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microRNA Expression and Biogenesis in Cellular Response to Ionizing Radiation

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Increasing evidence demonstrates that the expression levels of microRNAs (miRNAs) significantly change upon ionizing radiation (IR) and play a critical role in cellular response to IR. Although several radiation responsive miRNAs and their targets have been identified, little is known about how miRNAs expression and biogenesis is regulated by IR-caused DNA damage response (DDR). Hence, in this review, we summarize miRNA expression and biogenesis in cellular response to IR and mainly elucidate the regulatory mechanisms of miRNA expression and biogenesis from different aspects including ataxia-telangiectasia mutated (ATM) kinase, p53/p63/p73 family and other potential factors. Furthermore, we focus on ΔNp73, which might be a potential regulator of miRNA expression and biogenesis in cellular response to IR. miRNAs could effectively activate the IR-induced DDR and modulate the radiation response and cellular radiosensitivity, which have an important potential clinical application. Therefore, thoroughly understanding the regulatory mechanisms of miRNAs expression and biogenesis in radiation response will provide new insights for clinical cancer radiotherapy.

Introduction

MicroRNAs (miRNAs) are classes of noncoding RNA molecules with ~22 nucleotides in length, which regulate the stability and translation of mRNA by perfect or imperfect base pairing at the 3′ untranslated region (3′ UTR) of their target mRNA (Bartel, 2009). In the human genome, more than 1000 miRNAs have been identified and are predicted to target at least 60% of all protein coding genes (Friedman *et al.*, 2009). Not surprising, miRNAs, as critical gene regulators, can influence signaling pathways that alter multiple cellular processes, including the DNA damage response (DDR) after ionizing radiation (IR). Increasing evidence demonstrates that the expression levels of miRNAs significantly change upon IR, which suggests that miRNA play a critical role in the IR-caused DDR (Metheetrairut and Slack, 2013). Although several IR-induced DNA damage responsive miRNAs and their targets have been identified (Table 1), little is known about how miRNAs themselves respond to IR. Hence, in this review, we summarize miRNA expression and biogenesis in cellular response to IR in the current literatures and mainly elucidate the regulatory mechanisms of miRNA expression and biogenesis from different aspects including ataxia-telangiectasia mutated (ATM) kinase, p53/p63/p73 family, and other potential factors. To discuss how miRNA expression and biogenesis are regulated by the IR-caused DDR, the processes of miRNA biogenesis and the mechanism of action are primarily illustrated.

miRNAs Biogenesis and Mechanisms of Action

miRNAs biogenesis is a cellular process producing active 22-nt long miRNA from large transcripts coded by the cell genome. The process of miRNA biogenesis is comprised of transcription, processing/maturation, and degradation (Fig. 1). 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 (Lee *et al.*, 2004), whereas intronic miRNAs are transcribed together with their host genes from a common promoter (Kim and Kim, 2007). pri-miRNAs from intergenic miRNA are 5′ capped (m^7G) and 3′ polyadenylated, which are further cleaved into pre-miRNA by Drosha-DGCR8 microprocessor complexes (Han *et al.*, 2004), while the intronic miRNA is directly cleaved by spliceosome cooperating with Drosha-DGCR8 microprocessor complexes into pre-miRNA without affecting the splicing step of host gene (Kim and Kim, 2007).

Like smaller shuttling RNAs, pre-miRNAs are actively exported from nucleus to cytoplasm by exportin-5 in a Ran-GTP-dependent manner (Yi *et al.*, 2003; Lund *et al.*, 2004). In cytoplasm, Dicer, which acts in complex with the trans-activating response RNA-binding protein (TRBP), recognizes

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Table 1. Ionizing Radiation-Responsive miRNAs and Their Targets in Various Cell Lines

miRNA	*Response*	*Cell type*	*Target*	*Biological effects*	*Reference*
*let-7	Increase/decrease	Primary and cancer	K-Ras, CDC25a, CDK6	Overcome the radioresistance of cancer cells and improve radiotherapeutic effects Represses cell proliferation	Johnson *et al.* (2007), Weidhaas *et al.* (2007), Cha *et al.* (2009a), Chaudhry *et al.* (2010b), Oh *et al.* (2010)
miR-7	Decrease	Primary and cancer	EGFR, IGFR, PIK3CD, PAK1 LSH	Increase radiosensitivity of human cancer Repress tumorigenesis and revers the metastasis process of cancer cells	Ishii and Saito (2006), Ilnytskyy *et al.* (2008), Lee *et al.* (2011), Zhao *et al.* (2013)
miR-15a/16	Increase	Primary and cancer	Bcl-2, CDC25a, Cyclin E1/D1, Wip1, HuR, VEGF	Induce cancer cells apoptosis Control cell cycle Inhibit cancer stem cells proliferation and growth	Chaudhry (2009), Chaudhry *et al.* (2010b), Zhang *et al.* (2010c), Sun *et al.* (2013)
miR-17-92	Increase	Cancer	P21, CTGF, E2F, PTEN, Bim, TGFBR2	Enhance radioresistance of lymphoma cells	Jiang *et al.* (2010), Metheetrairut and Slack (2013)
miR-18a	Increase/ decrease	Primary and cancer	ATM	Affect DNA damage repair ability	Maes *et al.* (2008), Chaudhry (2009), Chaudhry *et al.* (2010a), Wagner-Ecker *et al.* (2010), Song *et al.* (2011)
*miR-21	Increase	Primary and cancer	PTEN, ERK, EGFR, CDC25a, PDCD4, Big-h3	Modulate radioresistance or radiosensitivity Induce tumor angiogenesis and metastasis	Zhang *et al.* (2010b), Zhou *et al.* (2010), Liu *et al.* (2011a)
miR-24	Increase/ decrease	Primary and cancer	H2AX, E2F2, Myc	Affect DNA repair ability	Lal *et al.* (2009a, 2009b), Metheetrairut and Slack (2013)
miR-26b	Increase/ decrease	Primary and cancer	ATF2, GATA4, COX-2, PTGS2, ATM, PTEN	Enhance the effect of IR Affect DNA damage repair ability	Simone *et al.* (2009), Ji *et al.* (2010), Arora *et al.* (2011), Dickey *et al.* (2011), Han *et al.* (2012), Lin *et al.* (2012), Palumbo *et al.* (2012), Li *et al.* (2013)
*miR-34s	Increase	Primary and cancer	E2F, SIRT1, CDK4, Myc, HDAC1, NOTCH, Bcl-2	Enhance radiosensitivity Induce apoptosis	Hermeking (2009), Liu *et al.* (2011b), Balca-Silva *et al.* (2012), Duan *et al.* (2013), Metheetrairut and Slack (2013)
miR-99a	Increase/ decrease	Primary and cancer	SNF2H, FGFR3, Akt	Influence the processes of NHEJ and HR in DNA damage repair	Mueller *et al.* (2012), Chakrabarti *et al.* (2013), Metheetrairut and Slack (2013)
miR-100	Increase/ decrease	Primary and cancer	ATM, DNA-PKcs, PLK1	Modulate radiosensitivity	Ng *et al.* (2010), Liu *et al.* (2012a), Metheetrairut and Slack (2013)

