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# Posttranscriptional regulation of miRNAs in the DNA damage response

Xinna Zhang[1] and Xiongbin Lu[2,*]

[1]Department of Gynecological Oncology; [2]Department of Cancer Biology; The University of Texas MD Anderson Cancer Center; Houston, TX USA

**DNA damage response is an elaborate process in which cells react to external or internal DNA damaging stress. An extensive network of signaling molecules, complexes and pathways has been identified in the DNA damage response. Emerging evidence indicates that microRNAs (miRNAs) play essential roles in the DNA damage and repair pathways. While much effort has been to predict in silico and verify miRNA target genes, little is known about how miRNAs themselves respond to DNA damage. Here we discuss recent studies showing whether and how miRNAs are regulated in the DNA damage response. MiRNA expression involves transcription of miRNA genes and maturation of the primary transcripts. Therefore, miRNA levels might be regulated in both transcription dependent and independent manners. While the DNA damage response is known to protect against tumorigenesis in vivo, a deficient response could contribute to tumorigenesis through miRNAs.**

**Key words:** DNA damage response, DNA repair, microRNA expression, processing, KSRP

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*Correspondence to: Xiongbin Lu;
Email: xlu2@mdanderson.org

## Introduction

To maintain genome stability, eukaryotic cells have evolved with the ability to detect and translate the initial signals of DNA damage to proper cellular responses. Different types of DNA damage are thus repaired through different DNA repair pathways that include base excision repair, nucleotide excision repair, mismatch repair, homologous recombination (HR) and non-homologous end-joining.[1] Meanwhile, DNA damage triggers a variety of cell activities that are collectively called DNA damage response. Cell cycle checkpoints are activated to arrest cell cycle, allowing cells to repair damaged DNA, or if the damage is too severe, apoptosis occurs to remove those "sick" cells, thereby preventing the transmittal of mutant DNA to daughter cells.

The key components of the DNA damage response are the phosphoinositide-3-kinase-related kinase (PIKK) family, which includes ATM (ataxia-telangiectasia mutated), ATR (ataxia-telangiectasia and Rad3-related), and DNA-PKcs (DNA dependent protein kinase catalytic subunit).[2] The ATM kinase initiates a major signaling pathway that responds particularly to double-strand DNA breaks (DSBs), whereas the ATR kinase is activated primarily by the disruption of DNA replication. These two kinases share substrate specificity by recognizing Ser-Gln (SQ) and Thr-Gln (TQ) motifs.[3,4] Recent work from the Elledge laboratory has unveiled a vast network of more than 700 ATM/ATR targets that function in a variety of cell activities.[4]

Genetic defects that perturb the DNA damage response inevitably cause severe syndromes that are characterized by tissue degeneration, sensitivity to specific DNA-damaging agents and predisposition to cancer. As examples, *ATM* mutations lead to the cancer-predisposing genetic disorder ataxia-telangiectasia (A-T),[2,5] and about a third of hereditary breast cancer is due to mutations in the *BRCA1* gene, which is another important component of the DNA damage response.[6] The direct functional roles of the DNA damage response in cancer resistance have been recently demonstrated. Activated oncogenes induce the stalling and collapse of DNA replication forks, leading to the formation of double-stranded breaks (DSBs). The persistent DNA damage

in precancerous lesions activates DNA damage response pathways, as indicated by upregulated levels of DNA damage responsive proteins, including the activated and phosphorylated forms of ATM, CHK2, p53 and H2AX. In addition, advanced tumors often show inactivation of DNA damage response markers, suggesting that silencing of the DNA damage response is an important prerequisite for cancer progression.[7-9]

## MiRNAs Target DNA Damage Signaling

MicroRNAs (miRNAs) are ~22 nt small non-coding RNAs that control gene expression at the posttranscriptional level through the translational inhibition and destabilization of their target mRNAs.[10] MiRNAs are transcribed as primary transcripts or pri-miRNAs by RNA polymerase II. The pri-miRNA is similar to mRNA in that it contains a 7-methylguanosine cap at the 5' end and a poly(A) tail at the 3' end. The RNase III enzyme Drosha digests pri-miRNAs to produce pre-miRNAs that are ~70 nt long and contain a characteristic stem-loop structure.[11] Drosha functions in a protein complex known as microprocessor that also includes the double-stranded RNA binding protein DGCR8, an essential component for Drosha activity.[11] Because the majority of mRNAs is found in the cytoplasm, miRNAs need to be exported from the nucleus, which is executed by a RanGTP-binding nuclear transporter, Exportin-5.[12] The final step for miRNA maturation is executed by another RNase III enzyme, Dicer, which cleaves pre-miRNAs into their mature forms.[13]

