Am J Cancer Res 2012;2(6):658-675
www.ajcr.us /ISSN:2156-6976/ajcr0000149

*Review Article*

# Non-coding RNAs in DNA damage response

Yunhua Liu, Xiongbin Lu

*Department of Cancer Biology, Metastasis Research Center, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA*

Received October 8, 2012; Accepted October 22, 2012; Epub November 20, 2012; Published November 30, 2012

**Abstract:** Genome-wide studies have revealed that human and other mammalian genomes are pervasively transcribed and produce thousands of regulatory non-protein-coding RNAs (ncRNAs), including miRNAs, siRNAs, piRNAs and long non-coding RNAs (lncRNAs). Emerging evidences suggest that these ncRNAs also play a pivotal role in genome integrity and stability via the regulation of DNA damage response (DDR). In this review, we discuss the recent finding on the interplay of ncRNAs with the canonical DDR signaling pathway, with a particular emphasis on miRNAs and lncRNAs. While the expression of ncRNAs is regulated in the DDR, the DDR is also subjected to regulation by those DNA damage-responsive ncRNAs. In addition, the roles of those Dicer- and Drosha-dependent small RNAs produced in the vicinity of double-strand breaks sites are also described.

**Keywords:** DNA damage response, ncRNAs, miRNAs, lncRNAs, crosstalk

## Introduction

Maintenance of the integrity of genomic information is critical to the survival and propagation of all organisms, avoiding propagation of mutations that could lead to genomic instability and cancer. DNA lesions can be caused by a variety of environmental and endogenous genotoxic insults, such as ultraviolet (UV) in sunlight or ionizing radiation (IR), numerous chemotherapeutic agents and by-products of normal cell metabolism, notably reactive oxygen species (ROS) [1]. In order to maintain the integrity of genomic DNA, eukaryotes have evolved a highly coordinated cellular system to sense and counteract these threads. Collectively, this system is known as the DNA damage response (DDR). Of the many types of DNA damage, DNA double strand break (DSB) is one of the most severe ones because it's lethal to the cell if the damage is not repaired. DSB can be repaired by homologous recombination (HR), which allows for error-free repair, and non-homologous end joining (NHEJ), which is an error-prone repair pathway, while other types of DNA damage are processed through the nucleotide excision repair (NER), base excision repair (BER) and mismatch repair (MMR) [2].

Almost all the DDR pathways encompass a similar set of tightly regulated steps: firstly the detection of DNA damage, the recruitment of DNA repair factors to damage site and finally the repair of DNA lesion. Accordingly, all these components in the transduction pathway can be functionally categorized into: sensors of damage, signal transducers and effectors [3]. The information collected and transmitted by these factors will be used in making cell fate decision - either arrest cell cycle to allow repair of damaged DNA and survival or apoptosis in case of severe damage [4]. However, previously all these factors are believed to be protein-coding genes. Recent studies point to the need for an expanded definition beyond just protein-coding genes to also include non-coding RNAs (ncRNAs).

Indeed, there is increasing evidence suggests that various ncRNAs could play an important role during initiation and progression of human diseases and in response environmental stimuli like stress [5]. The ncRNAs are those highly abundant and functionally important RNA molecules that are not translated into proteins and can be divided into a variety of groups. Except those housekeeping ribosomal and transfer

RNAs (rRNAs and tRNAs), ncRNAs are largely classified into 2 classes based on its size: small ncRNAs and long non-coding RNAs (lncRNAs, > 200 nt). The former class includes the well-documented microRNAs (miRNAs), small interfering RNAs (siRNAs), Piwi-interacting RNAs (piRNAs) as well as a collection of newly identified promoter-associated RNAs (PARs), enhancer RNAs (eRNAs) and DSB-induced RNAs (diRNAs or DDRNAs) [6-8]. Similar to protein-coding genes, the expression of miRNAs and lncRNAs genes following DNA damage appears to be modulated at transcriptional or post-transcriptional levels. On the contrary, accumulating data indicate that the core protein-coding components of DDR pathways are targets of miRNAs, and subjected to inhibition in damage response. In this review, we describe the characteristics and biological roles of these ncRNAs in DDR, with a particular emphasis on miRNAs, lncRNAs and the recently identified site-specific small RNA flanking DNA damage sites – DDRNAs [9, 10].

## microRNAs

The most well-studied group of ncRNAs are miRNAs, which are ~19- to 24-nt small ncRNAs with post-transcriptional regulatory functions by perfect or imperfect base-pairing, usually at the 3' untranslated region (3' UTR) of targeted mRNA [11]. Since the first discovery of *lin-4* and *let-7* in *C. elegans*, miRNAs are now found to exist in nearly all eukaryotic organisms and even in DNA viruses, indicating miRNA-like gene regulatory mechanism evolved early in the eukaryotic lineage [12]. To date, almost 2000 mature miRNAs have been annotated in human genome and involved in many cellular processes such as proliferation, differentiation, stress responses, apoptosis and development [13]. MiRNAs are encoded in diverse regions of the genome including both protein coding and non-coding transcription units. Approximately 40% of miRNAs are embedded in the introns or 3' UTR of protein-coding genes. These intronic or UTR-derived miRNAs share a common promoter with host genes and maybe generated by read-through transcription, whereas other intergenic miRNAs are transcribed independently from non-protein-coding genes [14, 15].

The biogenesis of miRNAs is a tightly regulated process involving two ordered endonucleolytic cleavages by the RNase III enzymes Drosha and Dicer [16, 17]. Following transcription by RNA polymerase II (RNA pol II), the primary miRNA transcript (pri-miRNA) are first processed by an RNase III enzyme Drosha and its co-factor DGCR8 into a ~60–100 nt hairpin structure termed the precursor-miRNA (pre-miRNA) [18]. Through the interaction with exportin-5, a Ran-GTP-binding nuclear transporter, the pre-miRNA is then transported into the cytoplasm, where it was further cleaved by Dicer and its cofactor, TRBP (Tar RNA binding protein), resulting in the production of mature ~22 nt RNA duplex [19]. One strand of the duplex is preferentially incorporated into Argonaute (Ago) family proteins to form the RNA-induced silencing complex (RISC), while miRNA* passenger strand is usually degraded. The RISC loaded with mature miRNAs are subsequently guided by miRNA to pair with target transcript at their 3' UTR and induce mRNA degradation or inhibition of translation [20]. Although our understanding of the basic mechanism of miRNA biogenesis has increased dramatically, the specific mechanisms that regulate miRNA expression remain elusive. As discussed below, during DDR each step of the general biogenesis pathway has been found to be differentially regulated to allow exquisite control of miRNA expression.

## Long noncoding RNAs

In addition to miRNAs, the most majority of ncRNAs are mRNA-like lncRNAs, range in length from 200 nt in length to ~100 kilobases (kb) lacking significant open reading frames. Most of them are transcribed by RNA polymerase II (RNA pol II) and polyadenylated [21], but this is not a hard and fast rule. In spite of their low levels of expression and poor conservation between species as compared to protein-coding genes or miRNAs, the expression of lncRNAs is tightly regulated with cell type and tissue specificity. The exact number of lncRNAs encoded within human genome is unknown. However, with the advent of RNA sequencing (RNA-seq) and computational methods for transcriptome reconstruction, thousands of lncRNAs have been identified in many different cell types and tissues in mammals [22, 23]. As the number of characterized long non-coding transcripts increased rapidly, so did the uncertainty regarding their putative function. How do lncRNAs exert their functions? At present only a

handful of lncRNAs have been well-characterized with distinctive biological roles, such as XIST or TSIX in X-chromosome inactivation [24, 25], H19 or AIR in genomic imprinting [26, 27], NRON in cytoplasmic-to-nuclear trafficking of NFAT transcriptional factor [28] and HORAIR or HOTTIP in trans-acting regulation of HOX gene family [29, 30]. Although only a minority has been studied in detail, it is now becoming evident that many lncRNAs, if not all, are important transcriptional outputs of the genome, but not transcription noise.

The lncRNAs include a heterogeneous group of long non-coding transcripts. According to their proximity adjacent protein-coding genes, lncRNAs may be broadly classified into 5 classes: sense, antisense, bidirectional, intronic and intergenic [31, 32]. The majority of them are antisense lncRNAs, which are transcribed from the opposite DNA strand of a protein-coding gene, and overlap in part with sense mRNA. Both ends of protein-coding genes may have the potential to encode antisense transcripts, and it's now clear that antisense transcription is a widespread feature of mammalian genomes, such as more than 70% of transcription units sequenced in mouse genome are antisense transcripts, most of them are lncRNAs [33, 34]. Usually, antisense transcript exerts its effect by modulating the expression of corresponding sense transcript positively or negatively. For instance, BACE1-AS, a conserved noncoding antisense transcript for β-secretase-1 (BACE1), is upregulated in patients with Alzheimer's disease. It regulates the expression of BACE1 by increasing BACE1 mRNA stability and generating additional BACE1 protein through a post-transcriptional feed-forward mechanism [35]. More recently, another group of lncRNAs, termed large or long intergenic ncRNAs (lincRNAs), have been identified by searching distinctive 'K4–K36' chromatin signature indicative of active transcription. These lincRNAs are exclusively intergenic and marked by trimethylation of lysine 4 of histone H3 (H3K4me3) at the promoter region and trimethylation of lysine 36 of histone H3 (H3K36me3) along the transcribed region. By searching for K4–K36 domains that do not overlap with known protein-coding genes, there are almost 1,600 regions in the mouse genome and 2,500 regions in the human genome, which show higher evolutionary conservation across mammals when compared to other types of lncRNAs [36-38]. For most of them, their biological functions are poorly defined, although they are presumably involved in transcription regulation by physically associating with different chromatin regulatory proteins. Using loss-of-function studies, dozens of lincRNAs has been found to play key roles in the circuitry controlling embryonic stem (ES) cell state *in trans*. Knockdown of these lincRNAs causes either exit from the pluripotent state or upregulation of lineage commitment programs, comparable to knockdown of well-known ES cell regulators, such as Oct4 and Nanog [39].