(*continued*)

Table 1. (Continued)

miRNA	*Response*	*Cell type*	*Target*	*Biological effects*	*Reference*
miR-101	Increase	Primary	DNA-PKcs, ATM	Enhance radiosensitivity of cancer cells	Yan *et al.* (2010), Chen *et al.* (2011), Templin *et al.* (2011b)
miR-106	Increase/ decrease	Primary and cancer	p21, E2F5	Promote cell cycling	Cha *et al.* (2009a), Dickey *et al.* (2011), Li *et al.* (2011), Gu and Zhang (2013), Metheetrairut and Slack (2013)
miR-143/145	Increase/ decrease	Primary and cancer	Myc, K-Ras, FHIT, Bcl-2	Modulate radiosensitivity of cancer cells Promote apoptosis	Josson *et al.* (2008), Chaudhry *et al.* (2010b), Lin *et al.* (2011), Kent *et al.* (2012), Liu *et al.* (2012b), Metheetrairut and Slack (2013)
*miR-181a	Decrease	Primary	ATM, K-Ras, Bcl-2	Affect DNA repair and promote tumor cells apoptosis	Ishii and Saito (2006), Chen *et al.* (2010), Wang *et al.* (2010), Shin *et al.* (2011), Chaudhry *et al.* (2013)
miR-185	decrease	Cancer	ATR, Six1	Inhibit cancer cells proliferation and enhance radiation-induced apoptosis	Imam *et al.* (2010), Wang *et al.* (2013)
miR-210	Increase/ decrease	Primary and cancer	RAD52, HIF	A component of radioresistance and promote a more efficient DSB repair	Marsit *et al.* (2006), Yang *et al.* (2012), Barjaktarovic *et al.* (2013), Grosso *et al.* (2013)
miR-221/222	Increase/ decrease	Primary and cancer cell	p27, PTEN, PUMA	Regulate cancer cell proliferation and radioresistance	Chun-Zhi *et al.* (2010), Zhang *et al.* (2010a), Metheetrairut and Slack (2013)
miR-335	Decrease	Cancer	CtIP, Rb1	Involve in the processes of HR in the DNA damage repair	Shi *et al.* (2012), Martin *et al.* (2013)
miR-421	Decrease	Cancer	ATM	Affect DNA damage repair	Hu *et al.* (2010)
*miR-449a	Increase	Cancer	HDAC1, NOTCH1, Bcl-2, CyclinD1	Enhance radiosensitivity Induce apoptosis	Josson *et al.* (2008), Noonan *et al.* (2009), Marcet *et al.* (2011), Hu *et al.* (2014)
miR-521	Decrease	Cancer	CSA	Affect DNA damage repair and modulate radiosensitivity of prostate cancer cells	Josson *et al.* (2008)
miR-663	Increase/ decrease	Primary and cancer	SMAD3, p21	Promotes the proliferation and tumorigenesis of nasopharyngeal carcinoma	Maes *et al.* (2008), Cha *et al.* (2009b), Yi *et al.* (2012)

*Of special interest.
ATM, ataxia-telangiectasia mutated; IR, ionizing radiation.

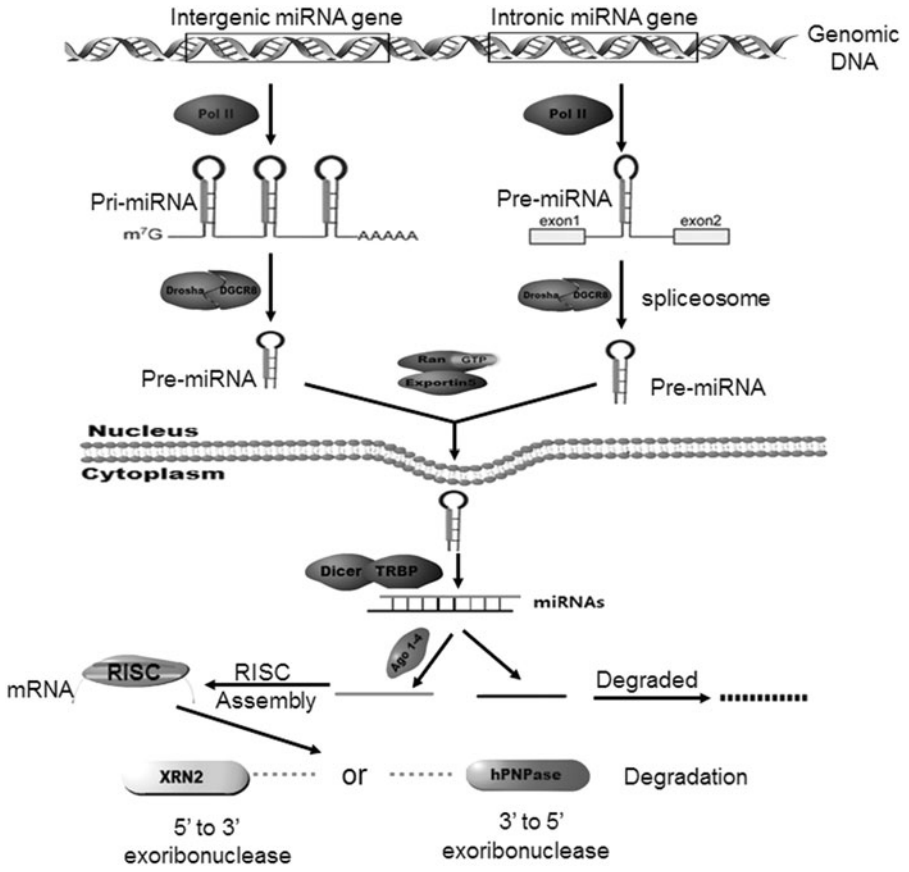

FIG. 1. miRNAs biogenesis and mechanisms of action. Briefly, miRNA genes are transcribed by RNA polymerase II as pri-miRNAs. pri-miRNAs with 5′ cap (m^7G) and 3′ polyadenylate are further cleaved into pre-miRNA by Drosha/DGCR8 complex. Pre-miRNAs are actively exported from nucleus to cytoplasm by exportin5/Ran-GTP. In cytoplasm, pre-miRNAs are recognized by Dicer/TRBP complex and cut to generate double-strand miRNAs. One of the two strands is selected as guide and assembled RISC to bind 3′ UTR of target genes and suppress its expression, while the complementary one is degraded. After completing its task, the mature miRNA is degraded by the 5′-3′ exoribonuclease or 3′-5′ exoribonucleases. miRNA, microRNAs; pri-miRNAs, primary miRNAs; RISC, RNA-induced silencing complex; TRBP, transactivating response RNA-binding protein; UTR, untranslated region.

