong></td>
<td>Regulation of FGF/FGFR, PI3K/AKT and MAPK signalling</td>
<td>S-phase specific expression</td>
<td>Upregulated in multiple cancers</td>
<td>[52]</td>
<td></td>
</tr>
<tr>
<td><strong>Linc00704 MIAT LUCAT1</strong></td>
<td>Regulation of gene expression to promote S-phase progression</td>
<td>S-phase specific expression</td>
<td>Upregulated in different types of cancer</td>
<td>[53]</td>
<td></td>
</tr>
<tr>
<td><strong>Linc00152</strong></td>
<td>Unknown</td>
<td>Unknown</td>
<td>Upregulated in multiple cancers</td>
<td>[58]</td>
<td></td>
</tr>
<tr>
<td><strong>Linc00899</strong></td>
<td>Repression of TPPP transcription</td>
<td>Not cell cycle-regulated</td>
<td>Downregulated in breast cancer</td>
<td>[56, 62]</td>
<td></td>
</tr>
<tr>
<td><strong>C1QTNF1-AS1</strong></td>
<td>Unknown</td>
<td>Not cell cycle-regulated</td>
<td>Downregulated in breast cancer</td>
<td>[56, 61]</td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>Function</td>
<td>Regulation</td>
<td>Reference(s)</td>
<td></td>
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</tr>
<tr>
<td>GiniR</td>
<td>High expression of GiniR perturbs the Cep112/BRCA1 interaction leading to mitotic defects</td>
<td>Developmental regulation leading to mitotic defects</td>
<td>[15]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LincRNA-RI</td>
<td>Binding to miRNA-210-3p and regulation of PLK1 mRNA stability</td>
<td>Induced by IR</td>
<td>[66]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APAL</td>
<td>Binding to PLK1 and Aurora A to promote PLK1 activity</td>
<td>Unknown</td>
<td>[67]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OIP5-AS1</td>
<td>Binding to GAK mRNA leading to decreased GAK levels</td>
<td>Unknown</td>
<td>[68, 70]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAM</td>
<td>Interaction with Sugu1 to regulate kinetochore assembly</td>
<td>Highly expressed in mouse myoblasts and satellite cells</td>
<td>[69]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MALAT1</td>
<td>Positive regulation of B-MYB expression</td>
<td>Elevated levels during mitosis</td>
<td>[44-46]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MA-linc1</td>
<td>Repression of Purα expression</td>
<td>E2F1-regulated</td>
<td>[41]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PAPAS</strong></td>
<td>Chromatin compaction by interacting with Suv4-20h2</td>
<td>Upregulated in G0</td>
<td>Unknown</td>
<td>[72]</td>
<td></td>
</tr>
<tr>
<td>---</td>
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<tr>
<td><strong>PDCD4-AS1</strong></td>
<td>Stabilization of PDCD4 mRNA by forming RNA duplexes</td>
<td>Upregulated in G0</td>
<td>Downregulated during breast cancer progression</td>
<td>[73]</td>
<td></td>
</tr>
<tr>
<td><strong>GAS5</strong></td>
<td>Negative regulation of glucocorticoid receptor transcriptional activity</td>
<td>Sox2-regulated during G0 phase</td>
<td>Involved in cancer stemness</td>
<td>[75, 76]</td>
<td></td>
</tr>
<tr>
<td><strong>DANCR</strong></td>
<td>Post-transcriptional regulation of β-catenin</td>
<td>Unknown</td>
<td>Involved in cancer stemness</td>
<td>[77]</td>
<td></td>
</tr>
<tr>
<td><strong>MIR222HG</strong></td>
<td>Interaction with ILF2/3 to positively regulate the stability of DNM3OS IncRNA</td>
<td>Induced upon serum stimulation</td>
<td>Upregulated in prostate cancer</td>
<td>[78, 141]</td>
<td></td>
</tr>
</tbody>
</table>

The table lists various non-coding RNAs and their functions in cell cycle progression and cancer. The table includes information on how these RNAs are upregulated or downregulated during G0 phase and their involvement in cancer stemness. References are provided for further reading.
Table 2. LncRNAs involved in the maintenance of genome stability