### The expression of ncRNAs is responsive to DDR

Eukaryotic cells respond to DNA damage by arresting the cell cycle and modulating gene expression to ensure efficient DNA repair. Tremendous progress has been achieved in elucidating the molecular regulators respond to diverse DNA damage. In addition to those protein-coding genes, expanding evidence shows that some ncRNAs, including miRNAs and lncRNAs, are also regulated by DDR and thought to be a new players in mediating the cellular response to damage response.

### Regulation of miRNAs in DNA damage response

It has been shown that treatment with different types of genotoxic agents, such as UV light, γ-irradiation, oxidative stress, and chemical mutagens, result in a global change on miRNAs expression in a variety of cell types [40-44]. Usually, the up- and down-regulation of miRNAs expression levels will happen in a few hours after DNA damage, and will return to basal levels in 24 hours. This response is slower than post-translational protein modification, such as phosphorylation, acetylation and ubiquitination, but faster than the transcriptional activation of p53 target genes such as CDC25a and p21. Therefore, it was postulated that miRNA-mediated gene silencing acts at the intermediate time points between the fast protein modification responses and the slow transcriptional reprogramming of genes.

For example, using miRNA microarrays and quantitative real-time PCR, there were 73 and 33 miRNAs being either up or down-regulated

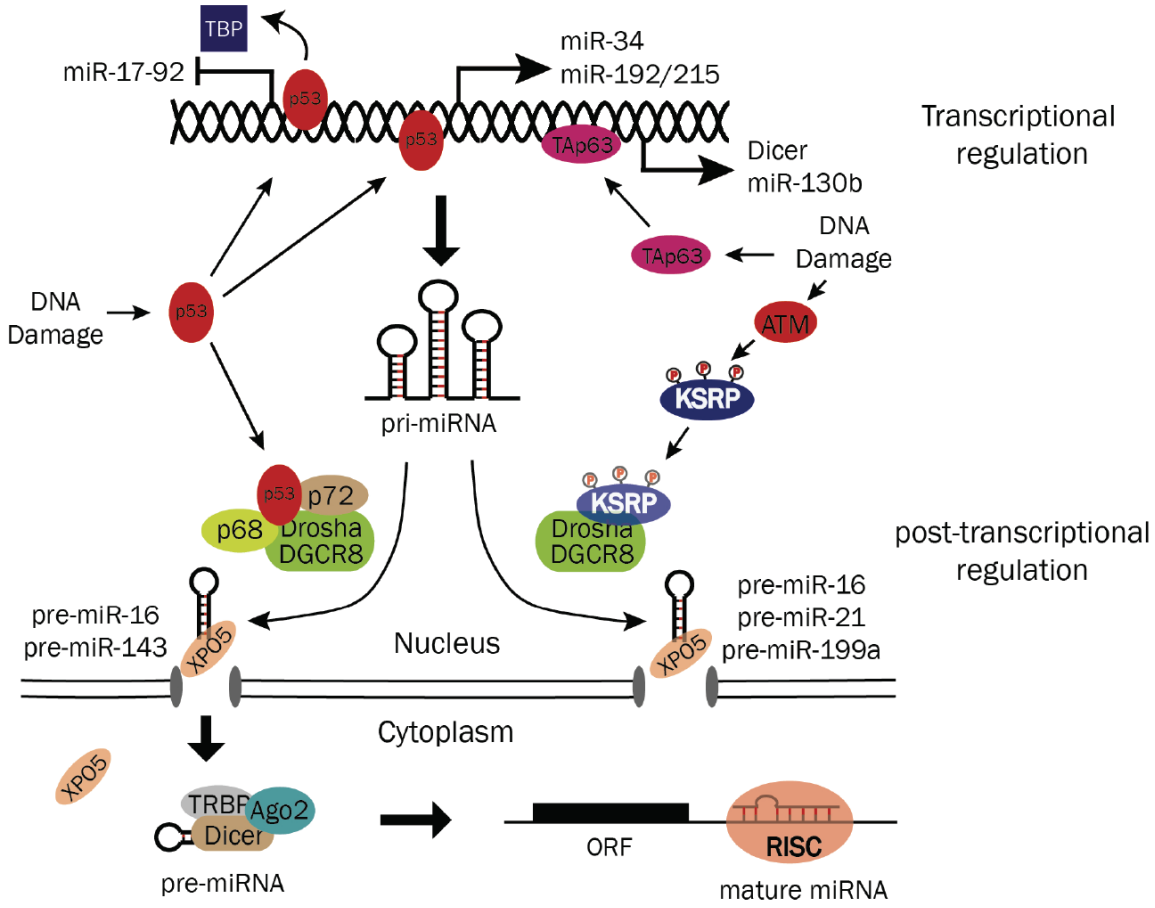


**Figure 1.** DNA damage modulates the biogenesis of miRNAs. In response to DNA damage, the expression of some specific miRNAs can be directly regulated by transcriptional factors, such as the tumor suppressor family members p53 and TAp63, which are activated upon genotoxic stress and, in turn, promote or inhibit the transcription of some miRNAs. In addition, DNA damage also regulates a subset of miRNAs at post-transcriptional level. p53 is functionally linked to Drosha/DGCR8 via direct interaction with p68/p72, promoting the processing of some pri-miRNAs to pre-miRNAs. The ATM kinase also facilitates the maturation of miRNAs through activating and phosphorylating KSRP, which in turn interact with Drosha/DGCR8 complex to enhance miRNAs processing.

(>2-fold) in 1 and 10 Gy-irradiated human lymphoblastic cells (IM9) respectively [40], indicating varying dose of DNA damage may lead to activation of different as well as common set of miRNAs.

Similar experiments have been done in other cell lines, including human fibroblast cells, dermal microvascular endothelial cells and non-small cell lung cancer cells [43, 45, 46]. However, there is no obvious overlap of IR-induced miRNA profiles in different cell lines upon the same treatment with IR, suggesting these IR-responsive miRNAs might be cell type-specific. Other DNA damage agents, such as UV light, etoposide, cisplatin and hydrogen peroxide, also resulted in similar but unique set of miRNAs even in the same type of cells [41, 44, 47]. Many of them were predicted to target those genes involved in DNA repair, cell cycle arrest or apoptosis, although there are some variations among DNA damage-responsive miRNAs. These variations between miRNA profiling points to the fact that miRNAs could be regulated by DNA damage in a mechanism based not only on the nature and intensity of DNA damage, but also on the type of cells where DNA damage occurred.

### DDR modulates miRNAs biogenesis transcriptionally

The expression of miRNAs can be directly regulated by transcriptional factors (**Figure 1**), such

as the tumor suppressor p53, a well-known transcriptional factors induced in DNA damage. In response to DNA damage, the ATM or ATR kinase activates p53, which in turn transactivates those genes in cell cycle regulation, senescence and apoptosis. The first discovery that connects p53 to the transactivation of miRNAs was the discovery of miR-34 family, which was found to be directly induced by p53 upon DNA damage and oncogenic stress [48]. Ectopic expression of miR-34a leads to G1 phase cell cycle arrest in both primary and tumor-derived cell lines likely through silencing a program of genes which promote cell cycle progression, suggesting of their tumor suppressive potentials. Moreover, miR-34a was reported to inhibit cell proliferation through the induction p53-mediated apoptosis [49]. MiR-34c, another member of the miR-34 family, was transcriptionally induced by p53 following DNA damage. However, in cells lacking p53, an alternative pathway exists to induce miR-34c although to a lesser extent. This pathway involves signaling through p38 MAPK to MK2 [50]. In addition to the miR-34 family, miR-192, miR-194, miR-215 and miR-17-92 cluster are other miRNAs found to be transcriptionally regulated by p53. Following genotoxic agents treatment, the expression levels of miR-192, miR-194 and miR-215 are upregulated and highly dependent on p53 activation. Ectopic expression of miR-192/215 induces cell-cycle arrest through targeting a number of transcripts that regulate G1/S and G2/M checkpoints [51, 52]. However, other studies revealed that miR-17-92 cluster was a novel repression target of p53, sensitizing the cells to apoptosis under hypoxia. The expression levels of miR-17-92 cluster were reduced in hypoxia-treated p53-proficient cells, but remained unchanged in p53-deficient cells. ChIP, Re-ChIP and gel retardation assays revealed that p53-mediated transcriptional repression of miR-17-92 cluster is function through preventing the TATA-binding protein (TBP) from binding to a TATA-box which overlap with the p53-binding sites within the miR-17-92 promoter. Notably, the expression levels of pri-miR-17-92 inversely correlated with p53 status in colorectal carcinomas, suggesting of their tumor-promoting role in cancers. These studies suggest that these p53-regulated miRNAs may act in concert with other p53 transcriptional protein-coding targets to modulate cellular response to DNA damage.

Similar to p53, its homologues p63 and p73 are also induced by and involved in DNA damage via apoptosis and cell cycle arrest. Computational analyses show that all three members of the p53 family could function as both positive and negative regulator of the major component of the miRNA processing machinery, such as Drosha-DGCR8, Dicer-TRBP2, and Argonaute proteins [53]. It has been reported that metastatic mouse and human tumors deficient in TAp63, the transactivation (TA) isoform of p63, express a very low level of Dicer and overexpression of Dicer and miR-130b markedly affected their metastatic potentials [54]. Further studies revealed that TAp63 binds to the promoters of Dicer and miR-130b and transactivates their expression, indicating the direct transcriptional regulation of Dicer and miR-130b by TAp63. In addition, there are a number of other DNA damage-responsive transcription factors have been identified, such as NF-κB, c-Myc, CREB and E2F1, which are known to modulate miRNA expression [55, 56]. However, the specific functions of those miRNAs in DNA damage and how much they contribute to the cellular response to DNA damage are still elusive and need further study.

