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MicroRNA response to DNA damage

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Abstract

Faithful transmission of genetic material in eukaryotic cells requires not only accurate DNA replication and chromosome distribution, but also the ability to sense and repair spontaneous and induced DNA damage. To maintain genomic integrity, cells undergo a DNA damage response using a complex network of signaling pathways, composed of coordinate sensors, transducers and effectors in cell cycle arrest, apoptosis and DNA repair. Emerging evidence has suggested that microRNAs (miRNAs) play a critical role in regulation of DNA damage response. Here, we discuss the recent findings on how miRNAs interact with the canonical DNA damage response and how miRNA expression is regulated after DNA damage.

Canonical DNA damage response

DNA damage in cells is caused by intrinsic and extrinsic genotoxic stresses, including ultraviolet light (UV), ionizing radiation (IR), chemo- and radiotherapeutic agents, and reactive oxygen species. DNA damage response is a functional network combining signal transduction, cell cycle regulation and DNA repair, which is conserved in many aspects from yeast to humans [1]. DNA damage response is initiated by early signaling events, including activation of the phosphoinositide-3 kinase (PI3K)-like kinases (ATM, ATR, DNA-PKcs), phosphorylation of histone H2AX and recruitment of the Mre11-Rad50-Nbs1 or the Rad9-Hus1-Rad1 complex to damage sites [2]. DNA double-strand breaks are repaired by homologous recombination and nonhomologous end-joining repair pathways, while other types of DNA damage are processed through the base excision repair, nucleotide excision repair or mismatch repair pathways [2].

The ATM kinase initiates a major signaling pathway that responds in particular to double-strand breaks, which are among the most severe genomic lesions. A genome-wide proteomic screen identified over 700 protein targets that are potentially phosphorylated by ATM[3]. The consensus phosphorylation motif in the ATM substrates is hydrophobic-X-hydrophobic-[S/T]-Q [3,4]. Once it is recruited to DNA damage sites, the ATM kinase rapidly activates many downstream target proteins through phosphorylation, causing checkpoint proteins to trigger cell cycle arrest and DNA repair enzymes to fix the damaged DNA. The ATM-mediated DNA damage response also involves many transcription factors or cofactors [3],

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which reset the gene transcription program to allow cells to properly respond to the DNA damage stress. Among a number of ATM targets, p53 is a transcription factor and a pivotal tumor suppressor in animals. Over half of human tumors contain a mutation or deletion of the *p53* gene [5]. p53 is activated in response to a myriad of stress types, which include but is not limited to DNA damage, oxidative stress, osmotic shock, ribonucleotide depletion and deregulated oncogene expression [6]. Activated p53 directs a transcriptional program that prevents the proliferation of genetically unstable cells. Inappropriate regulation of p53 results in a severe consequence for cells. While the loss of p53 function predisposes cells to tumorigenesis, errant p53 activation can lead to premature senescence or apoptosis.

MicroRNA expression and maturation

The DNA damage response involves a complex network of processes that detect and repair DNA damage, in which microRNAs (miRNAs), a class of small regulatory RNAs, could play important roles. However, very little is known about whether and how miRNA expression is regulated in the DNA damage response. To dissect mechanisms by which miRNA expression is regulated, it is important to first understand how miRNAs are transcribed and processed (Figure 1a). miRNAs are an evolutionarily conserved group of small non-coding RNA molecules (18-25 nucleotides in length) that regulate the stability and translation of mRNA by perfect or imperfect base pairing at the 3′ untranslated region (3′ UTR) of the mRNA [7]. Nucleotides 2-7 from the 5′ end of the miRNA are referred to as the seed sequence, are critical for recognition and hybridization of the miRNA targets. In the human genome, approximately 30% of genes are estimated to be targeted by miRNAs [8]. Through target mRNA degradation and translational inhibition, miRNAs function as key players in a variety of physiological and pathological processes, including differentiation, metabolism, embryonic development, and metabolism, as well as human diseases, such as diabetes, neurodegenerative disorders, and cancer [9]. For example, miR-1 and the related miR-133 arise from a common precursor RNA and function cooperatively to promote mesoderm differentiation of mouse and human embryonic stem cells [10]. The miRNA cluster *17-92* at 13q31 was identified as a region commonly amplified in lymphomas [11]. Furthermore, half of the known miRNAs are located inside or close to fragile sites and in minimal regions of loss of heterozygosity, minimal regions of amplifications, and common breakpoints associated with cancer [12].