<table>
<thead>
<tr>
<th>LncRNA</th>
<th>DNA damaging agents</th>
<th>DNA lesions</th>
<th>Mechanism</th>
<th>Regulation</th>
<th>Link to disease</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUNO1</td>
<td>Doxorubicin, Etoposide, HU</td>
<td>DNA breaks</td>
<td>Unknown</td>
<td>FOS, JUND and EGR1-regulated</td>
<td>High SUNO1 expression correlates with poor prognosis of colon cancer patients</td>
<td>[23]</td>
</tr>
<tr>
<td>PANDA</td>
<td>Doxorubicin, Etoposide</td>
<td>DNA breaks</td>
<td>Inhibition of apoptotic gene expression by sequestering NF-YA</td>
<td>p53-regulated</td>
<td>Deregulated in multiple cancers</td>
<td>[19, 102, 103]</td>
</tr>
<tr>
<td>DINO</td>
<td>Doxorubicin, Etoposide</td>
<td>DNA breaks</td>
<td>Binding and stabilization of p53; inducing p53 target genes</td>
<td>p53-regulated</td>
<td>Unknown</td>
<td>[96]</td>
</tr>
<tr>
<td>lincRNA-p21</td>
<td>Doxorubicin</td>
<td>DNA breaks</td>
<td>Repression of p21cip1 transcription by recruiting hnRNPK</td>
<td>p53-regulated</td>
<td>Downregulated in leukemia and colon cancer</td>
<td>[98, 104, 142]</td>
</tr>
<tr>
<td>SNHG1</td>
<td>Doxorubicin</td>
<td>DNA breaks</td>
<td>Stabilization of p53 by disruption of p53-hnRNPC interaction</td>
<td>Nuclear retention upon doxorubicin</td>
<td>Upregulated in various cancers and associated with poor prognosis</td>
<td>[12, 143]</td>
</tr>
<tr>
<td>LINC-PINT</td>
<td>Doxorubicin</td>
<td>DNA breaks</td>
<td>Silencing of genes involved in cell growth and proliferation by binding to PRC2</td>
<td>p53-regulated</td>
<td>Downregulated in multiple cancers</td>
<td></td>
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<td>---------------------------------------------------------------------------------</td>
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<td></td>
</tr>
<tr>
<td>NEAT1</td>
<td>Doxorubicin, UV, ROS</td>
<td>DNA breaks, DNA adducts, SSBs, oxidized bases</td>
<td>Modulation of ATR signalling and attenuation of oncogene-dependent activation of p53</td>
<td>p53-regulated</td>
<td>Deregulated in multiple cancers</td>
<td></td>
</tr>
<tr>
<td>LincRNA-RoR</td>
<td>Doxorubicin, UV</td>
<td>DNA breaks, DNA adducts</td>
<td>Suppression of p53 translation through binding to hnRNP-I</td>
<td>p53-regulated</td>
<td>Overexpressed in breast cancer; high expression of lincRNA-ROR associates with poor prognosis</td>
<td></td>
</tr>
<tr>
<td>MEG3</td>
<td>Doxorubicin</td>
<td>DNA breaks</td>
<td>Binding to p53 and activation of p53 target genes</td>
<td>Maternal expression</td>
<td>Downregulated in multiple cancers and during cardiac remodeling</td>
<td></td>
</tr>
<tr>
<td>DDSR1</td>
<td>Camptothecin, Etoposide, Neocarzinostatin, Bleomycin</td>
<td>DNA breaks</td>
<td>Binds to BRCA1 and regulates BRCA1/RAP80 recruitment to laser-induced DSBs to promote HR; controls expression of DDR genes</td>
<td>ATM-NF-κB mediated upregulation upon DNA damage</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>

References:
[106, 107], [108-111], [112-114], [120]
<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>DNA Damage Induction</th>
<th>Mechanism</th>
<th>Regulation</th>
<th>Biological Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALAT1</td>
<td>PARP inhibitor, bortezomib</td>
<td>Site-specific induction of DSBs</td>
<td>Binding to PARP1 and Ligase III to regulate alt-NHEJ</td>
<td>MALAT1 inhibition induces DNA damage</td>
<td>Overexpressed in multiple myeloma</td>
</tr>
<tr>
<td>ANRIL</td>
<td>Neocarzinostatin, Etoposide, Bleomycin</td>
<td>DNA breaks</td>
<td>Suppression of INK4/ARF locus; regulation of cell cycle, apoptosis and HR</td>
<td>ATM-E2F1 regulated</td>
<td>Deregulated in multiple cancers and cardiovascular diseases</td>
</tr>
<tr>
<td>CUPID1</td>
<td>IR</td>
<td>DNA breaks</td>
<td>Regulation of RAD51 recruitment at DSBs to promote HR and control end resection</td>
<td>Estrogen-regulated</td>
<td>Genomic locus affected by the breast cancer risk variant at 11q13</td>
</tr>
<tr>
<td>CUPID2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HITT</td>
<td>Doxorubicin, Etoposide, Bleomycin</td>
<td>DNA breaks</td>
<td>Binding and inhibition of ATM activity to restrain HR</td>
<td>EGR1-regulated</td>
<td>Downregulated in colon cancer</td>
</tr>
<tr>
<td>PRLH1/</td>
<td>Adriamycin</td>
<td>DNA breaks</td>
<td>Binding and stabilization of RNF169 to promote HR</td>
<td>p53-regulated</td>
<td>Upregulated in hepatocellular carcinoma</td>
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<tr>
<td>linc01419</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LINP1</td>
<td>Doxorubicin, IR</td>
<td>DNA breaks</td>
<td>Recruitment of Ku70/80 and DNA-PKcs at DSBs to promote NHEJ; replacement of PAXX function</td>
<td>EGF-regulated</td>
<td>Overexpressed in multiple cancers</td>
</tr>
<tr>
<td>Gene</td>
<td>Stimuli</td>
<td>DNA Damage</td>
<td>Regulation of BRCA1/BARD1 complex at DSBs to promote HR and control end resection</td>
<td>Recruited to DNA damage site after laser-induced DSBs</td>
<td>Negative correlation of BGL3 expression with survival rate of breast cancer patients</td>
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</tr>
<tr>
<td>BGL3</td>
<td>Camptothecin, Etoposide, IR, HU</td>
<td>DNA breaks</td>
<td>Regulation of BRCA1/BARD1 complex at DSBs to promote HR and control end resection</td>
<td>Recruited to DNA damage site after laser-induced DSBs</td>
<td>Negative correlation of BGL3 expression with survival rate of breast cancer patients</td>
</tr>
</tbody>
</table>