### DDR regulates miRNAs processing and maturation

DNA damage also regulates miRNA expression through post-transcriptional processing, leading to increased levels of some pre-miRNAs and mature miRNAs without significant changes in their primary transcripts (**Figure 1**). The first evidence of post-transcriptional regulation of certain miRNAs maturation came from the study of Miyazono group [57]. This study demonstrated that several miRNAs, including miR-16-1, miR-143 and miR-145, were post-transcriptionally upregulated in a p53-dependent and p68/p72-dependent manner upon genotoxic stress. DEAD box RNA helicases p68 (DDX5) and p72 (DDX17) were identified as subunits of the Drosha complex and required for recognition and processing of a subset of primary miRNAs [58, 59]. In HCT116 and WI-38 cells, p53 interacts with the Drosha processing complex through direct interaction with p68 and, in turn, facilitates the processing of pri-miRNAs to pre-miRNAs. However, inactive p53 mutants disrupt a functional assembly between

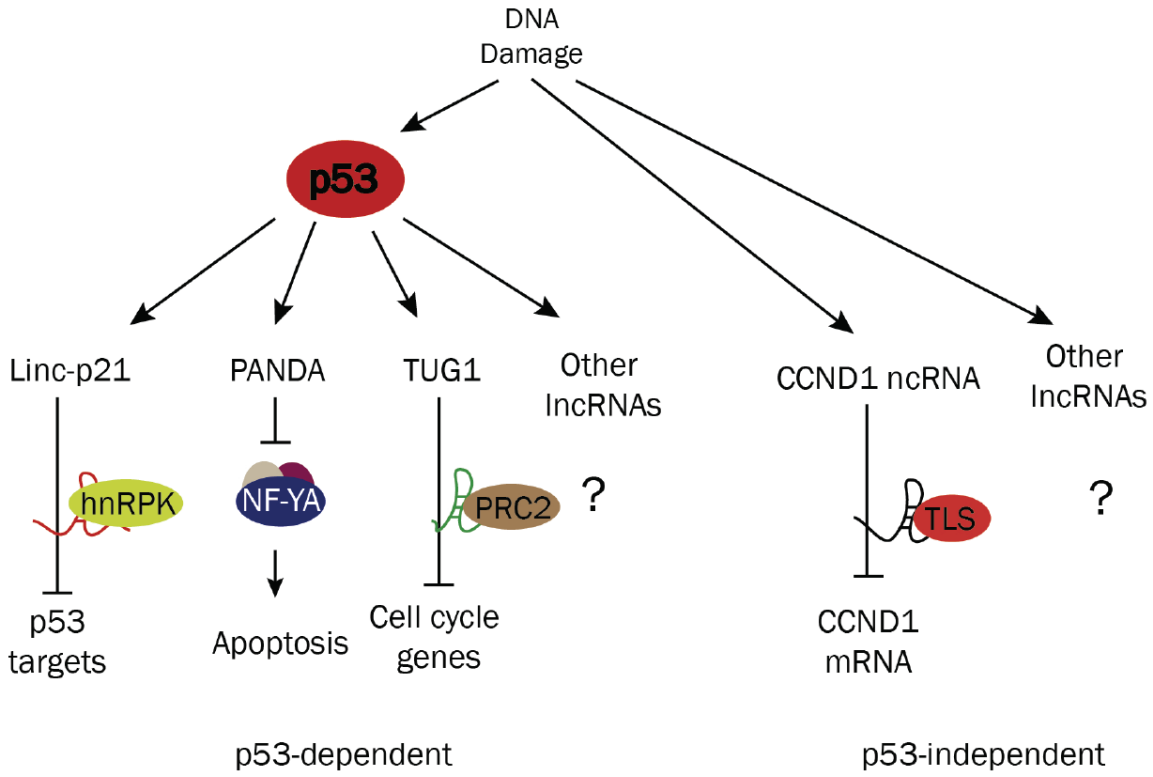


**Figure 2.** DNA damage regulates the expression of lncRNAs. Similar to those protein-coding genes, the expression levels of lncRNAs are regulated globally by the tumor suppressor p53 or other transcriptional factors after DNA damage. These lncRNAs, once activated, participate in the DNA damage response by modulating downstream gene expression via interacting with their protein partners.

the Drosha complex and p68, resulting in attenuation of miRNA processing activity. These findings indicate that p53, apart from functions as a sequence-specific transcription factor, plays an important role in the post-transcriptional modulation of miRNA biogenesis and processing. Remarkably, p53 mutations are frequently observed in cancers and most of them are located in a domain that is required for both the miRNA processing function and transcriptional activity [60]. Loss of p53 functions in transcription and processing of specific miRNAs might act in concert and contribute to tumor progression. As mentioned before, the major components of the miRNAs processing machinery including Dicer are predicted to be transcriptional targets of p53 and its homolog p63 and p73. Interestingly, the guardians of genome, p53/p63/p73, appears to modulate the processing of a group of miRNAs, including the tumor suppressor miRNAs, let-7, miR-34, miR-15/16a, miR-145, miR-26, miR-29, and miR-146a [61]. It was also predicted that many components in the miRNA processing complexes are targeted by p53-regulated miRNAs, suggesting a negative feedback effect also exists to maintain physiological levels of miRNAs in response to DNA damage.

Recent data from our lab show that miRNA biogenesis is globally induced in an ATM-dependent manner [62]. As many as one-fourth of miRNAs were significantly upregulated upon DNA damage in ATM-proficient human fibroblasts, while a small group of miRNAs were decreased. Among these induced miRNAs, a cohort of miRNAs associated with the KH-type splicing regulatory protein (KSRP, also known as KHSRP), an AU-rich element binding protein that mediates mRNA decay. Previous studies revealed that KSRP serves as a critical component of both Drosha and Dicer complexes and regulates the biogenesis of a subset of miRNAs [63]. The complex pattern of post-translational modifications on KSRP determines its interaction with a wide spectrum of RNA target

sequences, as well as with other RNA-binding proteins and adaptor proteins [64]. The ATM gene encodes a DNA damage-inducible kinase, initiating the DNA damage signaling. Our results show that DNA damage activates the ATM kinase which directly binds to and phosphorylates KSRP, leading to enhanced interaction between KSRP and pri-miRNAs and increased KSRP activity in miRNA processing [62]. This activity results in the activation of the pri-miRNA processing by Drosha microprocessors and stimulation of miRNA maturation. However, mutations of those sites phosphorylated by ATM on KSRP impaired its activity in regulating miRNAs biogenesis. These findings strongly support the hypothesis that ATM functions as a major regulator of KSRP in miRNA processing, and that KSRP acts as a molecular gatekeeper that promote the production of a subset of miRNAs that in turn act in concert with other protein coding effectors to regulate cellular response to DNA damage. However, we also noticed that the expression levels of some miRNAs after DNA damage were significantly reduced in an ATM-dependent manner, indicating that ATM may also participate in inhibitory signaling that down-regulate the expression of certain miRNAs. Further studies will be required to elucidate whether ATM-dependent or -independent kinases involved in the regulation of miRNA biogenesis following genotoxic treatment.

## Regulation of lncRNAs in DNA damage response

Interestingly, the earliest report describing functional lncRNAs known as H19 and XIST, which play an important role in imprinting and X-chromosome inactivation separately, was published in 1990s, predating the discovery of miRNA. However, the discovery of miRNA *lin-14* in *C. elegans* dramatically shifted the focus of research of ncRNAs from lncRNAs to miRNAs. In the past decade, a number of publications have documented the emerging functions of lncRNAs in a variety of biological processes, including nuclear organization, nuclear-cytoplasmic trafficking, dosage compensation, regulation of gene expression and epigenetic modification [5, 21, 65]. More recently, a few of lncRNAs have been examined experimentally, indicating they are indispensable players in DNA damage (**Figure 2**).

### CCND1 ncRNAs

In 2008, a series of lncRNAs, named CCND1 ncRNAs, were generated in the upstream of CCND1 promoter when subjected to genotoxic stress and this was the first example of lncRNAs found to be responsive to DDR [66]. These lncRNAs, pol II-regulated and polyadenylated but not capped, vary in length from 200 to 330 nt or even bigger and always show multiple or diffuse bands when detected using Northern blotting analyses. Surprisingly, these CCND1ncRNAs are, remarkably, present at low copy number (2-4 copies/cell) following IR treatment and are associated with chromatin epigenetic modifications, functioning *in cis* as "selective ligands" to recruit and modulate the activity of TLS (translocated in liposarcoma), an RNA-binding protein with RNA-binding domains at its C-terminus. However, the N-terminal of TLS, glutamine-rich domain, is responsible for the interaction with two well-known histone acetyltransferases, CBP and p300. The interaction between TLS and CBP/p300 results in the substrate-specific inhibition of the HAT activities of CBP/p300 [66]. In response to genotoxic stress, the expression of CCND1ncRNAs was induced while the CCND1 mRNA is down-regulated. The DDR-responsive lncRNAs, CCND1ncRNAs, localized to the regulatory regions of CCND1 gene, cooperatively recruit TLS and cause a close-to-open conformation change in TLS that licenses its interaction with CBP/p300, resulting in substrate-specific inhibition of their HAT enzymatic activities, and thus establishing the hypo-acetylation status of the chromatin and repressing of the CCND1 mRNA expression [66, 67]. Surprisingly, in the same region of CCND1 promoter a number of antisense ncRNA transcripts were also found to be upregulated following IR treatment, but the functions of these promoter-derived ncRNAs and how they were modulated by genotoxic stress are currently unknown and need further study.