the pre-miRNA and cuts both strands of the imperfect duplex at about two helical turns (~22 nucleotides) from the base of the hairpin and generates a short RNA duplex with 3′ 2-nucleotide overhang ends (MacRae *et al.*, 2006). Only one of the two strands is selected as guide strand and is incorporated into RNA-induced silencing complex (RISC) to bind 3′ UTR of target genes and suppress its expression, while the complementary one is usually subjected to degradation (Rand *et al.*, 2005; Diederichs and Haber, 2007). After completing its task, the mature miRNA is degraded by the 5′-3′ exoribonuclease (Chatterjee and Großhans, 2009) or 3′-5′ exoribonucleases (Das *et al.*, 2010).

The Regulation of miRNA Expression and Biogenesis in Cellular Response to IR

DSBs caused by IR trigger the DDR and activate multiple intracellular signal transduction pathways involved in gene transcriptional and post-transcriptional regulation. miRNA expression and biogenesis are critical components of DDR, which should be tightly controlled by the IR-caused DDR. In the next sections, we will discuss the regulatory mechanisms of miRNA expression and biogenesis in cellular response to IR from different aspects that include ATM kinase, p53 family, and other potential factors.

ATM kinase regulates miRNAs expression and biogenesis in cellular response to IR

ATM serine/threonine kinase plays a critical role in the cellular responses to IR-caused DNA damage (Canman *et al.*, 1998) and transduces the DNA damage signal to downstream proteins (Lavin, 2008). Over 700 proteins are identified as its targets (Matsuoka *et al.*, 2007). Recently, several studies have revealed that many miRNAs are significantly induced by DSBs in an ATM-dependent manner (Liu *et al.*, 2011b; Zhang *et al.*, 2011; Zhang and Lu, 2011; Martin *et al.*, 2013). IR mainly caused DSBs (Han and Yu, 2010) and ATM responds in particular to DSBs. Therefore, ATM kinase must play a critical role in the regulation of micriRNAs expression and biogenesis in cellular response to IR.

Martin *et al.* (2013) provided direct evidence that miR-335 was downregulated by IR-induced DSBs in an ATM-dependent manner. IR-activated ATM phosphorylated cAMP response element-binding protein (CREB), which was responsible for a large portion of miR-335 expression by binding to the promoter region. ATM-dependent miR-335 downregulated CtIP, which was an important trigger of DNA end resection in homologous recombination repair (HRR), and likely modulated the initiation of DNA end resection and repair (Martin *et al.*, 2013).

p53 is also one substrate of ATM. ATM phosphorylates p53 leads to its dissociation from MDM-2, an inhibitor of p53, and activation of p53 (Lee and Paull, 2007). Activated p53 induces the expression of some miRNAs, such as miR-34s (He *et al.*, 2007; Zenz *et al.*, 2009), miR-192, and miR-215 (Georges *et al.*, 2008; Maes *et al.*, 2008; Song *et al.*, 2008; Shin *et al.*, 2009) in cellular response to IR. Ectopic expression of miR-34a induces cell cycle arrest and promote apoptosis (Chang *et al.*, 2007; Tarasov *et al.*, 2007; Cole

et al., 2008), whereas miR-192 and miR-215 downregulate transcription of CDC7 and MAD2L1 that are involved in cell proliferation (Georges *et al.*, 2008). The regulatory mechanism of p53 in miRNA expression upon IR will be discussed in detail in other sections.

As a key component of the DDR, ATM kinase not only phosphorylates transcription factors, such as CREB and p53, to regulate some miRNAs expression at the transcriptional level but phosphorylates breast cancer 1 (BRCA1) and KH-type splicing regulatory protein (KSRP), key components of both Drosha and Dicer complex, to induce miRNA biogenesis at the post-transcriptional level in the IR-caused DDR (Fig. 2).

Cortez (1999) indicated that ATM was required for phosphorylation of BRCA1 in cellular response to IR. Recently, Kawai and Amano elucidated that BRCA1 regulated miRNAs biogenesis through the Drosha microprocessor complex and Smad3/p53/DHX9. BRCA1 could be directly associated with Drosha and DDX5 of the Drosha microprocessor complex and interacted with Smad3, p53, and DHX9 RNA helicase to accelerate the processing of pri-miRNAs (Kawai and Amano, 2012). So we argue that BRCA1, activated by ATM upon IR-induced DNA damage, facilitates the processing of some pri-miRNAs through direct interaction with Drosha microprocessor complex.

Zhang *et al.* (2011) described a critical link between ATM kinase and miRNA biogenesis, whereby, ATM kinase induced miRNA biogenesis through phosphorylation of KSRP. KSRP associates with both Drosha and Dicer and post-transcriptionally regulates the biogenesis of miRNAs (Liu *et al.*, 2011b). 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 miRNAs processing. Mutations of the ATM phosphorylation sites of KSRP impair its activity in regulating miRNAs (Liu *et al.*, 2011b; Zhang *et al.*, 2011). Although the authors used a radiomimetic drug, neocarzinostain, not directly used IR, to generate DSBs and there is no direct evidence that ATM kinase regulate miRNA biogenesis in IR-induced DDR, the study illuminated that ATM kinase regulates many miRNA biogenesis in cellular response to DSBs. IR mainly caused DSBs (Han and Yu, 2010) and ATM responds in particular to DSBs. Moreover, ATM-dependent phosphorylation of KSRP enhances processing of many miRNAs, including miR-21, which is consistently upregulated upon IR in a variety of normal and cancer cell lines (Simone *et al.*, 2009; Chaudhry *et al.*, 2010b, 2012; Wagner-Ecker *et al.*, 2010; Arora *et al.*, 2011; Templin *et al.*, 2011b; Vincenti *et al.*, 2011). Therefore, it is proposed that ATM-dependent phosphorylation of KSRP could play a critical role in the regulation of miRNA biogenesis in cellular response to IR.