miRNAs play an important role in gene regulation and link multiple facets of cell activities. miRNA production and maturation are assumed to be regulated by transcription, processing and nucleus to cytoplasm transportation [13,14]. Depending on their genomic locations, miRNA genes can be transcribed from two different pathways: intergenic miRNAs are transcribed by RNA polymerase II as primary miRNAs (pri-miRNAs) with independent transcription units [15], whereas intronic or exonic miRNAs are transcribed together with their host genes from a common promoter [16]. Pri-miRNAs from intergenic miRNA are long transcripts of variable size with 5′ caps and 3′ poly(A) tails. The pri-miRNA is recognized and cleaved in the nucleus by the Drosha-DGCR8 (Microprocessor) to generate a hairpin-shaped precursor form called pre-miRNA [17]. Pre-miRNAs are approximately 70-nt RNAs with 3′ overhangs, 25-30 base pair stems, and relatively small loops [17]. Exportin-5, a RanGTP-binding nuclear transporter, is responsible for export of pre-miRNAs from the nucleus to cytoplasm where Dicer, an endoribonuclease in the RNase III family, cleaves pre-miRNAs into a transient duplex around 20-25 nt in size, consisting of the functional miRNA strand and the passenger strand [18,19]. Mature miRNAs and Argonaute (Ago) proteins form the RNA-induced silencing complex (RISC) that mediates post-transcriptional gene silencing. The mature miRNA guides the RISC complex to repress gene expression by inhibiting translation and inducing mRNA degradation [20,21]. Recent evidence showed that cytoplasmic pre-miRNA processing and RISC assembly are coupled

by the RISC loading complex (RLC), which is composed of Dicer, TRBP (Tar RNA binding protein) and PACT (protein activator of PKR), and Ago2 [22]. After completing its task, the mature miRNA is degraded by the 5′-3′ exoribonuclease XRN2 [23] or 3′-5′ exoribonucleases such as human polynucleotide phosphorylase [24] and nuclear exosome [25]. In this review, we discuss recent studies that shed light on how multiple steps in the miRNA biogenesis pathway are regulated to modulate miRNA function in the DNA damage response.

miRNAs regulate DNA damage response through target genes

In addition to being regulated by the canonical signalling pathways, increasing evidence shows that the DNA damage response is also epigenetically regulated. First, the chromatin structure needs to be reorganized to accommodate the binding of the involved transcription factors in gene transcription and replication. During DNA repair, chromatin remodeling occurs to allow DNA repair proteins to reach damaged DNA. Two mechanisms are involved in the chromatin remodeling: posttranslational histone modifications (phosphorylation, methylation, ubiquitination and acetylation) and displacement of histones or entire nucleosomes [26]. With more and more predicted and validated miRNA targets, it is becoming clear that DNA damage responsive genes have been subject to inhibition by miRNAs. MicroRNA-421 (miR-421) was shown to attenuate ATM expression by direct binding to the 3′ UTR of the ATM mRNA, leading to altered S phase cell cycle checkpoint and increased radiosensitivity in cells [27]. miR-421 expression was upregulated in neuroblastoma and large B cell lymphoma cell lines in an N-myc dependent manner. N-myc is a proto-oncogene frequently amplified in neuroblastoma. Higher expression of miR-421 along with N-myc has been reported in the ataxia-telangiectasia phenotype, which might inhibit ATM expression and regulate the disease onset and progression [27]. Histone variant H2AX, which regulates DNA repair, replication, recombination and cell cycle, is a target of miR-24. Overexpression of miR-24 was shown to downregulate H2AX, resulting in higher sensitivity to IR and reduced repair capacity [28].

While it has long been known that tumor suppressor p53 directly transactivates miRNA genes expressing miR-34a and miR-34b/c [29], recent reports showed that several miRNAs are able to inhibit the expression of p53 and its related family member p63. miR-504 and miR-125b act as negative regulators of human p53 through their direct binding to specific sites in the 3′ UTR of p53 mRNA [30,31]. Overexpression of these miRNAs reduced p53 protein levels and promoted tumorigenecity of cells *in vivo*. miR-302 was reported to target p63 and reduce its expression in germ cells, and miR-92 might increase proliferation of myeloid cells by modulating the abundance of p63 isoforms [32,33]. A large body of literature has shown that many critical genes in the DNA damage response are regulated by their specific miRNAs (Table 1).