Extensive efforts have been made in the last two decades to dissect the components and regulators involved in the DNA damage response. Kinases/phosphatases, ubiquitin ligases/deubiquitinases, and protein adaptors and effectors have been identified in the network of the DNA damage signaling pathways.[1] It is estimated that miRNAs may directly regulate the expression of at least 30% of human genes that contain miRNA-targeting sequences in their 3' untranslated regions (3'-UTR).[14] Not surprisingly, miRNAs were first realized to be part of the DNA damage response when their targets were identified in the signaling pathways. For example, the human miRNA-421 was found to suppress ATM expression by targeting the 3'-UTR of ATM transcripts.[15] In neuroblastomas, overexpression of the oncogenic transcription factor, N-Myc induces miR-421 expression, which consequently downregulates ATM expression and possibly inhibits the ATM-p53 signaling pathway. In addition, the Chowdhury group reported that miR-24 inhibits the expression of the histone variant H2AX, which has a key role in the repair of DSBs.[16] Because miR-24 is upregulated during post-mitotic differentiation of hematopoietic cell lines, this finding helps explain why terminally differentiated hematopoietic cells have a reduced capacity to repair double-stranded breaks. The same group also recently showed that miR-182 downregulates BRCA1 levels in human breast cancer cells, which results in the impairment of homologous recombination DNA repair and sensitizes the cells to ionizing radiation and chemotherapeutic agent PARP.[17]

MiRNA expression is a critical component of the DNA damage response as well. Knocking down Dicer or Ago2 significantly reduces cell survival and the checkpoint response to UV damage. The Jones group reported that depletion of Dicer in mice and the loss of mature miRNAs induced a premature senescence phenotype in mouse embryonic fibroblasts, developing limb and adult skin.[18] Loss of Dicer resulted in increased levels of DNA damage, p53 activity and premature senescence in cells, suggesting that loss of miRNA biogenesis activates DNA damage checkpoints. As more miRNA targets are identified, the regulatory roles of miRNA in the DNA damage response will become clearer.

## How is miRNA Expression Regulated in the DNA Damage Response?

An initial report from the Persengiev group showed that UV damage triggered a change in miRNA expression profiling. Caffeine, a non-specific ATM/ATR inhibitor, failed to abrogate the UV-induced change in miRNA expression.[19] They also observed a cell cycle-dependent relocalization of Ago2 into stress granules, which may contribute to the function of miRNAs in the RNA-induced silencing complex (RISC). In our laboratory, we examined DNA damage-responsive miRNAs in $Atm^{+/+}$ and $Atm^{-/-}$ littermate mouse embryonic fibroblasts (MEFs). The two types of MEFs were treated with a radiomimetic drug, neocarzinostatin (NCS) that generates double-stranded DNA breaks and activates the ATM-dependent DNA damage response. A wide-spectrum induction of miRNAs was observed, among which 71 miRNAs had a greater than 2-fold induction in an ATM-dependent manner. This observation suggested that ATM may play a major role in regulating miRNA expression.[20]

While ATM is known as a central player in the DNA damage response that controls many signaling pathways, it is extremely important to identify a direct target of the ATM kinase in the miRNA regulation. A recent study from Trabucchi and colleagues has provided a clue.[21] In their study, KH-type splicing regulatory protein (KSRP) promoted the processing of a subset of 29 miRNAs. KSRP was originally thought to primarily control messenger RNA decay, but the authors showed convincing evidence that KSRP regulates the maturation of this subset of miRNAs through its interaction with Drosha and Dicer. In our studies, all of the KSRP-promoted miRNAs were also induced after DNA damage in an ATM-dependent manner, but no notable induction occurred as we measured the levels of these pri-miRNAs after DNA damage.[20] What, then, does ATM do to these miRNAs?

In a genome-wide search for the phosphorylation targets of the ATM/ATR kinases, Matsuoka and colleagues identified KSRP as a potential ATM target among more than 700 other targets.[4] Once the ATM-mediated phosphorylation of KSRP was verified in vitro and in vivo in our laboratory, we examined if the ATM phosphorylation of KSRP had functional consequences in miRNA biogenesis.[20] Mutating three ATM phosphorylation sites on KSRP virtually abolished its activity in promoting miRNA expression. In cells stressed by DNA damage, KSRP is

localized primarily in the nucleus, and the phosphorylation of KSRP enhances its recruitment of pri-miRNAs to the Drosha microprocesser. While we observed no notable change in the binding capacity of KSRP with Drosha in the presence or absence of DNA damage, the interaction between KSRP and its associated pri-miRNAs was remarkably enhanced after DNA damage had occurred.[20] As a consequence, increased amounts of the pri-miRNAs were processed by the Drosha complex and more pre-miRNAs were produced. This functional link between the ATM kinase and the Drosha complex can explain the DNA damage-mediated induction for the KSRP-associated miRNAs.