These findings strongly suggest that ATM functions as a major switch for the activity of CREB, p53, BRCA1, or KSRP in miRNAs expression and biogenesis, and that CREB, p53, BRCA1, or KSRP act as molecular gatekeepers that up- or downregulates the production of a subset of miRNAs in cellular response to IR (Fig. 2). Multiple

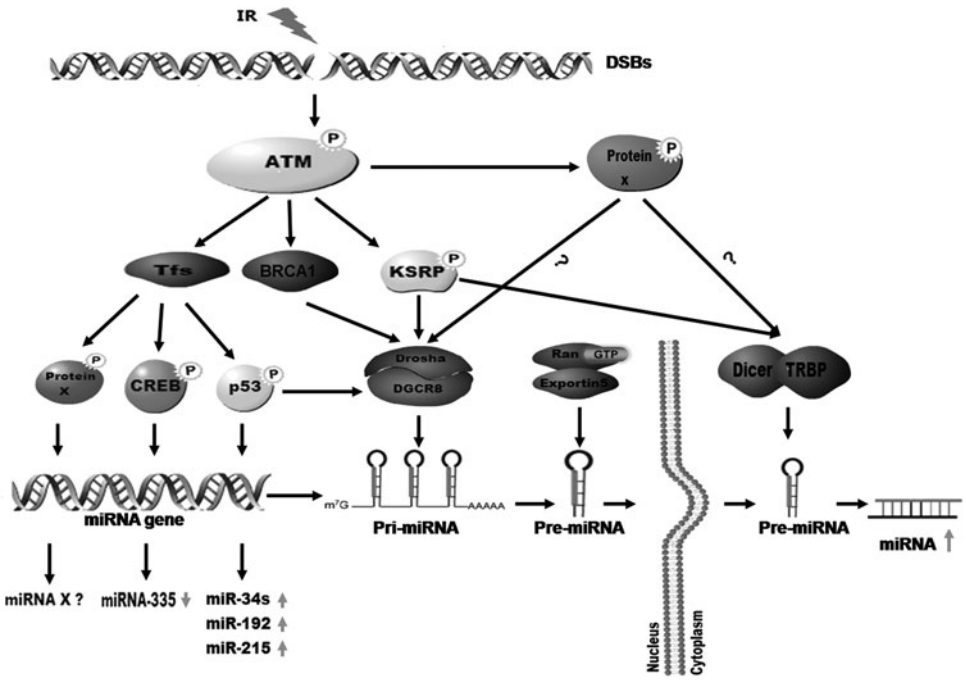

FIG. 2. ATM kinase regulates miRNA biogenesis in cellular response to IR. Briefly, IR-induced DSBs activate ATM. Activated ATM kinase phosphorylates transcription factors, such as CREB and p53, which are responsible for a large portion of miRNAs expression by binding to the promoter region. In addition, activated ATM kinase also can induce many miRNAs biogenesis through phosphorylation of BRCA1 and KSRP, which associates with both Drosha and Dicer and post-transcriptionally regulates the biogenesis of miRNAs. Over 700 proteins are identified as ATM kinase's targets, so, apart from CREB, p53, BRCA1, and KSRP, there must be other proteins that regulate miRNAs expression and biogenesis in cellular response to IR. ATM, ataxia-telangiectasia mutated; BRCA1, breast cancer 1; IR, ionizing radiation; CREB, cAMP response element-binding protein; KSRP, KH-type splicing regulatory protein; Tfs, transcription factors.

phosphorylation sites have been identified or predicted on Drosha and Dicer protein, some of which might be phosphorylated by ATM or its downstream kinases (Chendrimada *et al.*, 2005; Dephoure *et al.*, 2008). Apart from CREB, p53, BRCA1, and KSRP, there must be other proteins that regulate miRNA expression and biogenesis in cellular response to IR (Fig. 2).

p53 family regulates miRNA expression and biogenesis in cellular response to IR

p53 mainly exerts its function through transcriptional regulation of its targets. In response to various stress signals, including the DDR after IR, ATM phosphorylates p53 leads to its dissociation from MDM-2, an inhibitor of p53, and accumulation of p53 (Lee and Paull, 2007). The accumulation of p53 leads to the transcriptional activation of its target genes and initiates various cellular responses (Stiewe, 2007). Several studies have demonstrated that miRNA expression and biogenesis is controlled by p53 in cellular response to IR (Fig. 3). The first discovery connecting p53 to the regulation of miRNAs expression is the identification of the miR-34 family. miR-34s can be induced by IR *in vitro* (Josson *et al.*, 2008; Nikiforova *et al.*, 2011; Girardi *et al.*, 2012) and *in vivo* (Kato *et al.*, 2009) and whose expression is precisely correlated with p53 status (He *et al.*, 2007). Predicted gene structure for miR-34 family shows that the promoter regions included a palindromic sequence that matched the canonical p53-binding sites (He *et al.*, 2007). The induction of miR-34s by p53 in cellular response to IR is further confirmed by other groups (Chang *et al.*, 2007; Tarasov *et al.*, 2007; Bhatt *et al.*, 2010; Liu *et al.*, 2011b; Balca-Silva *et al.*, 2012).

Several miRNA profiling studies reveal that the expression levels of *let*-7 family are altered by IR [reviewed by Metheetrairut and Slack (2013)]. The *let*-7 family are other miRNAs that are regulated by p53. Saleh *et al.* describe that *let*-7*a* and *b* are transcriptionally repressed by p53 after IR. p53 can directly bind the region upstream of *let*-7*a* and *b*, leading to its expressional repress. The expression of *let*-7*a* and *b* not only depends on functional p53, but also depends on IR-induced ATM signaling upstream of p53 (Saleh *et al.*, 2011). However, there are inconsistencies among various cell lines as to whether *let*-7 miRNAs were up- or down-regulated upon IR. In some cases, the expression of some miRNAs appear inconsistent. The detected differential expression of these miRNAs might be explained by the following facts. On the one hand, miRNAs are expressed in a tissue- or cell type-specific manner, the differences in species, model system, cell type, and irradiation conditions, that is, they are differentially expressed at radiation types, time points, and/or doses that are quite different from each other. On the other hand, some miRNAs may belong to multiple ''response networks'' that are activated by different cellular stimuli. Moreover, the limited power of our analysis to detect differentially expressed miRNAs with low fold changes may have prevented the detection of these miRNAs in additional irradiation conditions. These studies suggest that p53, activated by DSBs caused by IR, plays a critical role in the regulation of *let*-7 family miRNAs expression.