Regulation of miRNA expression in DNA damage response

Treatment with different types of DNA damaging agents has been shown to result in differential activation of miRNAs. Varying doses of DNA damage seemingly lead to activation of unique as well as common sets of miRNAs, suggesting that miRNAs regulate the DNA damage response by a mechanism based on the nature and intensity of DNA damage [34]. Although several DNA damage responsive miRNAs and their targets have been identified, many remain to be discovered. Complex interconnections between miRNAs and their DNA damage response targets need to be established. Furthermore, many factors, such as the binding capacity of miRNAs to target mRNAs and target mimicry, are shared by several miRNAs. In addition, miRNA-protein interactions regulate the extent of miRNA control of expression of different target proteins. In the next two sections, we focus on

recent findings regarding how miRNA expression is transcriptionally and posttranscriptionally regulated by the DNA damage response.

Transcriptional regulation of miRNA in DNA damage

DNA damage can regulate miRNA expression at the transcriptional level. Similar to regular genes, miRNA gene transcription is controlled by transcription factors. The tumor suppressor p53 is well known as a DNA damage-induced transcription factor. The first discovery connecting p53 to the regulation of miRNAs was the identification of the miR-34 family, a direct transcriptional target of p53, whose induction by DNA damage and oncogenic stress pervades in diverse aspects of the DNA damage response pathway [29]. Ectopic expression of miR-34 genes causes a G1 phase cell cycle arrest and downregulates a group of genes promoting cell cycle progression [28,29,31]. Introduction of miR-34 genes into primary and tumor-derived cell lines induces cellular senescence, a permanent form of cell cycle arrest. Transactivation of miR-34a also induces p53-mediated apoptosis. The induction of miR-34 genes allows p53 to regulate expression of a large number of proteins in DNA damage response, even after their transcripts have already been synthesized (Figure 1b).

miR-192, miR-194, miR-215 and miR-17-92 clusters are other miRNAs that appear to be transcriptionally regulated by p53 [35-38]. DNA damage promotes the p53-dependent upregulation of miR-192, miR-194 and miR-215 (Figure 1b). The genomic region around the miR-194/miR-215 cluster contains a putative p53-binding element, indicating that these miRNAs are transcriptionally activated by p53. Ectopic expression of miR-192/215 induces cell cycle arrest and targets on a number of transcripts that regulate the G1/S and G2/M checkpoints [36,39]. The miR-17-92 cluster was repressed under hypoxic conditions via a p53-dependent mechanism, leading to sensitization to hypoxia-induced apoptosis [37]. The repression of miR-17-92 mediated by p53 is modulated through preventing the TATA-binding protein (TBP) transcriptional factor from binding to a TATA box that overlaps with the p53-binding site.

Two other transcription factors that play major roles in the DNA damage-induced cell cycle checkpoints, Myc and E2F, induce expression of several miRNAs [40]. Both induce transcription of the miR-17-92 cluster that, in turn, inhibits E2F expression, forming an autoregulatory feedback loop. Moreover, E2F transcription factors are also repressed by several other miRNAs, including members in the miR-106a-92 and miR-106b-25 clusters, miR-210, miR-128, miR-34, and miR-20. TAp63, a major transcript of the p63 gene, has an important role in suppression of tumorigenesis and metastasis. Similar to p53, TAp63 is induced by DNA damage and other stresses. Overexpression of Dicer and miR-130b markedly affected the metastatic potential of cells lacking TAp63 [41]. Further studies revealed that TAp63 binds to and transactivates the promoters of Dicer and miR-130b, demonstrating direct regulation of miRNA expression by TAp63.

While not much is currently known about how the miRNA gene expression is transcriptionally regulated due to lack of basic information regarding their gene structure, global prediction and verification of promoter regions of miRNA genes would allow us to further explore the functional interaction of transcriptional machinery and epigenetic miRNA regulation.

Posttranscriptional regulation of miRNA in DNA damage

Equally as important as miRNA gene transcription, posttranscriptional processing of miRNAs is also regulated in the DNA damage response. We recently found that DNA damage led to increased levels of some pre-miRNAs and mature miRNAs without

significant changes of levels of their primary transcripts, suggesting posttranscriptional mechanism(s) could contribute to the induction of certain miRNAs under DNA damage stress [42]. There appears to be functional connections between DNA damage response and miRNA processing and maturation.