Beyond KSRP, other regulators for miRNA biogenesis in the DNA damage response are poorly known. p53 is a hallmark tumor suppressor that responds to a variety of stresses, including DNA damage.[22] In mammalian cells, p53 is primarily regulated in a p53-Mdm2 autoregulatory feedback loop. It is rapidly stabilized and induced after DNA damage due to the destabilization of Mdm2 and the inhibition of the Mdm2-p53 interaction. A recent study on p53 has revealed a connection between the p53 tumor suppressor and miRNA biogenesis. p53 promotes the processing of pri-miRNAs to pre-miRNAs through its interaction with DEAD-box RNA helicase p68 and Drosha complex.[23] It is yet to be determined if other direct or indirect mechanisms are involved in the DNA damage-induced changes in miRNA expression. From miRNA expression profilings in our study, a small group of miRNAs (19 mouse miRNAs) had ATM-dependent reduction of expression after DNA damage.[20] It is likely that ATM triggers an inhibitory pathway to specifically suppress these miRNAs.

## Other Potential miRNA Regulatory Pathways

Although mature miRNAs are non-coding small RNAs that differ from mRNAs and other types of RNAs, the primary transcripts of miRNAs (that are pri-miRNAs) share similar characteristics of mRNAs. Therefore, certain pathways may regulate both mRNAs and pri-miRNAs. For example, like mRNAs, pri-miRNAs may be degraded or stabilized in response to various types of stress. It is noteworthy that KSRP not only promotes miRNA expression, but also is an mRNA decay factor.[24,25] Other RNA-binding proteins probably participate in the miRNA regulation. For certain miRNAs transcriptional regulation confers a specific effect. To understand miRNA transcription, greater effort is needed to identify the promoter sequence of each pri-miRNA. The sublocalization of miRNAs may also be involved in miRNA processing or stability. For example, Pothof and colleagues mentioned potential functions of stress granules in regulating miRNAs.[19] It will be of great interest to explore this possibility.

Other questions regarding the miRNA regulatory pathway include the flowwing: (1) What other proteins in miRNA expression machinery are direct or indirect targets of the ATM/ATR DNA damage signaling pathways? (2) How many regulatory proteins interact with the miRNA transcription and processing machinery? (3) Are miRNAs regulated specifically in response to different types of DNA damage stress? (4) Is miRNA transportation regulated after DNA damage? (5) Are there unknown post-transcriptional modifications of miRNAs? (6) Is miRNA stability regulated after DNA damage, as other types of RNA molecules are?

## Regulatory Defects of miRNAs in Human Cancer

Recent reports suggest that miRNA expression is deregulated in human cancer. There appears to be a global repression of miRNA expression in cancer tissues and cells.[26] Dicer mutation or dysregulation contributes to reduced miRNA expressions. Dicer functions as a haplo-insufficient tumor suppressor in vivo.[27,28] Impaired miRNA processing accelerates tumor formation and increased tumor invasion. While Dicer knockout mice are embryonic lethal, conditional deletion of Dicer1 in a K-Ras-induced lung cancer model markedly enhanced tumor progression.[28] Lanbertz and co-workers discovered that monoallelic loss of Dicer1 had no effect on the normal retinal development of mice, but remarkably accelerated retinoblastoma formation.[29] Frequent hemizygous deletions of DICER1 have been observed in human cancers as well.[29] In addition, Su et al. reported that the tumor suppressor p63, a member of the p53 family, binds to the promoter region of the *Dicer* gene and activates its transcription. Knockout of p63 dampens tumor metastasis in mice.[30] Promoter hypermethylation and oncogene-mediated transrepression may also suppress the transcription of miRNA genes in human cancer.[31] Finally, defects in miRNA post-transcriptional regulation have been found as a possible mechanism for the miRNA repression in cancer cells. A recent study identified a C-terminal truncate of Exportin-5 in human colorectal cancer cells which fails to transport pre-miRNA to cytoplasm, thereby trapping them in the nucleus.[32]

DNA damage checkpoints are considered as an anti-cancer barrier to tumorigenesis. Hyperactivation of these checkpoints occurs in the early stages of tumorigenesis and in precancerous lesions. However, inactivation of these checkpoints often accompanies the evolution of the cancer. In the late stages of tumor development, cells fail to properly respond to the internal DNA damage caused by aberrant DNA replication, resulting in genomic instability and the accumulation of DNA mutations that favor cancer cells.[9] Given the fact that the ATM gene is frequently mutated or deregulated in many types of tumors,[2] our finding that ATM promotes miRNA maturation provides another important mechanism to explain why cancer cells have a global miRNA silencing.[20]


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