Moreover, miR-192, miR-194, and miR-215 are other miRNAs that appeared to be regulated by p53 in cellular response to IR. Several studies reveal that miR-192, miR-194, and miR-215 are significantly upregulated by IR in different normal and cancer cell lines (Maes *et al.*, 2008; Shin *et al.*, 2009; Iizuka *et al.*, 2012). IR-caused DNA damage promotes the p53-dependent upregulation of miR-192, miR-194, and miR-215 (Braun *et al.*, 2008; Georges *et al.*, 2008; Yan *et al.*, 2009; Pichiorri *et al.*, 2010). The

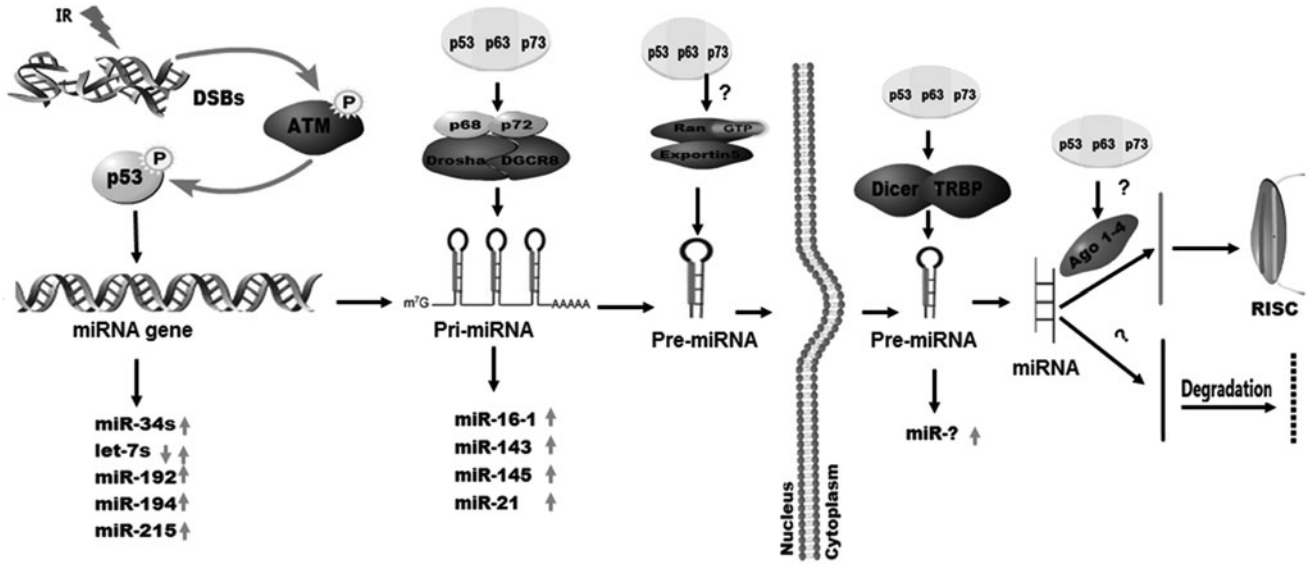

FIG. 3. p53 family regulates miRNA expression and biogenesis in cellular response to IR. Briefly, p53, as a transcriptional factor and target of ATM, not only exerts its function through transcriptional regulation of its targets, but also regulates a subset of miRNAs by modulating the processing and maturation of miRNA biogenesis. IR-induced DSBs directly activate p53 or ATM phosphorylates p53 to mediate transcription of miRNAs by binding the promoter regions of miRNA genes. p53/p63/p73 also can interact with Drosha/DGCR8 complex through p68 and p72 to enhance the expression of miRNAs. Whether p53/p63/p73 influence transportation of miRNAs, degradation, and RISC assembly is unclear and needs further investigation.

genomic region around the miR-194/miR-215 cluster contains a putative p53-binding element, which suggest that the cluster is activated by p53 at transcriptional level (Braun *et al.*, 2008; Georges *et al.*, 2008).

p53, as a transcriptional factor and target of ATM, not only regulates miRNA expression at transcriptional level but regulates miRNA biogenesis at post-transcriptional level in cellular response to IR (Fig. 3). Some of miRNAs, including miR-16-1, miR-143, and miR-145, are upregulated in a p53-dependent and p68/p72-dependent manner in the DDR (Suzuki *et al.*, 2009; Zhang *et al.*, 2013). p53 can interact with the Drosha/DGCR8 processing complex through an association with RNA helicase p68 (DDX5) and p72 (DDX17). A direct interaction between p53 and p68/p72 facilitates p53 promoting of miRNA processing from pri-miRNAs to pre-miRNAs. p53 mutants disrupt a functional assembly between Drosha complex and p68, resulting in attenuation of miRNA processing activity (Suzuki *et al.*, 2009). Similar to p53, TAp63 could bind to and transactivate the promoters of Dicer and miR-130b, and direct regulated the biogenesis and expression of miRNAs (Su *et al.*, 2010). p63/p73 is also noted that function as both positive and negative regulators of the miRNA transcription and processing components and regulate the expression and biogenesis of multiple miRNAs (Boominathan, 2010; Ory and Ellisen, 2011; Tucci *et al.*, 2012).

Although several studies have shown that the tumor suppressors p53/p63/p73 are regulators of miRNA processing complex, it currently remains largely unknown whether and how p53 family regulate the miRNA biogenesis in cellular response to IR. For instance, there is no direct evidence that confirms p53/p63/p73 regulates miRNA processing and maturation in cellular response to IR. Whether and how p53/p63/p73 regulates miRNAs processing in cellular response to IR? Moreover, whether and how p53/p63/p73 modulate the transportation of pre-miRNAs from the nucleus to cytoplasm after IR? Whether IR-induced DNA damage affects the degradation or modification of miRNAs? How p53/p63/p73 regulates the degradation of miRNAs? (Fig. 3). These questions should be further confirmed and elucidated in the future research.

Furthermore, we recently discovered that there was a differential ΔNp73 expression in response to different LET radiations, and downregulated ΔNp73 expression play a critical role in promoting cycle arrest and apoptosis in Hela cells (Di *et al.*, 2012, 2013). ΔNp73, as an antagonist to p53/p63/TAp73, whether and how the downregulated ΔNp73 expression affects the miRNA biogenesis in cellular response to IR remains unknown. It will be interesting to clarify the relationship of ΔNp73 expression and miRNA biogenesis in cellular response to different LET irradiation. We should play close attention to discover the effect of different LET irradiation on miRNAs expression and biogenesis and the regulatory mechanism of biogenesis employing the HIRFL (Heavy Ion Research Facility of Lanzhou, Institute of Modern Physics, Lanzhou, China).

Other potential factors regulate miRNAs expression and biogenesis in cellular response to IR

Besides many miRNAs responding to the IR-caused DNA damage is in ATM-dependent, a subset of miRNAs expression is DNA-PKcs or ATR-dependent. For example, after IR treatment, miR-17-3p, miR-17-5p, miR-19a/b, miR-142-3p, and miR-142-5p are upregulated in both DNA-PKcs-deficient (M059J) and-proficient (M059K) glioma cells lines, whereas miR-15a, miR-16, miR-143, miR-155, and miR-21 are upregulated only in DNA-PKcs-proficient cells, suggesting that their upregulation is dependent upon DNA-PKcs (Chaudhry *et al.*, 2010b). miR-709 is upregulated by IR-induced DNA damage in mouse testes through ATR-dependent upregulation of Rfx-1, the host gene of miR-709 (Tamminga *et al.*, 2008). Although ATR and DNA-PKcs may share some substrates with ATM, they may differentially regulate other substrates and the expression of some miRNAs in cellular response to IR.