### LincRNA-p21

Another example of a lncRNA involved in DNA damage and cell cycle control is the long intergenic ncRNA p21 (lincRNA-p21), which was identified in an attempt to unravel those lincRNAs regulated by p53. Previously, by searching for the specific K4–K36 methylation domains, indicator of RNA polymerase II active

transcription, reside outside known gene loci, more than thousands of lincRNAs were identified in mouse genome [36]. When $p53^{+/+}$ and $p53^{-/-}$ MEF cells exposed to a DNA damaging agent (doxorubicin), 39 lincRNAs were significantly induced in $p53^{+/+}$ but not $p53^{-/-}$ cells. Interestingly, most of them bear the consensus p53 *cis*-regulatory binding element in their promoters and reside in the cluster associated with p53-mediated damage response, indicating that p53, an important tumor suppressor gene in maintaining genome integrity, may exert its function in part by directly activating some lincRNAs, which in turn regulate downstream transcriptional repression in damage response. Indeed, numerous lincRNAs are identified to be induced by the p53 tumor-suppressor pathway using microarray analysis in two independent cell systems. In particular, one of them is lincRNA-p21 , a ~3 kb transcript located in the proximity of the cell cycle regulator gene, CDKN1A [68]. The lincRNA-p21 is directly induced by p53 to play a critical role in the p53 transcriptional response. Unlike CCND1 ncRNA which acts locally by regulating nearby coding gene epigenetically, lincRNA-p21acts globally as an inhibitor of the p53-dependent transcriptional response by repressing the transcription of genes that interfere with apoptosis. Interestingly, lincRNA-p21 mediates gene repression by physically interacting with ribonucleoprotein K (hnRNP-K), which in turn be recruited to the promoters of genes known to be repressed in a p53-dependent manner. Knockdown of lincRNA-p21 results in hnRNP-K mislocalization at promoters of p53-repressed genes, in reverse loss of hnRNP-K leads to derepression of these shared targets. A 780 nt region at the 5′ end of lincRNA-p21 is necessary for interacting with hnRNP-K and RNA folding analyses of this region predict a highly stable 280 nt structure with deep evolutionary conservation [68]. However, the factors that determine the targeting of lincRNA-p21 to specific loci are poorly understood.

## PANDA

Recently a diversity of small ncRNAs ranging from 18 to 200 nt, which have been variously named promoter-associated small RNAs (PASRs), transcription-initiation RNAs (tiRNAs) and transcription start site associated RNAs (TSSa-RNAs), have been identified in human, mouse, chicken and Drosophila [69-71]. However, it remains uncertain whether these small ncRNAs are functional or just as cleavage products of larger capped (m)RNAs. In addition to these small ncRNAs, a few long noncoding transcripts as mentioned above have been identified in promoters and implicated in transcriptional regulation, considering the pervasive noncoding transcription proximal to transcription start sites (TSS) is a widespread phenomenon at eukaryotic promoters. Inspired by these examples, Hung and his colleagues, taking advantage of ultrahigh-resolution microarray technology, identified 216 putative lncRNAs encoded in the genomic loci of 56 cell-cycle genes (cyclins, cyclin-dependent kinases (CDKs), CDK inhibitors (CDKIs)) [72]. Similar to their neighboring coding partners, the expression levels of these lncRNAs fluctuate during cell cycle progression, stem cell differentiation, neoplastic transformation as well as DNA damage. Among them, 12 lncRNAs showed at least 2-fold change in response to p53 activation via DNA damage using tilling array and qRT-PCR. Notably, there is another lncRNA, coined PANDA (P21 Associated ncRNA DNA damage Activated), locates approximately 5 kb upstream on the antisense strand between the protein-coding CDKN1A gene and lincRNA-p21 [72]. Interestingly, PANDA was specifically induced by p53 in response to genotoxic stress. Knockdown of PANDA selectively promoted the expression of p53-regulated pro-apoptotic genes such as FAS and APAF1, whereas its depletion had no effect on p21 expression, suggesting it is a p53 effector that acts independently of p21. Using RNA chromatography and chromatin immunoprecipitation, they found that PANDA delimits DNA damage-induced apoptosis by physically interacting with the transcription factor NF-YA, not other chromatin modification complexes known to binding with lncRNAs, such as LSD1 or EZH2. NF-YA is a subunit of heterotrimeric CCAAT-binding complex (NF-Y), which has been shown to function as a trans-activator of a subset of p53 targets, such as FAS [73]. Knockdown of PANDA promotes NF-YA occupancy at the promoter regions of p53-dependent pro-apoptotic target genes, such as FAS, PUMA, CCNB1 and NOXA, which in turn leading to increased cell death in response to DNA damage. However, the specific mechanism of the pro-survival effects of PANDA dur-

ing DNA damage is not yet entirely clear and need to be determined in future.

### Site-specific small RNAs induced in DNA damage response

More recently, Francia et al have uncovered an unexpected additional layer of crosstalk between the RNA interference (RNAi) pathway and DNA damage. A novel and unsuspected class of small RNAs, named DDR-regulating RNAs (DDRNAs), have been identified in the vicinity of double strand breaks (DSB), one of the most lethally damaging effects after exposure to ionizing radiation [10]. It was known that the RNAi pathway giving rise to double-stranded RNA products are evolutionary conserved, and its components are thought to have evolved to preserve genome integrity against naturally occurring transposons and viruses. Conditional deletion of a single copy of Dicer led to enhanced tumor development and reduced survival on several mouse models and inactivation of various components of Dicer and Drosha complexes stimulate cell transformation and tumorigenesis [74, 75]. In addition, piRNAs and QDE-2 interacting RNAs (qiRNAs) in the filamentous fungus *Neurospora crassa* have been implicated in maintenance of genomic integrity [76, 77]. These findings inspired them to examine the possibility that factors involved in small RNA biogenesis or processing may have some direct role in the control of DDR activation at the site of DNA damage. Inactivation of Dicer or Drosha, not the downstream components of RNAi pathway, significantly reduced the number of cell positive for DDR foci and impaired the DNA-damage-induced G1/S and G2/M cell cycle checkpoints in cultured human and mouse cells and even in various tissues of living zebrafish larvae. In addition, these DDR foci are lost in irridiated cells following RNase A treatment and can be robustly restored when incubation RNase-A-treated cells with of exogenous RNA, even if generated by chemical synthesis or processed by recombinant Dicer *in vitro*, suggesting these so call DDRNAs are locally generated and favor the assembly of DDR foci. Indeed deep sequencing analysis confirmed the presence of site-specific small RNAs arising from the integrated exogenous locus upon cleavage with SceI. Almost at the same time, another group reported the presence of Dicer-dependent small RNAs (named DSB-induced RNAs, diRNAs) arising from the sequences flanking DSBs in plant and even in human cells [9]. In *Arabidopsis,* the biogenesis of diRNAs requires the PI3K-related kinase ATR, RNA polymerase IV (Pol IV), and Dicer-like proteins (DCLs) and mutations in these proteins significantly impair the repair efficiency of DSBs. The diRNAs exert their functions by interaction with Ago2 and Ago2 is important for their accumulation, while it is unclear whether DDRNAs also rely on the Argonaute protein family members. Although at present how these site-specific small RNAs act to control DDR activation has not fully understood, these findings indicate that the presence of DSB-derived site-specific small RNAs may be a universal phenomenon in DNA damage, implicated in recruiting of chromatin-modifying complexes to sites of damage or orchestrating DNA repair signaling.

### The roles and mechanisms of ncRNAs in DNA damage response

Efficient repair of DNA damage requires a coordinated response between the factors that sense DNA damage and those that mediate repair. Upon recognition of DNA damage, transducers, such as ATM (ataxia-telangiectasia mutated), ATR (ATM- and Rad3-related) and DNA-PKcs((DNA-dependent protein kinase catalytic subunit) relay and amplify the damage signal to effectors proteins like Chk1 and Chk2 that control cell cycle progression, DNA repair and apoptosis. In addition to the expression of ncRNAs is responsive to DNA damage as mentioned above, it is becoming more and more clear that those factors (sensors, transducers and effectors) in DNA damage are subjected to regulation by those DDR-responsive ncRNAs, including miRNAs and lncRNAs. In the following section, we will summarize our current understanding on the emerging and potential roles of these ncRNAs in the DDR pathway.

### MiRNAs-mediated gene silencing in DNA damage response

Because the transcription and maturation of miRNAs changes in response to DNA damage, it was not surprising that miRNAs are involved in the regulation of genes related to DNA damage, implying bidirectional communication signals between miRNAs and DDR. One of the first clues that implicated miRNAs in the regulation

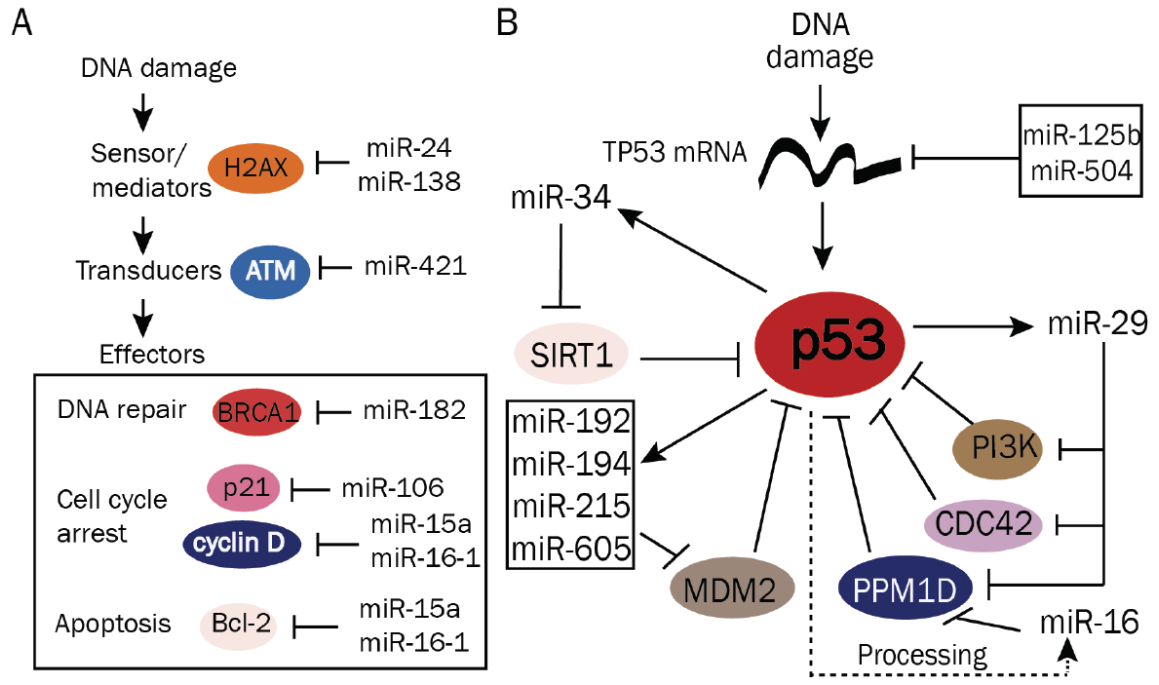


**Figure 3.** The roles of miRNAs in DNA damage response. A. Direct regulation. Nearly all primary components of the DDR signaling pathway are subjected to miRNAs-mediated gene silencing during DNA damage, including sensors or mediators of damage, signal transducers and effectors. B. Indirect regulation. miRNAs exert their functions by fine-tuning the expression of critical components of the DNA damage response indirectly, such as p53, which was subjected to indirect regulation by miRNAs via down-regulation of upstream regulators of p53.