The Persengiev group first provided evidence showing that UV damage triggered a cell cycle-dependent relocalization of Ago2 into stress granules and promoted miRNA expression in a partially ATM/ATR-independent manner [43]. Stress granules are protein-RNA aggregates that form in stressed cells. These granules are postulated to regulate mRNA metabolism and their formation might inhibit translation of many housekeeping mRNAs in stressed cells [44]. In addition to mRNA and transcriptional and translational factors, other RNA-binding proteins were also identified in stress granules, including tristetraprolin (TTP), AU-rich element-binding protein (HuR), T-cell-restricted intracellular antigen 1 (TIA-1) and TIA-1-related protein (TIAR). Following UV damage, the intracellular relocalization of Ago2 to stress granules and miRNA expression changes suggest that miRNA-mediated gene silencing is an integral part of the DNA damage response. It remains to be elucidated whether stress granules are directly involved in miRNA processing and whether they mediate the function of miRNAs in translational inhibition.

A subsequent study demonstrated that several miRNAs, including miR-16-1, miR-143 and miR-145, were posttranscriptionally upregulated in a p53-dependent and p68/p72-dependent manner after DNA damage [45] (Figure 1c). DEAD box RNA helicases p68 (DDX5) and p72 (DDX17) were identified in the Drosha complex and found to contribute to the efficient processing of a subset of pri-miRNAs into the corresponding mature miRNAs [46,47]. A direct interaction between p53 and p68/p72 facilitates p53 promoting of miRNA processing [45]. Wild-type p53 interacts with the Drosha-DGCR8 processing complex through its association with p68. Inactive p53 mutants disrupt a functional assembly between the Drosha complex and p68, resulting in attenuation of miRNA processing activity. Overexpression of these p53-induced miRNAs (miR-16, miR-103, miR-143, miR-145, miR-26a and miR-206) decreases the rate of cell proliferation. Most p53 mutations found in cancers are located in a domain that is required for both the miRNA processing function and transcriptional activity [45,48]. Loss of p53 functions in transcription and processing of specific miRNAs might together contribute to tumor progression. A complex network of transcriptional regulation and posttranscriptional regulation has been examined by comparative and computational genomic analyses, showing that the tumor suppressors p53, p63 and p73 could function as both positive and negative regulators of the miRNA processing components. Promoters of several components of the miRNA processing machinery, including Dicer and P2P-R, contain p53-responsive elements, indicating that they could be direct transcriptional targets of p63, p73 and p53 [49]. It was also predicted that most of the components in the miRNA processing complexes are targeted by p53-induced miRNAs. Thus, a feedback effect could help maintain physiological levels of miRNAs.

Recent studies provide direct evidence that as many as one fourth of miRNAs are significantly induced upon DNA damage in an ATM-dependent manner [42]. Among these induced miRNAs, a cohort of miRNAs associated with KSRP, a key component of both the Drosha and Dicer complexes, were identified [50] (Figure 1d). KSRP is an AU-rich element binding protein that regulates mRNA decay. The complex pattern of posttranslational 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 [51]. Trabucchi and colleagues presented compelling evidence that KSRP promotes maturation of a select group of miRNA precursors [50]. KSRP binds with high affinity to the terminal loop of these miRNAs and interacts with both Drosha and Dicer. As a key kinase in initiation of the

DNA damage signaling cascade, ATM directly binds to and phosphorylates KSRP, leading to enhanced interaction between KSRP and pri-miRNAs and increased KSRP activity in miRNA processing. Mutations of the ATM phosphorylation sites of KSRP impaired its activity in regulating miRNAs [42]. These findings strongly support the hypothesis that ATM functions as a major switch for the activity of KSRP in miRNA biogenesis, and that KSRP acts as a molecular gatekeeper that accelerates the production of a subset of miRNAs that regulate cell activities in response to DNA damage.

Protein phosphorylation is a major event in DNA damage signaling pathways. In addition to initiating PI3K kinases such as ATM and ATR, many downstream kinases are activated after DNA damage and they might also modulate the activity of miRNA processing. Liu and co-workers identified phosphorylated TRBP in the Dicer complex [52]. Phosphorylation of TRBP, which is mediated by the mitogen-activated protein kinase (MAPK) Erk, stabilizes the Dicer-TRBP complex and increased mature miRNA production. A family of growth promoting miRNAs (miR-17, miR-20a and miR-92a) is upregulated by phosphorylated TRBP. Interestingly, opposite effects were observed on levels of the *let-7* tumor suppressor miRNA family. These results suggest a mitogenic miRNA expression profile, including coordinated upregulation of pro-growth miRNAs and downregulation of anti-growth miRNAs in response to phosphorylation of TRBP. Interestingly, Erk and other MAPKs are phosphorylated and activated after DNA damage [53]. Further studies will be required to elucidate whether and how DNA damage signaling targets these kinases to regulate the miRNA processing machinery and to achieve biological responses.