**GiniR**

- **None**
- **Unknown**
- **Perturbation of Cep112/Brca1 interaction**
- **Developmental regulation**
- **Ginir overexpression induces oncogenic transformation**

**dilncRNAs**

- **None**
- **Site-specific induction of DSBs**
- **Precursors for small ncRNAs to promote DDR**
- **Expressed upon DSBs**
- **Unknown**

**NORAD**

- **Doxorubicin, Camptothecin, UV**
- **DNA breaks, DNA adducts**
- **Decoy for PUMILIO; NORAD forms also a ribonucleoprotein complex**
- **Induced by DNA damage and indirectly regulated by p53**
- **Loss of NORAD is associated with premature aging**

**CCAT2**

- **Bleomycin, 5-FU, Oxaliplatin**
- **DNA breaks**
- **Binding to and stabilization of BOP1**
- **WNT-regulated**
- **Upregulated in colon cancer**

**GUARDIN**

- **Doxorubicin, IR**
- **DNA breaks**
- **Sponging with miR-23a to regulate TRF2; RNA scaffold for BRCA1/BARD1 complex to promote HR and NHEJ**
- **p53-regulated**
- **Downregulated in colon cancer with p53 mutation**
HU, Hydroxyurea; UV, Ultraviolet radiation; Reactive oxygen species, ROS; Double strand breaks, DSBs; Single strand breaks, SSBs; IR, Ionizing radiation; 5-Flurouracil, 5-FU.
5 **Figure legends:**

**Box 1.** An overview of the mammalian cell cycle progression. The cell cycle is regulated by different cyclin/CDK complexes and CDK inhibitors throughout the different stages of the cell cycle.

**Box 4.** The components of the DNA damage response pathway.

**Figure 1, Key Figure.** LncRNA-mediated control of the cell cycle progression and genome stability.

**Figure 2.** Emerging mechanisms how IncRNAs mediate DDR to maintain genome stability.

(A) *DDSR1* modulates HR by interacting with *hnRNPUL1* and *BRCA1*. Loss of *DDSR1* or *hnRNPUL1* leads to aberrant recruitment of *BRCA1* and its recruitment factor RAP80 at laser-induced DSBs.

(B) In response to DSBs, *Ku70/80* recruits DNA-PKcs to the damage site. Depletion of *LINP1* leads to reduction in chromatin-associated DNA-PKcs. *LINP1* promotes NHEJ repair by acting as a scaffold for DNA-PKcs and *Ku70/Ku80*.

(C) At early time points after DNA damage, *BGL3* is recruited to damaged sites by interacting with *PARP1*. Later on, *BGL3* binds *BARD1* to control HR. Depletion of *BGL3* leads to compromised recruitment of *BRCA1/BARD1* and its downstream factors (*Rad51*, *RPA70*) at DSBs.

(D) DSBs can induce synthesis of dilncRNAs that act as precursors for small ncRNAs. Both classes of ncRNAs contribute to DNA damage signalling and repair by forming a scaffold for their recruitment to DNA damage site.

(E) Upon DNA damage, *NORAD* localizes more to the nucleus (i) or in the cytoplasm (ii). *NORAD* can form a higher-order ribonucleoprotein complex by binding to proteins involved in DNA replication and repair (i,[13]), or can sequester PUMILIO proteins, which repress genes necessary for genome integrity (ii, [133-135]). Inactivation of *NORAD* leads to chromosome segregation errors and aneuploidy-induced genome instability.
Cytoplasmic *GUARDIN* can sequester miR-23a to stabilise TRF2 expression and prevents chromosome end-to-end fusion. Simultaneously, *GUARDIN* acts as a scaffold for BRCA/BARD1 complex and regulates HR and NHEJ. It is not clear whether *GUARDIN* is involved in the formation of BRCA/BARD1 complex in the nucleus to regulate DNA repair.
References

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