of DNA damage was knockdown of the essential component of miRNA-processing pathway, such as Dicer and Ago2, which result in a significant decrease in cell survival and altered checkpoint response after exposure to DNA-damaging agents UV and cisplatin [44, 78]. These studies indicate that miRNA-mediated gene silencing may have physiological relevance in the responses of DNA damage and repair. It was known that miRNAs are known to control gene expression post-transcriptionally by binding to complementary sequences in target mRNAs, thereby leading to mRNA degradation or translational inhibition. In silico prediction and experimental validation have identified that miRNAs regulate multiple aspects of DNA damage, including directly regulating the expression of diverse components of the DDR pathway and indirectly fine-tune the expression of master regulatory proteins such as p53 through cross-talking with other signaling pathways (**Figure 3**).

### Direct regulation

By analyzing the 3′UTR of 142 genes that implicated in DDR using 2 different miRNA target prediction algorithms, more than half of them predicted to contain conserved miRNA target sites [79]. With more and more prediction and then validation of these miRNA targets, it's now clear that almost all principle components of the DDR signaling pathway are subjected to miRNAs-mediated gene silencing during DNA damage, including sensors or mediators of damage, signal transducers and effectors.

For example, histone variant H2AX, an initial sensor protein for the DDR and rapidly phosphorylated (Ser139) by PI3K-related protein kinases (PIKKs) following DNA damage, was found to be a target of miR-24 [80]. Overexpression of miR-24 dramatically down-regulated H2AX in terminal differentiated human blood cells, rendering them hypersensitive to gamma-irradiation, deficient in DSB repair, and susceptible to chromosomal instability. Further studies revealed that H2AX was also targeted by miR-138 via directly binding to its 3' UTR [81]. Ectopic expression of miR-138 repressed γ-H2AX foci formation, inhibited homologous recombination and enhanced cellular sensitivity to multiple DNA-damaging

agents in a human osteosarcoma cell line U2OS.

ATM, a serine/threonine kinase that transfers the DNA damage signals to downstream pathways, was also reported to be attenuated by miR-421 upon DNA damage. Ectopic expression of miR-421 results in a deficient S-phase cell cycle checkpoint and an increased sensitivity to IR, while blocking the interaction between miR-421 and ATM with antisense morpholino oligonucleotides rescued this defective phenotype [82]. Interestingly, the expression of miR-421 was significantly induced in N-Myc-amplified neuroblastoma and large B cell lymphoma cell lines, implying the interaction between miR421 and ATM might contribute to N-Myc-induced tumorigenesis. Until now, there are a large body of literature has revealed that many crucial genes in DDR are regulated by miRNAs and a complete list of miRNAs involved in DNA damage has been summarized in our another review [83].

### Indirect regulation

In addition to direct regulation of diverse components in the DDR signaling pathway, miRNAs exert their functions by fine-tuning the expression of critical components of the DNA repair pathways indirectly, such as p53, which was subjected to indirect regulation by miRNAs via down-regulation of upstream regulators of p53. The tumor suppressor p53 plays a central role in the maintenance of genomic integrity and tumor suppression and, thus, its expression and activity are under tight surveillance [84]. Although it has long been known that p53 directly transactivates miRNA expression, including the miR34 family miR15a/16-1, and the miR-192/194/215 clusters, recent studies shown that p53 activity was widely regulated by a number of miRNAs. For example, miR-125b and miR-504 were reported to repress p53 by binding to specific responsive element (MRE) located in the p53 3′ UTR, respectively. Functional studies revealed that overexpression of these miRNAs reduces endogenous p53 level and suppresses apoptosis in human cells, whereas loss of them had the opposite effect [85, 86]. In addition to direct binding to the 3' UTR of p53, miRNAs were found to regulate p53 activity indirectly by modulating p53-associated factors. Mdm2 is an E3 ubiquitin ligase and a known negative regulator of p53. The interaction of p53 and Mdm2 was disrupted following DNA damage, which leads to the rapid activation of p53. The studies from Wang group showed that miR-605 is a new component in the p53 regulatory network, being transcriptionally activated by p53 and post-transcriptionally repressing Mdm2 [87]. Transactivated miR-605 directly decreases the expression of Mdm2 and thus indirectly enhances the transcriptional activity of p53. A similar feedback loop was also described for miR-192, miR-194 and miR-215, which are also induced by p53 and then negatively regulate MDM2 expression [88].

miR-34 also functions as a downstream effector to amplify the p53 signal by modulating multiple cell cycle-related transcripts, including silent information regulator 1 (SIRT1) [89]. Inhibition of SIRT1 by miR-34 results in an increase in the acetylation and activity of p53, which in turn promote the expression of its targets p21 and PUMA that regulate the cell cycle and apoptosis, respectively. Moreover, it was shown that miR-122 is involved in the up-regulation of p53 activity [90]. By modulating cyclin G1, ectopic expression of miR-122 inhibits the recruitment of the PP2A phosphatase to MDM2, resulting in decreased MDM2 activity and increased p53 levels and activity, as well as increases sensitivity to doxorubicin challenge in hepatocellular carcinoma-derived cell lines. In the meanwhile, our own studies revealed that miR-16, induced immediately after DNA damage, targets the expression of PPM1D, which is a negative regulator of p53, leading to p53 induction [91]. Further studies described a similar loop between the miR-29 family (miR-29a, b and c) and p53 [92, 93]. miR-29 upregulates the expression levels of p53 and induce apoptosis in a p53-dependent manner by suppressing p85 (the regulatory subunit of PI3 kinase) and CDC42 (a Rho family GTPase), both of which negatively regulate p53. Furthermore, similar to miR-16, in DNA damage the transcriptionally activated miR-29 also found to represses Ppm1d phosphatase. Taken together, these results revealed p53 activity and expression are controlled by a dense network of miRNAs, which forms a positive feedback loop that ensures rapid accumulation of p53 after DNA damage.

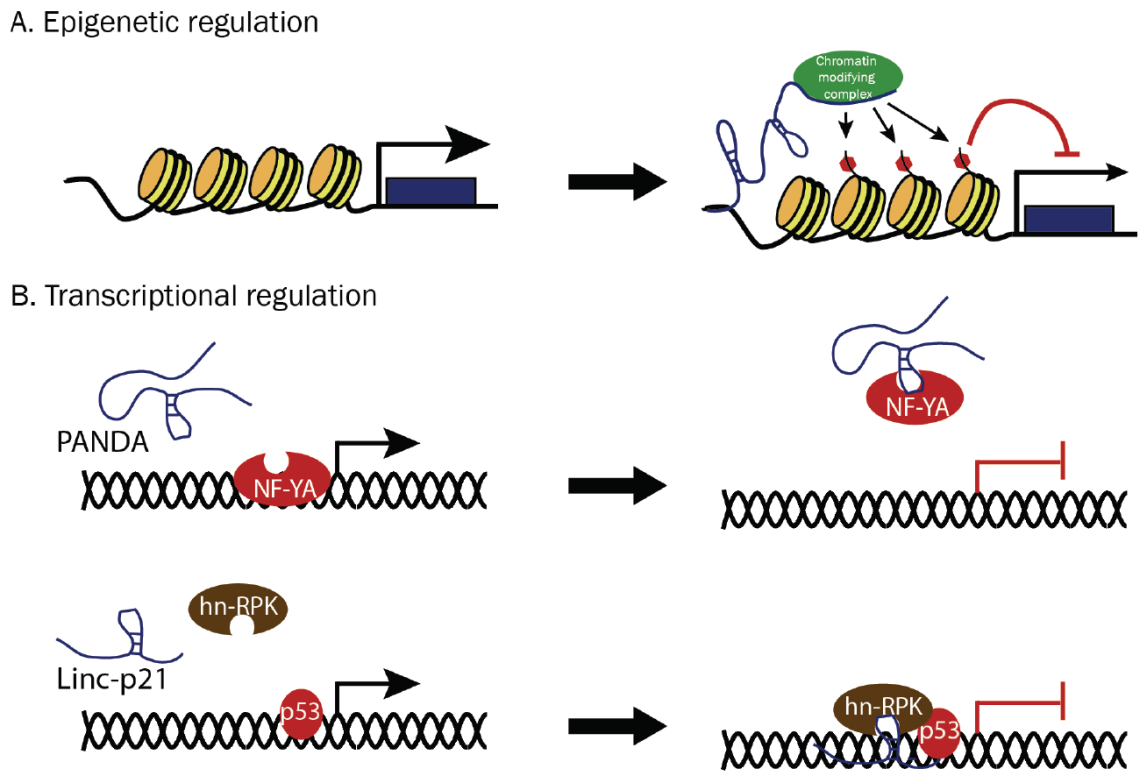


**Figure 4.** The roles of lncRNAs in DNA damage response. A. Epigenetic regulation. lncRNAs such as CCND1-ncRNA and Tug1 can function as 'GPS devices' to interact with chromatin-remodeling or histone-modifying protein complexes and then guide them to specific genomic loci to regulate specific genes expression. B. Transcriptional regulation. Linc-p21 physically interacts with hn-RPK and modulates its localization at repressed genes, regulating p53-mediated apoptosis. By contrast, the interaction of PANDA with the transcription factor NF-YA prevents its binding to chromatin, which impedes the expression of pro-apoptotic genes and facilitates cell-cycle arrest.