Concluding remarks and future directions

Taken together, although several studies have shown that miRNA expression is regulated transcriptionally and posttranscriptionally in the DNA damage response, there are still many important questions to be addressed. In particular, it remains largely unknown how miRNA biogenesis responds to DNA damage for p53- or KSRP-independent miRNAs. There should be other potential mechanisms to account for the induction of those miRNAs. Further studies on the following aspects might give insights into the molecular mechanisms by which DNA damage signaling is linked to miRNA biogenesis. (i) In the DNA damage response, biochemical activity and intracellular localization of Drosha and Dicer might be regulated by posttranslational modifications. Multiple phosphorylation sites have been identified or predicted on Drosha and Dicer proteins, some of which might be phosphorylated by ATM or its downstream kinases [54,55]. For example, phosphorylation of Drosha at Ser300 or Ser302 was found to be necessary for its nuclear localization and pri-miRNA processing [56]. (ii) Transportation of pre-miRNAs from the nucleus to cytoplasm is possibly stimulated by DNA damage. Recent proteomic analyses revealed a complex network of exportin-5-interacting proteins whose levels or binding activity could be altered after DNA damage [57]. A recent report showed that an inactivating mutant of exportin-5 traps pre-miRNAs in the nucleus and thus reduces miRNA processing [58]. It would be of great interest to determine whether DNA damage affects the nucleus-cytoplasm shuffling of exportin-5. (iii) Transcription of some pri-miRNAs is promoted by other transcriptional factors that are involved in the stress response pathways, such as NF-κB, CREB and E2F1 [59-61]. These transcription factor-regulated miRNAs could also contribute to the complex cellular response to DNA damage. To this end, a genome-wide identification of miRNA gene promoters and their associated transcription factors is required. (iv) DNA damage-induced miRNAs have feedback effects on the DNA damage response. In Table 1, some miRNAs are induced by DNA damage [42] and in turn regulate DNA damage response. As an example, miR-16 is immediately induced after DNA damage and specifically targets the mRNA of Wip1 that is a master inhibitor for the ATM-p53 signaling pathway. mir-16 suppression of Wip1 prevents a premature inactivation of the ATM signaling and allows a

functional completion of the early DNA damage response [62]. Extensive studies on DNA damage-induced miRNAs will reveal their functions in cellular responses such as cell cycle arrest, DNA damage repair and apoptosis. (v) Cell metabolic changes in the DNA damage response might also affect miRNA production. As a major energy currency molecule of the cell**,** ATP facilitates RISC loading of small-RNA duplexes in miRNA-mediated suppression [63]. miRNA biogenesis is energy-dependent at each step. For example, exportin-5-mediated pre-miRNA transportation uses another energy carrier, GTP, which binds to the Ran proteins in the exportin-5 complex [19]. It is postulated that overall level of cell metabolism will have negative or positive effects on this process.

As many questions remain, it is essential that we continue to decipher the molecular and cellular mechanisms involved in miRNA expression and maturation. Such work will not only lead to a better understanding on the functional roles of DNA damage response, but provide new insight into many human diseases with DNA damage-processing defects.