In addition, the relocalization of Ago2 into stress granules (SG) promotes miRNAs expression in an ATM-independent manner in cellular response to UV irradiation (Pothof *et al.*, 2009). The miRNA-expression profile at 4h after UV irradiation, when Ago2 relocalization and SG formation, is most prominent. Ago2 fast relocalization into SG to regulate the miRNA expression after UV treatment provided a mechanism for the model of fast regulation of miRNA expression after DNA damage. The fast regulation of miRNA expression is required for proper cell cycle checkpoint control and allows a functional completion of the early DDR, eventually leading to increased survival of cells. As an example, miR-16 is immediately induced after DNA damage and specifically targets the mRNA of CDC25a and Wip1. CDC25a mRNA is downregulated already 1 h after UV, as a central mediator of G1-S cell cycle checkpoint, which is required for cell cycle checkpoint control (Pothof *et al.*, 2009). Wip1 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 DDR (Zhang *et al.*, 2010c). SGs are protein-RNA aggregates that form in stressed cells to regulate mRNA metabolism and inhibit translation of many mRNA (Anderson and Kedersha, 2008). As UV irradiation, IR also mainly causes DSBs and triggers the accumulation of SGs. However, there is a surprising lack of evidence of Ago2 or heterogeneous ribonuclear proteins (hnRNP) relocalization to the cytoplasm or SGs following IR treatment (Haley *et al.*, 2009). It remains to be elucidated whether the relocalization of Ago2 or other hnRNPs is directly involved in miRNAs expression in cellular response to IR.

IR also modulates the activity of other transcription factors, such as NF-κB, Myc, and E2F, which are known to induce expression of several miRNAs in the processes of DDR (Sawey *et al.*, 1987; Brach *et al.*, 1991; Huang *et al.*, 1997; Tusher *et al.*, 2001). For example, miR-125b was significantly induced in an NF-κB-dependent manner (Tan *et al.*, 2012) and miR-449a and 449b, and their host gene CDC20B, were strongly upregulated by E2F1 in the DDR (Lize *et al.*, 2009). In addition, overexpression of N-Myc induced miR-17-92 cluster and miR-421expression and increased sensitivity of cell to IR (Aguda *et al.*, 2008; Hu *et al.*, 2010). Moreover, the interaction of transcription factors and miRNAs was bidirectional. These transcription factor-regulated miRNAs also contributed to the cellular response to IR.

Besides these transcription factors, the chromatin remodeling and protein post-transcriptional modification (phosphorylation, methylation, ubiquitination, and acetylation) also were important events in the DDR after IR, such as, protein phosphorylation (Matsuoka *et al.*, 2007; Huertas *et al.*, 2009). A previous study had suggested that phosphorylation of TRBP stabilizes the Dicer-TRBP complex and increased mature miRNA production (Chendrimada *et al.*, 2005). Interestingly, phosphorylation of TRBP was mediated by the mitogen-activated protein kinase (MAPK) Erk, which were phosphorylated and activated after IR (Dent *et al.*, 2003). Further studies will be required to elucidate whether and how IR modulate these kinases to regulate the miRNA processing machinery and to achieve biological responses.

Furthermore, cell metabolic changes after IR might also affect miRNA production. miRNA biogenesis is energy-dependent at each step. For example, as a major energy currency molecule of the cell, ATP facilitates RISC loading of small-RNA duplexes in miRNA-mediated suppression (Yoda *et al.*, 2010). Exportin-5-mediated pre-miRNA transportation uses another energy carrier, GTP, which binds to the Ran proteins in the exportin-5 complex (Yi *et al.*, 2003). It is proposed that the changes of cell metabolism after IR will have negative or positive effects on the miRNA expression and biogenesis.

Interestingly, the DDR has been considered exclusively a protein-made signaling cascade, with no direct contributions of RNA species to its activation. But in recent studies, a new class of small RNAs, other than miRNAs, is identified and also control the DDR. Wei *et al.* (2012) described that a new class of Dicer-processed small noncoding RNAs, called DSB-induced small RNAs (diRNAs), can be generated at DNA damage sites and are responsible for the activation of HRR. Franci *et al.* (2012) further demonstrated that site-specific DICER- and DROSHA RNA products, named DNA damage response RNA (DDRNAs), are required for efficient foci formation of several DDR proteins, including phosphor-ATM, 53BP1, MDC1, and ATM/ATR substrates. diRNAs and DDRNAs are DICER- and DROSHA-dependent RNA products with the sequence of the damaged site after IR, which control the DDR activation and affects ATM phosphorylation. In turn, as described above, DDR activation and ATM phosphorylation is needed to regulate the miRNAs expression and biogenesis in cellular response to IR. Therefore, diRNAs and DDRNAs, as a novel class of ncRNAs, might play a critical role in the regulation of miRNAs expression and biogenesis.

miRNAs in the IR Response and in Radiotherapy

Radiotherapy is an important modality in tumor combinational treatment and is widely used for treating multiple tumors. The radiation response of tumor is the determining factor of the radiotherapeutic effect. How to improve tumor radiosensitivity and reduce the acute and late damage of normal tissue is a hot topic in the tumor radiotherapeutic field.

miRNAs have an intimate relationship with the cell cycle and apoptosis and play a critical role in tumor radiation response, which can effectively control tumor radiosensitivity by regulating DDR, cell cycle checkpoint, apoptosis, radio-related signal transduction pathways, and tumor microenvironment [reviewed by Hu and Gatti (2011); Halimi *et al.* (2012); Zhao *et al.* (2012, 2013)]. Therefore, miRNAs are promising agents for improving the efficacy of conventional cancer radiotherapy. For example, repression of ATR pathway by miR-185 enhances radiation-induced apoptosis and proliferation inhibition (Wang *et al.*, 2013). Inhibition of ATM or DNA-PKcs by miR-101, miR-100, and miR-421 causes increased cellular sensitivity to IR (Hu *et al.*, 2010; Ng *et al.*, 2010; Yan *et al.*, 2010). Inhibition of H2AX expression by either miR-24 (Lal *et al.*, 2009b) or miR-138 (Wang *et al.*, 2011b) promotes cellular sensitivity to IR. Several other IR-responsive miRNAs, such as miR-34s (Balca-Silva *et al.*, 2012; Maki *et al.*, 2012; Duan *et al.*, 2013), miR-181a (Chen *et al.*, 2010), miR-449a (Liu *et al.*, 2013), let-7 (Oh *et al.*, 2010), and miR-7 (Lee *et al.*, 2011), can also modulate radiosensitivity by targeting the DDR, cell cycle checkpoint, or apoptosis genes. Based on the fact that miRNAs can target multiple genes involved in the IR-caused DDR, modulating the endogenous miRNAs expression or biogenesis may be a promising strategy to overcome radioresistance and improving the radiotherapeutic effect.