## Regulation of DNA damage response by lncRNAs

Similar to coding genes, there is considerable variability in the functions of lncRNAs. They are involved in a surprisingly wide variety of cellular processes and these processes typically related to transcriptional regulation or mRNA processing, which is a reminiscent of miRNAs. However, unlike miRNAs, lncRNAs participate in a wide spectrum of biological contexts which show greater complexity to their functions. Here we will briefly discuss the potential mechanisms by which those lncRNAs exerts their effects in response to DNA damage, although much remains to be learned about the detailed mechanism of action in each case (**Figure 4**).

### Epigenetic regulation

Many lncRNAs such as XIST, ANRIL, HOTAIR, H19, KCNQ1OT1 and AIR have been shown to be involved in epigenetic regulation of target genes [24, 26, 30, 94-97]. These lncRNAs interact with chromatin-remodeling or histone-modifying protein complexes and guide them to specific genomic loci to exert their functions. The most notable protein partners of lncRNAs are the polycomb repressive complex 1 and 2 (PRC1 and PRC2), which usually transfer repressive posttranslational modifications to specific amino acid positions on histone tail proteins, thereby facilitating chromatin compaction and heterochromatin formation. In support of this model, a recent study shows that approximately 20% of lncRNAs expressed in a given cell associated with chromatin-modifying complexes such as PRC2 and many of these lncRNAs interact with multiple chromatin factors [38]. For example, the lncRNA CCND1ncRNAs, which was abundantly induced from the 5′ regulatory regions of CCND1 upon DNA damage, functions as 'GPS devices' to guide other cellular compo-

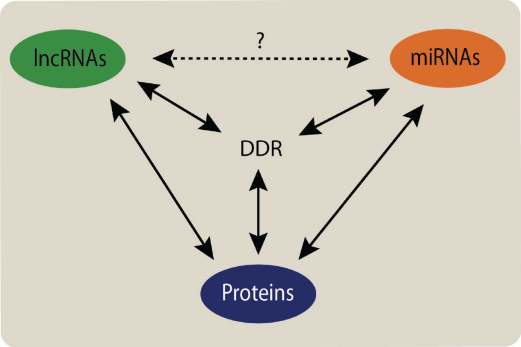


**Figure 5.** The crosstalk of proteins, miRNAs and lncRNAs in DNA damage response.

nents to the sites of action [66]. It functions as a ligand to mediate histone modifications through interaction with RNA binding protein TLS. The CCND1ncRNA-TLS complex effectively binds to CBP/p300 and inhibits the substrate-specific HAT activities of CBP/p300, resulting in repression of CCND1 expression. Another example is the lncRNA Tug1, which was originally identified as a transcript upregulated by taurine, involving in the mouse retinal development [98]. Further study shows that Tug1 was one of those 39 lncRNAs specifically induced in p53-wild type, but not p53-mutant cells, likely through the binding of p53 to the conserved binding sites in promoter [38]. Unlike CCND1 ncRNA which acts locally, Tug1 acts globally as a downstream repressor of the p53-dependent transcriptional response through interaction with chromatin-modifying complex PRC2.

## Transcriptional regulation

In addition to facilitating chromatin regulation by serving as molecular scaffold, emerging evidence suggests that some lncRNAs are induced in response to specific stimuli, and in turn activate or repress specific transcriptional programs, which allow cells respond to these stimuli. Several lncRNAs such as linc-p21and PANDA are found to be direct targets of the tumor-suppressor protein p53 in response to DNA damage [72, 99]. Subsequently, these lncRNAs regulate downstream gene expression by distinct pathways. LincRNA-p21, located in the promoter of CDKN1A gene, was found to act as a transcriptional repressor in the canonical p53 pathway and to play a role in triggering apoptosis through its binding to and modulation of hnRNP-K localization [99]. By contrast, the lncRNA PANDA, which is also transcribe from CDKN1A promoter, interacts with the transcription factor NF-YA. The binding of PANDA to NF-YA may evict or prevent NF-YA binding to chromatin, which impedes the expression of pro-apoptotic genes and facilitates cell-cycle arrest [72], suggesting ncRNAs may act as key regulatory nodes in multiple transcriptional pathways.

## Conclusions and perspectives

The DDR signaling pathway encompasses a set of tightly regulated steps: sense the DNA damage, transduce the signal and initiate the repair of damaged DNA. The information collected and transmitted by these factors will be used in making cell fate decision - either arrest cell cycle to allow repair of damaged DNA and survival or apoptosis in case of severe damage [4]. In addition to those well-studied protein-coding genes, recent studies point to the need for an expanded definition to also include ncRNAs, such as miRNAs and lncRNAs. In the last decade, the crosstalk of miRNAs with DDR is extensively studied, although many questions remain to be resolved. Available evidences show that DNA damage signaling participates in the regulation of miRNA biogenesis at both the transcriptional and post-transcriptional levels. On the other hand, the DDR-responsive miRNAs in turn act in concert with others protein factors to modulate the DDR signaling pathway via miRNA-mediated gene silencing. By contrast, the roles of lncRNAs in DNA damage are poorly understood but beginning to emerge. So far only a few lncRNAs in DDR signaling pathway have been evaluated experimentally, although thousands of mRNA-like lncRNAs without significant protein-coding capacity have been predicted in human genome. Similar to miRNAs, these DDR-responsive lncRNAs in turn work cooperatively with proteins by forming ribonucleoprotein complexes (RNPs) to modulate the DDR signaling pathway. lncRNAs not only interact with chromatin-remodeling or histone-modifying protein complexes and guide them to specific genomic loci to exert their functions post-transcriptionally, but also function as molecular scaffold to activate or repress specific programs transcriptionally. In addition to the interplays of proteins with miRNAs and lncRNAs,

recent evidences suggest that some lncRNAs may also regulate gene expression post-transcriptionally by directly binding to miRNAs, acting as a "sponge" to prevent specific miRNAs from binding to their target mRNAs [100]. These findings revealed a novel layer of regulation that distinct classes of ncRNAs interplay with each other and cooperate to regulate gene expression. However, it is unclear whether this kind of crosstalk between miRNAs and lncRNAs also exist in DDR signaling pathway. Based on the interplays of proteins, miRNAs and lncRNAs in DNA damage response, we proposed a four-component model to depict the coding and noncoding genes' interactions (**Figure 5**). In this model, proteins, miRNAs and lncRNAs are 3 players and mediators that regulate each other's expression by various means in DNA damage response. More recently, a new class ncRNAs, Dicer- and Drosha-dependent small RNAs, was identified in the vicinity of DNA double-strand break sites in human and mouse cell lines, as well as in plant [9, 10], indicating that the presence of DSB-derived site-specific small RNAs may be a universal phenomenon in DNA damage response. These results are very intriguing since they demonstrated a novel ncRNA player in DDR pathway and we are sure more of them are on the way and yet to be determined in future.

## Acknowledgements

We thank every member in the Lu laboratory for critical discussion and revision on the manuscript. This research was supported by grants to X.L. from the National Institutes of Health (R01CA136549 and R03CA142605), the American Cancer Society (119135-RSG-10-185-01-TBE), University of Texas Star Plus award, and the Start-up fund from MD Anderson Cancer Center.

**Address correspondence to:** Dr. Xiongbin Lu, Department of Cancer Biology, Unit-173, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA. Tel: 713-745-6247; E-mail: xlu2@mdanderson.org

## References

[1] Ciccia A and Elledge SJ. The DNA damage response: making it safe to play with knives. Mol Cell 2010; 40: 179-204.

[2] Lord CJ and Ashworth A. The DNA damage response and cancer therapy. Nature 2012; 481: 287-294.

[3] Harper JW and Elledge SJ. The DNA Damage Response: Ten Years After. Molecular Cell 2007; 28: 739-745.

[4] Shiloh Y. ATM and ATR: networking cellular responses to DNA damage. Current Opinion in Genetics & Development 2001; 11: 71-77.

[5] Prensner JR and Chinnaiyan AM. The emergence of lncRNAs in cancer biology. Cancer Discov 2011; 1: 391-407.

[6] Wei W, Ba ZQ, Gao M, Wu Y, Ma YT, Amiard S, White CI, Danielsen JMR, Yang YG and Qi YJ. A Role for Small RNAs in DNA Double-Strand Break Repair. Cell 2012; 149: 101-112.

[7] Francia S, Michelini F, Saxena A, Tang D, de HM, Anelli V, Mione M, Carninci P and di Fagagna FD. Site-specific DICER and DROSHA RNA products control the DNA-damage response. Nature 2012.

[8] Kaikkonen MU, Lam MT and Glass CK. Non-coding RNAs as regulators of gene expression and epigenetics. Cardiovasc Res 2011; 90: 430-440.

[9] Wei W, Ba Z, Gao M, Wu Y, Ma Y, Amiard S, White CI, Rendtlew Danielsen JM, Yang YG and Qi Y. A role for small RNAs in DNA double-strand break repair. Cell 2012; 149: 101-112.

[10] Francia S, Michelini F, Saxena A, Tang D, de HM, Anelli V, Mione M, Carninci P and di Fagagna FD. Site-specific DICER and DROSHA RNA products control the DNA-damage response. Nature 2012.

[11] Bartel DP. MicroRNAs: Target Recognition and Regulatory Functions. Cell 2009; 136: 215-233.

[12] Grimson A, Srivastava M, Fahey B, Woodcroft BJ, Chiang HR, King N, Degnan BM, Rokhsar DS and Bartel DP. Early origins and evolution of microRNAs and Piwi-interacting RNAs in animals. Nature 2008; 455: 1193-1U15.

[13] Mendell JT and Olson EN. MicroRNAs in Stress Signaling and Human Disease. Cell 2012; 148: 1172-1187.

[14] Saini HK, Griffiths-Jones S and Enright AJ. Genomic analysis of human microRNA transcripts. Proceedings of the National Academy of Sciences of the United States of America 2007; 104: 17719-17724.

[15] Davis BN and Hata A. Regulation of MicroRNA Biogenesis: A miRiad of mechanisms. Cell Communication and Signaling 2009; 7.