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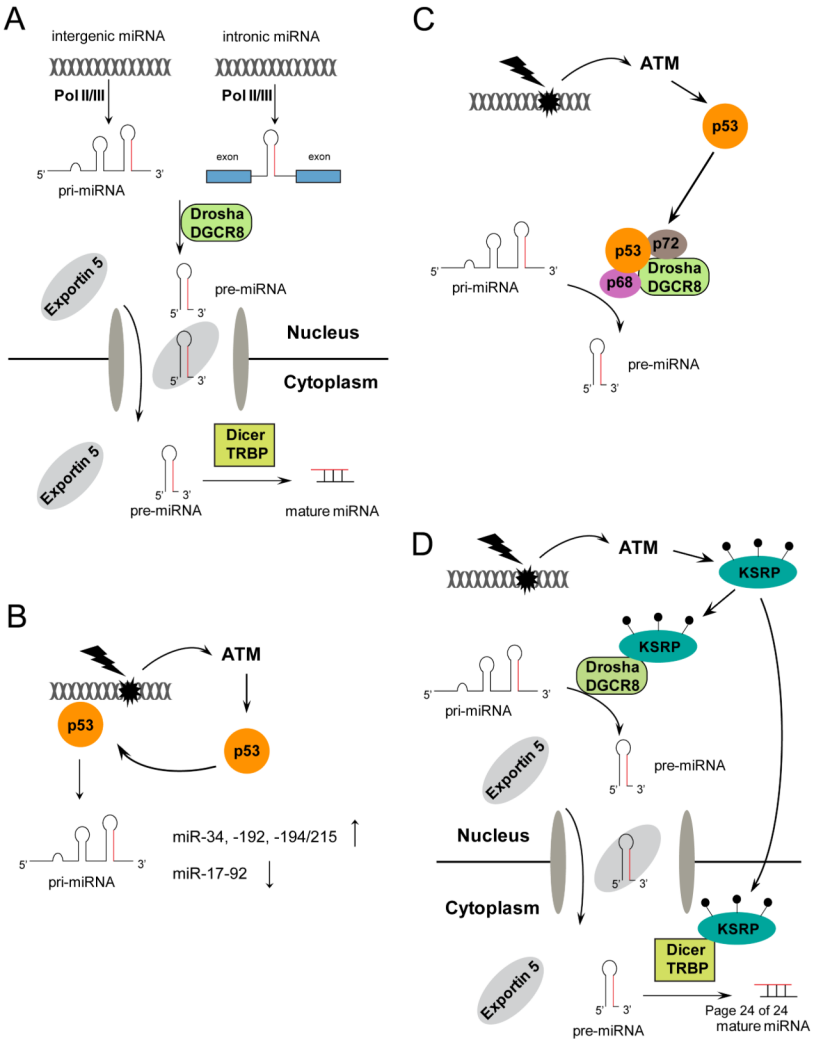

Figure 1.

miRNA biogenesis in the DNA damage response

(a) miRNA biogenesis without DNA damage stress. Intergenic miRNA genes are transcribed by RNA polymerase II/III (Pol II/III) into pri-miRNAs while intronic miRNAs are transcribed together with their host genes. The Drosha-DGCR8 complex recognizes and cleaves pri-miRNAs into pre-miRNAs in the nucleus. Pre-miRNA is exported to the cytoplasm by exportin-5 in a RanGTP-dependent manner, and then is further processed by Dicer/TRBP complex into the mature miRNA duplex. The mature miRNA incorporates into Ago2 complex to repress gene expression. (**b)** p53 transcriptionally activate miRNA genes after DNA damage. **(c)** ATM-induced p53 promotes the posttranscriptional processing of pri-miRNAs via p68 and p72 after DNA damage. **(d)** The ATM kinase upregulates miRNA maturation by phosphorylating and activating KSRP. ATM-mediated phosphorylation of KSRP enhances the activity of KSRP in the processing of miRNAs by the Drosha and Dicer complexes.

Table 1

miRNAs target key genes involved in the DNA damage response

Targets	Function in DNA damage response	miRNAs	Refs.
ATM	Mediator/transducer	miR-421	[27]
H2AX	Mediator, DNA repair	miR-24	[28]
RAD52	DNA repair	miR-210, miR-373	[64]
RAD23B	DNA repair	miR-373	[64]
MSH2	DNA mismatch repair	miR-21	[65]
BRCA1	DNA repair	miR-182	[66]
p53	Cell cycle checkpoint, apoptosis	miR-504, miR-125b	[30,31]
p63	Transcription factor	miR-92, miR-302	[32,33]
E2F	Transcription factor	miR-17-92, miR-20a, miR-34a	[67,68]
p21	Cell cycle	miR-17, miR-20a/b, miR-106a/b, miR-93, miR-215, miR-192	[39,69]
CDK2	Cell cycle	miR-124a, miR-885-5p	[70,71]
CDK6	Cell cycle	miR-124a, miR-29, miR-449a/b	[72-74]
Cdc25A	Cell cycle checkpoint	miR-21, miR-449a/b	[74,75]
Cdc42	Cell cycle checkpoint	miR-29	[76]
Cyclin E	Cell cycle	miR-15a, miR-16	[77,78]
Cyclin D	Cell cycle	miR-15a, miR-16	[79]
Cyclin G1	Cell cycle	miR-122	[80]
Wee1	Cell cycle checkpoint	miR-195	[81]
p27	Cell cycle	miR-221/222, miR-181	[82,83]
p57	Cell cycle	miR-221/222	[82]
Wip1	Cell cycle checkpoint	miR-16	[62]
Bcl-2	Apoptosis	miR-15a, miR-16-1	[84]