Furthermore, in recent years, it has become increasingly evident that miRNA signatures describe cell and tissue status very precisely. Most diagnostic and prognostic expression profiling of miRNAs has been conducted using samples from tumor tissues (Bartels and Tsongalis, 2009) and body fluid (Kosaka *et al.*, 2010). miRNA signatures induced by IR are radiation type- and radiation dose-specific (Templin *et al.*, 2011a). In several studies, miRNA expression profiles have been shown to have signatures related to tumor radiation response. For example, IR changes the miRNA expression profiles of normal human fibroblasts (Simone *et al.*, 2009) and immortalized cell lines (Shin *et al.*, 2009; Chaudhry *et al.*, 2010b; Niemoeller *et al.*, 2011). Moreover, Templin *et al.* (2011b) demonstrated that IR leads to the upregulation of the expression of a considerable proportion of the human miRNAs of peripheral blood cells of radiotherapy patients. Wang *et al.* (2011a) found 12 differently expressed miRNAs in radiotherapy sensitive and resistant NSCLC patients. These miRNA expression signatures upon IR can be used as important biomarkers of radiation exposure and radiotherapy. The intrinsic ability of miRNAs to act as the biomarker for radiation exposure allows them to have the ability to predict the radiation response of each patient and determine the personalized radiation dose for optimizing the therapeutic effect.

Conclusions and Perspectives

In summary, miRNA expression and biogenesis are regulated in cellular response to IR-caused DNA damage at either the transcriptional or post-transcriptional level. The regulation is ATM kinase-dependent or -independent. Some of these miRNAs can regulate the expression of a wide range of DDR/DNA repair genes and modulate cellular sensitivity to IR. The regulatory role of miRNAs in tumor radiosensitivity and the intrinsic ability of miRNA to act as biomarker allow them to have the potential to be useful in the clinical radiotherapy, manipulating the radiation response to enhance susceptibility to or protect cells from

radiation. Therefore, it will be of great importance to discover the regulatory mechanism of miRNA biogenesis in cellular responds to IR.

It is now clear that the expression level of miRNA is altered in cellular response to IR. IR-induced DDR modulates miRNA expression and biogenesis by either promoting the transcription of miRNA genes or directly interacting with the processing and maturation machinery of miRNA. Although the recent advances have been achieved in understanding miRNA expression and biogenesis in the DDR after IR, there are still many outstanding questions to be addressed. In particular, for ATM or p53-independent miRNAs, little is known about the regulatory mechanisms. The distinct mechanisms by which DDR signaling is linked to miRNA expression and biogenesis in cellular response to IR should be further elucidated in the future studies. Thorough understanding of miRNA expression and biogenesis in cellular response to IR will provide new insights for clinical cancer radiotherapy to improve the efficiency of current cancer radiotherapy.

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Disclosure Statement

No potential conflicts of interest were disclosed.

References

Aguda, B.D., Kim, Y., Piper-Hunter, M.G., Friedman, A., and Marsh, C.B. (2008). 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 **105,** 19678–19683.

Anderson, P., and Kedersha, N. (2008). Stress granules: the Tao of RNA triage. Trends Biochem Sci **33,** 141–150.

Arora, H., Qureshi, R., Park, A.-K., and Park, W.-Y. (2011). Coordinated regulation of ATF2 by miR-26b in γ-irradiated lung cancer cells. PLoS One **6,** e23802.

Balca-Silva, J., Neves, S.S., Goncalves, A.C., Abrantes, A.M., Casalta-Lopes, J., Botelho, M.F., Sarmento-Ribeiro, A.B., and Silva, H.C. (2012). Effect of miR-34b overexpression on the radiosensitivity of non-small cell lung cancer cell lines. Anticancer Res **32,** 1603–1609.

Barjaktarovic, Z., Anastasov, N., Azimzadeh, O., Sriharshan, A., Sarioglu, H., Ueffing, M., Tammio, H., Hakanen, A., Leszczynski, D., and Atkinson, M.J. (2013). Integrative proteomic and microRNA analysis of primary human coronary artery endothelial cells exposed to low-dose gamma radiation. Radiat Environ Biophys **52,** 87–98.

Bartel, D.P. (2009). MicroRNAs: target recognition and regulatory functions. Cell **136,** 215–233.

Bartels, C.L., and Tsongalis, G.J. (2009). MicroRNAs: novel biomarkers for human cancer. Clin Chem **55,** 623–631.

Bhatt, K., Zhou, L., Mi, Q.-S., Huang, S., She, J.-X., and Dong, Z. (2010). MicroRNA-34a is induced via p53 during cisplatin nephrotoxicity and contributes to cell survival. Mol Med **16,** 409.

Boominathan, L. (2010). The tumor suppressors p53, p63, and p73 are regulators of microRNA processing complex. PLoS One **5,** e10615.

Brach, M., Hass, R., Sherman, M., Gunji, H., Weichselbaum, R., and Kufe, D. (1991). Ionizing radiation induces expression and binding activity of the nuclear factor kappa B. J Clin Invest **88,** 691.

Braun, C.J., Zhang, X., Savelyeva, I., Wolff, S., Moll, U.M., Schepeler, T., Ørntoft, T.F., Andersen, C.L., and Dobbelstein, M. (2008). p53-Responsive micrornas 192 and 215 are capable of inducing cell cycle arrest. Cancer Res **68,** 10094–10104.

Canman, C.E., Lim, D.-S., Cimprich, K.A., Taya, Y., Tamai, K., Sakaguchi, K., Appella, E., Kastan, M.B., and Siliciano, J.D. (1998). Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. Science **281,** 1677–1679.

Cha, H.J., Seong, K.M., Bae, S., Jung, J.H., Kim, C.S., Yang, K.-H., Jin, Y.-W., and An, S. (2009a). Identification of specific microRNAs responding to low and high dose γ-irradiation in the human lymphoblast line IM9. Oncol Rep **22,** 863–868.