[16] Kim VN. MicroRNA biogenesis: Coordinated cropping and dicing. Nature Reviews Molecular Cell Biology 2005; 6: 376-385.

[17] Kim VN, Han J and Siomi MC. Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol 2009; 10: 126-139.

[18] Liu Q and Paroo Z. Biochemical principles of small RNA pathways. Annu Rev Biochem 2010; 79: 295-319.
[19] Bohnsack MT, Czaplinski K and Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. Rna-A Publication of the Rna Society 2004; 10: 185-191.
[20] Gregory RI, Chendrimada TP, Cooch N and Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell 2005; 123: 631-640.
[21] Gibb EA, Brown CJ and Lam WL. The functional role of long non-coding RNA in human carcinomas. Mol Cancer 2011; 10: 38.
[22] Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A and Rinn JL. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes & Development 2011; 25: 1915-1927.
[23] Chu C, Qu K, Zhong FL, Artandi SE and Chang HY. Genomic Maps of Long Noncoding RNA Occupancy Reveal Principles of RNA-Chromatin Interactions. Molecular Cell 2011; 44: 667-678.
[24] Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R and Willard HF. A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. Nature 1991; 349: 38-44.
[25] Lee JT, Davidow LS and Warshawsky D. Tsix, a gene antisense to Xist at the X-inactivation centre. Nat Genet 1999; 21: 400-404.
[26] Sotomaru Y, Katsuzawa Y, Hatada I, Obata Y, Sasaki H and Kono T. Unregulated expression of the imprinted genes H19 and Igf2r in mouse uniparental fetuses. J Biol Chem 2002; 277: 12474-12478.
[27] Brannan CI, Dees EC, Ingram RS and Tilghman SM. The product of the H19 gene may function as an RNA. Mol Cell Biol 1990; 10: 28-36.
[28] Willingham AT, Orth AP, Batalov S, Peters EC, Wen BG, Aza-Blanc P, Hogenesch JB and Schultz PG. A strategy for probing the function of noncoding RNAs finds a repressor of NFAT. Science 2005; 309: 1570-1573.
[29] Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, Lajoie BR, Protacio A, Flynn RA, Gupta RA, Wysocka J, Lei M, Dekker J, Helms JA and Chang HY. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. Nature 2011; 472: 120-124.
[30] Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, Goodnough LH, Helms JA, Farnham PJ, Segal E and Chang HY. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 2007; 129: 1311-1323.
[31] Mercer TR, Dinger ME and Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet 2009; 10: 155-159.
[32] Ponting CP, Oliver PL and Reik W. Evolution and Functions of Long Noncoding RNAs. Cell 2009; 136: 629-641.
[33] Katayama S, Tomaru Y, Kasukawa T, Waki K, Nakanishi M, Nakamura M, Nishida H, Yap CC, Suzuki M, Kawai J, Suzuki H, Carninci P, Hayashizaki Y, Wells C, Frith M, Ravasi T, Pang KC, Hallinan J, Mattick J, Hume DA, Lipovich L, Batalov S, Engstrom PG, Mizuno Y, Faghihi MA, Sandelin A, Chalk AM, Mottagui-Tabar S, Liang Z, Lenhard B and Wahlestedt C. Antisense transcription in the mammalian transcriptome. Science 2005; 309: 1564-1566.
[34] Faghihi MA and Wahlestedt C. Regulatory roles of natural antisense transcripts. Nat Rev Mol Cell Biol 2009; 10: 637-643.
[35] Faghihi MA, Modarresi F, Khalil AM, Wood DE, Sahagan BG, Morgan TE, Finch CE, St LG, III, Kenny PJ and Wahlestedt C. Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase. Nat Med 2008; 14: 723-730.
[36] Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabili MN, Jaenisch R, Mikkelsen TS, Jacks T, Hacohen N, Bernstein BE, Kellis M, Regev A, Rinn JL and Lander ES. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 2009; 458: 223-227.
[37] Guttman M, Garber M, Levin JZ, Donaghey J, Robinson J, Adiconis X, Fan L, Koziol MJ, Gnirke A, Nusbaum C, Rinn JL, Lander ES and Regev A. Ab initio reconstruction of cell type-specific transcriptomes in mouse reveals the conserved multi-exonic structure of lincRNAs. Nat Biotechnol 2010; 28: 503-510.
[38] Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea MD, Thomas K, Presser A, Bernstein BE, van OA, Regev A, Lander ES and Rinn JL. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci U S A 2009; 106: 11667-11672.
[39] Guttman M, Donaghey J, Carey BW, Garber M, Grenier JK, Munson G, Young G, Lucas AB, Ach R, Bruhn L, Yang X, Amit I, Meissner A, Regev A, Rinn JL, Root DE and Lander ES. lincRNAs act in the circuitry controlling pluripotency and differentiation. Nature 2011; 477: 295-300.
[40] Cha HJ, Shin S, Yoo H, Lee EM, Bae S, Yang KH, Lee SJ, Park IC, Jin YW and An S. Identification of ionizing radiation-responsive microRNAs in

the IM9 human B lymphoblastic cell line. Int J Oncol 2009; 34: 1661-1668.
[41] Faraonio R, Salerno P, Passaro F, Sedia C, Iaccio A, Bellelli R, Nappi TC, Comegna M, Romano S, Salvatore G, Santoro M and Cimino F. A set of miRNAs participates in the cellular senescence program in human diploid fibroblasts. Cell Death Differ 2012; 19: 713-721.
[42] Galluzzi L, Morselli E, Vitale I, Kepp O, Senovilla L, Criollo A, Servant N, Paccard C, Hupe P, Robert T, Ripoche H, Lazar V, Harel-Bellan A, Dessen P, Barillot E and Kroemer G. miR-181a and miR-630 regulate cisplatin-induced cancer cell death. Cancer Res 2010; 70: 1793-1803.
[43] Josson S, Sung SY, Lao K, Chung LW and Johnstone PA. Radiation modulation of microRNA in prostate cancer cell lines. Prostate 2008; 68: 1599-1606.
[44] Pothof J, Verkaik NS, van IW, Wiemer EA, Ta VT, van der Horst GT, Jaspers NG, van Gent DC, Hoeijmakers JH and Persengiev SP. MicroRNA-mediated gene silencing modulates the UV-induced DNA-damage response. EMBO J 2009; 28: 2090-2099.
[45] Shin S, Cha HJ, Lee EM, Lee SJ, Seo SK, Jin HO, Park IC, Jin YW and An S. Alteration of miRNA profiles by ionizing radiation in A549 human non-small cell lung cancer cells. Int J Oncol 2009; 35: 81-86.
[46] Wagner-Ecker M, Schwager C, Wirkner U, Abdollahi A and Huber PE. MicroRNA expression after ionizing radiation in human endothelial cells. Radiat Oncol 2010; 5: 25.
[47] Galluzzi L, Morselli E, Vitale I, Kepp O, Senovilla L, Criollo A, Servant N, Paccard C, Hupe P, Robert T, Ripoche H, Lazar V, Harel-Bellan A, Dessen P, Barillot E and Kroemer G. miR-181a and miR-630 regulate cisplatin-induced cancer cell death. Cancer Res 2010; 70: 1793-1803.
[48] He L, He X, Lim LP, de SE, Xuan Z, Liang Y, Xue W, Zender L, Magnus J, Ridzon D, Jackson AL, Linsley PS, Chen C, Lowe SW, Cleary MA and Hannon GJ. A microRNA component of the p53 tumour suppressor network. Nature 2007; 447: 1130-1134.
[49] Welch C, Chen Y and Stallings RL. MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. Oncogene 2007; 26: 5017-5022.
[50] Cannell IG, Kong YW, Johnston SJ, Chen ML, Collins HM, Dobbyn HC, Elia A, Kress TR, Dickens M, Clemens MJ, Heery DM, Gaestel M, Eilers M, Willis AE and Bushell M. p38 MAPK/MK2-mediated induction of miR-34c following DNA damage prevents Myc-dependent DNA replication. Proc Natl Acad Sci U S A 2010; 107: 5375-5380.
[51] Georges SA, Biery MC, Kim SY, Schelter JM, Guo J, Chang AN, Jackson AL, Carleton MO, Linsley PS, Cleary MA and Chau BN. Coordinated regulation of cell cycle transcripts by p53-Inducible microRNAs, miR-192 and miR-215. Cancer Res 2008; 68: 10105-10112.
[52] Braun CJ, Zhang X, Savelyeva I, Wolff S, Moll UM, Schepeler T, Orntoft TF, Andersen CL, and Dobbelstein M. p53-Responsive micrornas 192 and 215 are capable of inducing cell cycle arrest. Cancer Res 2008; 68: 10094-10104.
[53] Boominathan L. The tumor suppressors p53, p63, and p73 are regulators of microRNA processing complex. PLoS One 2010; 5: e10615.
[54] Su X, Chakravarti D, Cho MS, Liu L, Gi YJ, Lin YL, Leung ML, El-Naggar A, Creighton CJ, Suraokar MB, Wistuba I and Flores ER. TAp63 suppresses metastasis through coordinate regulation of Dicer and miRNAs. Nature 2010; 467: 986-990.
[55] Niu J, Shi Y, Tan G, Yang CH, Fan M, Pfeffer LM and Wu ZH. DNA damage induces NF-kappaB-dependent microRNA-21 up-regulation and promotes breast cancer cell invasion. J Biol Chem 2012; 287: 21783-21795.
[56] Aguda BD, Kim Y, Piper-Hunter MG, Friedman A and Marsh CB. MicroRNA regulation of a cancer network: consequences of the feedback loops involving miR-17-92, E2F, and Myc. Proc Natl Acad Sci U S A 2008; 105: 19678-19683.
[57] Suzuki HI, Yamagata K, Sugimoto K, Iwamoto T, Kato S and Miyazono K. Modulation of microRNA processing by p53. Nature 2009; 460: 529-533.
[58] Fukuda T, Yamagata K, Fujiyama S, Matsumoto T, Koshida I, Yoshimura K, Mihara M, Naitou M, Endoh H, Nakamura T, Akimoto C, Yamamoto Y, Katagiri T, Foulds C, Takezawa S, Kitagawa H, Takeyama K, O'Malley BW and Kato S. DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. Nat Cell Biol 2007; 9: 604-611.
[59] Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N and Shiekhattar R. The Microprocessor complex mediates the genesis of microRNAs. Nature 2004; 432: 235-240.
[60] Junttila MR and Evan GI. p53--a Jack of all trades but master of none. Nat Rev Cancer 2009; 9: 821-829.
[61] Boominathan L. The guardians of the genome (p53, TA-p73, and TA-p63) are regulators of tumor suppressor miRNAs network. Cancer Metastasis Rev 2010; 29: 613-639.
[62] Zhang X, Wan G, Berger FG, He X and Lu X. The ATM kinase induces microRNA biogenesis in the DNA damage response. Mol Cell 2011; 41: 371-383.

[63] Trabucchi M, Briata P, Garcia-Mayoral M, Haase AD, Filipowicz W, Ramos A, Gherzi R and Rosenfeld MG. The RNA-binding protein KSRP promotes the biogenesis of a subset of microRNAs. Nature 2009; 459: 1010-1014.
[64] Briata P, Chen CY, Giovarelli M, Pasero M, Trabucchi M, Ramos A and Gherzi R. KSRP, many functions for a single protein. Front Biosci 2011; 16: 1787-1796.
[65] Moran VA, Perera RJ and Khalil AM. Emerging functional and mechanistic paradigms of mammalian long non-coding RNAs. Nucleic Acids Res 2012; 40: 6391-6400.
[66] Wang X, Arai S, Song X, Reichart D, Du K, Pascual G, Tempst P, Rosenfeld MG, Glass CK and Kurokawa R. Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription. Nature 2008; 454: 126-130.
[67] Wang XT, Song XY, Glass CK and Rosenfeld MG. The Long Arm of Long Noncoding RNAs: Roles as Sensors Regulating Gene Transcriptional Programs. Cold Spring Harbor Perspectives in Biology 2011; 3.
[68] Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, Kenzelmann-Broz D, Khalil AM, Zuk O, Amit I, Rabani M, Attardi LD, Regev A, Lander ES, Jacks T and Rinn JL. A Large Intergenic Noncoding RNA Induced by p53 Mediates Global Gene Repression in the p53 Response. Cell 2010; 142: 409-419.
[69] Taft RJ, Glazov EA, Cloonan N, Simons C, Stephen S, Faulkner GJ, Lassmann T, Forrest AR, Grimmond SM, Schroder K, Irvine K, Arakawa T, Nakamura M, Kubosaki A, Hayashida K, Kawazu C, Murata M, Nishiyori H, Fukuda S, Kawai J, Daub CO, Hume DA, Suzuki H, Orlando V, Carninci P, Hayashizaki Y and Mattick JS. Tiny RNAs associated with transcription start sites in animals. Nat Genet 2009; 41: 572-578.
[70] Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, Stadler PF, Hertel J, Hackermuller J, Hofacker IL, Bell I, Cheung E, Drenkow J, Dumais E, Patel S, Helt G, Ganesh M, Ghosh S, Piccolboni A, Sementchenko V, Tammana H and Gingeras TR. RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science 2007; 316: 1484-1488.
[71] Fejes-Toth K, Sotirova V, Sachidanandam R, Assaf G, Hannon GJ, Kapranov P, Foissac S, Willingham AT, Duttagupta R, Dumais E and Gingeras TR. Post-transcriptional processing generates a diversity of 5'-modified long and short RNAs. Nature 2009; 457: 1028-1032.
[72] Hung T, Wang Y, Lin MF, Koegel AK, Kotake Y, Grant GD, Horlings HM, Shah N, Umbricht C, Wang P, Wang Y, Kong B, Langerod A, Borresen-Dale AL, Kim SK, van de Vijver M, Sukumar S, Whitfield ML, Kellis M, Xiong Y, Wong DJ and Chang HY. Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. Nat Genet 2011; 43: 621-629.
[73] Morachis JM, Murawsky CM and Emerson BM. Regulation of the p53 transcriptional response by structurally diverse core promoters. Genes Dev 2010; 24: 135-147.
[74] Kumar MS, Lu J, Mercer KL, Golub TR and Jacks T. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet 2007; 39: 673-677.
[75] Kumar MS, Pester RE, Chen CY, Lane K, Chin C, Lu J, Kirsch DG, Golub TR and Jacks T. Dicer1 functions as a haploinsufficient tumor suppressor. Genes Dev 2009; 23: 2700-2704.
[76] Lee HC, Chang SS, Choudhary S, Aalto AP, Maiti M, Bamford DH and Liu Y. qiRNA is a new type of small interfering RNA induced by DNA damage. Nature 2009; 459: 274-277.
[77] Girard A and Hannon GJ. Conserved themes in small-RNA-mediated transposon control. Trends Cell Biol 2008; 18: 136-148.
[78] Bu Y, Lu C, Bian C, Wang J, Li J, Zhang B, Li Z, Brewer G and Zhao RC. Knockdown of Dicer in MCF-7 human breast carcinoma cells results in G1 arrest and increased sensitivity to cisplatin. Oncol Rep 2009; 21: 13-17.
[79] Wouters MD, van Gent DC, Hoeijmakers JH and Pothof J. MicroRNAs, the DNA damage response and cancer. Mutat Res 2011; 717: 54-66.
[80] Lal A, Pan Y, Navarro F, Dykxhoorn DM, Moreau L, Meire E, Bentwich Z, Lieberman J and Chowdhury D. miR-24-mediated downregulation of H2AX suppresses DNA repair in terminally differentiated blood cells. Nat Struct Mol Biol 2009; 16: 492-498.
[81] Wang Y, Huang JW, Li M, Cavenee WK, Mitchell PS, Zhou X, Tewari M, Furnari FB and Taniguchi T. MicroRNA-138 modulates DNA damage response by repressing histone H2AX expression. Mol Cancer Res 2011; 9: 1100-1111.
[82] Hu H, Du L, Nagabayashi G, Seeger RC and Gatti RA. ATM is down-regulated by N-Myc-regulated microRNA-421. Proc Natl Acad Sci U S A 2010; 107: 1506-1511.
[83] Wan G, Mathur R, Hu X, Zhang X and Lu X. miRNA response to DNA damage. Trends in Biochemical Sciences 2011; 36: 478-484.
[84] Hermeking H. MicroRNAs in the p53 network: micromanagement of tumour suppression. Nat Rev Cancer 2012; 12: 613-626.
[85] Le MT, Teh C, Shyh-Chang N, Xie H, Zhou B, Korzh V, Lodish HF and Lim B. MicroRNA-125b is a novel negative regulator of p53. Genes Dev 2009; 23: 862-876.
[86] Hu W, Chan CS, Wu R, Zhang C, Sun Y, Song JS, Tang LH, Levine AJ and Feng Z. Negative regu-

lation of tumor suppressor p53 by microRNA miR-504. Mol Cell 2010; 38: 689-699.
[87] Xiao J, Lin H, Luo X, Luo X and Wang Z. miR-605 joins p53 network to form a p53:miR-605:Mdm2 positive feedback loop in response to stress. EMBO J 2011; 30: 524-532.
[88] Pichiorri F, Suh SS, Rocci A, De LL, Taccioli C, Santhanam R, Zhou W, Benson DM Jr, Hofmainster C, Alder H, Garofalo M, Di LG, Volinia S, Lin HJ, Perrotti D, Kuehl M, Aqeilan RI, Palumbo A and Croce CM. Downregulation of p53-inducible microRNAs 192, 194, and 215 impairs the p53/MDM2 autoregulatory loop in multiple myeloma development. Cancer Cell 2010; 18: 367-381.
[89] Yamakuchi M, Ferlito M and Lowenstein CJ. miR-34a repression of SIRT1 regulates apoptosis. Proc Natl Acad Sci U S A 2008; 105: 13421-13426.
[90] Fornari F, Gramantieri L, Giovannini C, Veronese A, Ferracin M, Sabbioni S, Calin GA, Grazi GL, Croce CM, Tavolari S, Chieco P, Negrini M and Bolondi L. MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res 2009; 69: 5761-5767.
[91] Zhang X, Wan G, Mlotshwa S, Vance V, Berger FG, Chen H and Lu X. Oncogenic Wip1 phosphatase is inhibited by miR-16 in the DNA damage signaling pathway. Cancer Res 2010; 70: 7176-7186.
[92] Ugalde AP, Ramsay AJ, de la Rosa J, Varela I, Marino G, Cadinanos J, Lu J, Freije JM and Lopez-Otin C. Aging and chronic DNA damage response activate a regulatory pathway involving miR-29 and p53. EMBO J 2011; 30: 2219-2232.
[93] Park SY, Lee JH, Ha M, Nam JW and Kim VN. miR-29 miRNAs activate p53 by targeting p85 alpha and CDC42. Nat Struct Mol Biol 2009; 16: 23-29.
[94] Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S and Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010; 464: 1071-1076.
[95] Yap KL, Li S, Munoz-Cabello AM, Raguz S, Zeng L, Mujtaba S, Gil J, Walsh MJ and Zhou MM. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. Mol Cell 2010; 38: 662-674.
[96] Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R and Fraser P. The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. Science 2008; 322: 1717-1720.
[97] Pandey RR, Mondal T, Mohammad F, Enroth S, Redrup L, Komorowski J, Nagano T, Mancini-Dinardo D and Kanduri C. Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. Mol Cell 2008; 32: 232-246.
[98] Young TL, Matsuda T and Cepko CL. The noncoding RNA taurine upregulated gene 1 is required for differentiation of the murine retina. Curr Biol 2005; 15: 501-512.
[99] Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, Kenzelmann-Broz D, Khalil AM, Zuk O, Amit I, Rabani M, Attardi LD, Regev A, Lander ES, Jacks T and Rinn JL. A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. Cell 2010; 142: 409-419.
[100] Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, Tramontano A and Bozzoni I. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell 2011; 147: 358-369.