Cha, H.J., Shin, S., Yoo, H., Lee, E.-M., Bae, S., Yang, K.-H., Lee, S.-J., Park, I.-C., Jin, Y.-W., and An, S. (2009b). Identification of ionizing radiation-responsive microRNAs in the IM9 human B lymphoblastic cell line. Int J Oncol **34,** 1661–1668.

Chakrabarti, M., Banik, N.L., and Ray, S.K. (2013). Photofrin based photodynamic therapy and miR-99a transfection inhibited FGFR3 and PI3K/Akt signaling mechanisms to control growth of human glioblastoma in vitro and in vivo. PLoS One **8,** e55652.

Chang, T.-C., Wentzel, E.A., Kent, O.A., Ramachandran, K., Mullendore, M., Lee, K.H., Feldmann, G., Yamakuchi, M., Ferlito, M., and Lowenstein, C.J. (2007). Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell **26,** 745–752.

Chatterjee, S., and Großhans, H. (2009). Active turnover modulates mature microRNA activity in *Caenorhabditis elegans*. Nature **461,** 546–549.

Chaudhry, M.A. (2009). Real-time PCR analysis of micro-RNA expression in ionizing radiation-treated cells. Cancer Biother Radiopharm **24,** 49–56.

Chaudhry, M.A., Kreger, B., and Omaruddin, R.A. (2010a). Transcriptional modulation of micro-RNA in human cells differing in radiation sensitivity. Int J Radiat Biol **86,** 569–583.

Chaudhry, M.A., Omaruddin, R.A., Brumbaugh, C.D., Tariq, M.A., and Pourmand, N. (2013). Identification of radiation-induced microRNA transcriptome by next-generation massively parallel sequencing. J Radiat Res **54,** 808–822.

Chaudhry, M.A., Omaruddin, R.A., Kreger, B., de Toledo, S.M., and Azzam, E.I. (2012). Micro RNA responses to chronic or acute exposures to low dose ionizing radiation. Mol Biol Rep **39,** 7549–7558.

Chaudhry, M.A., Sachdeva, H., and Omaruddin, R.A. (2010b). Radiation-induced micro-RNA modulation in glioblastoma cells differing in DNA-repair pathways. DNA Cell Biol **29,** 553–561.

Chen, G., Zhu, W., Shi, D., Lv, L., Zhang, C., Liu, P., and Hu, W. (2010). MicroRNA-181a sensitizes human malignant glioma U87MG cells to radiation by targeting Bcl-2. Oncol Rep **23,** 997–1003.

Chen, S., Wang, H., Ng, W.L., Curran, W.J., and Wang, Y. (2011). Radiosensitizing effects of ectopic miR-101 on non–small-cell lung cancer cells depend on the endogenous miR-101 Level. Int J Radiat Oncol Biol Phys **81,** 1524–1529.

Chendrimada, T.P., Gregory, R.I., Kumaraswamy, E., Norman, J., Cooch, N., Nishikura, K., and Shiekhattar, R. (2005). TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature **436,** 740–744.

Chun-Zhi, Z., Lei, H., An-Ling, Z., Yan-Chao, F., Xiao, Y., Guang-Xiu, W., Zhi-Fan, J., Pei-Yu, P., Qing-Yu, Z., and Chun-Sheng, K. (2010). MicroRNA-221 and microRNA-222 regulate gastric carcinoma cell proliferation and radioresistance by targeting PTEN. BMC Cancer **10,** 367.

Cole, K.A., Attiyeh, E.F., Mosse, Y.P., Laquaglia, M.J., Diskin, S.J., Brodeur, G.M., and Maris, J.M. (2008). A functional screen identifies miR-34a as a candidate neuroblastoma tumor suppressor gene. Mol Cancer Res **6,** 735–742.

Cortez, D. (1999). Requirement of ATM-dependent phosphorylation of Brca1 in the DNA damage response to double-strand breaks. Science **286,** 1162–1166.

Das, S.K., Sokhi, U.K., Bhutia, S.K., Azab, B., Su, Z.-Z., Sarkar, D., and Fisher, P.B. (2010). Human polynucleotide phosphorylase selectively and preferentially degrades microRNA-221 in human melanoma cells. Proc Natl Acad Sci U S A **107,** 11948–11953.

Dent, P., Yacoub, A., Fisher, P.B., Hagan, M.P., and Grant, S. (2003). MAPK pathways in radiation responses. Oncogene **22,** 5885–5896.

Dephoure, N., Zhou, C., Villén, J., Beausoleil, S.A., Bakalarski, C.E., Elledge, S.J., and Gygi, S.P. (2008). A quantitative atlas of mitotic phosphorylation. Proc Natl Acad Sci U S A **105,** 10762–10767.

Di, C., Yang, L., Zhang, H., Ma, X., Zhang, X., Sun, C., Li, H., Xu, S., An, L., and Li, X. (2013). Mechanisms, function and clinical applications of DNp73. Cell Cycle **12,** 1861–1867.

Di, C.-X., Yang, L.-N., Zhang, H., An, L.-Z., Zhang, X., Ma, X.-F., Sun, C., Wang, X.-H., Yang, R., and Wu, Z.-h. (2012). Effects of carbon-ion beam or X-ray irradiation on anti-apoptosis ΔNp73 expression in HeLa cells. Gene **515,** 208–213.

Dickey, J.S., Zemp, F.J., Martin, O.A., and Kovalchuk, O. (2011). The role of miRNA in the direct and indirect effects of ionizing radiation. Radiat Environ Biophys **50,** 491–499.

Diederichs, S., and Haber, D.A. (2007). Dual role for argonautes in microRNA processing and posttranscriptional regulation of microRNA expression. Cell **131,** 1097–1108.

Duan, W., Xu, Y., Dong, Y., Cao, L., Tong, J., and Zhou, X. (2013). Ectopic expression of miR-34a enhances radiosensitivity of non-small cell lung cancer cells, partly by suppressing the LyGDI signaling pathway. J Radiat Res **54,** 611–619.

Francia, S., Michelini, F., Saxena, A., Tang, D., de Hoon, M., Anelli, V., Mione, M., Carninci, P., and d'Adda di Fagagna, F. (2012). Site-specific DICER and DROSHA RNA products control the DNA-damage response. Nature **488,** 231–235.

Friedman, R.C., Farh, K.K.-H., Burge, C.B., and Bartel, D.P. (2009). Most mammalian mRNAs are conserved targets of microRNAs. Genome Res **19,** 92–105.

Georges, S.A., Biery, M.C., Kim, S.-Y., Schelter, J.M., Guo, J., Chang, A.N., Jackson, A.L., Carleton, M.O., Linsley, P.S., and Cleary, M.A. (2008). Coordinated regulation of cell cycle transcripts by p53-Inducible microRNAs, miR-192 and miR-215. Cancer Res **68,** 10105–10112.

Girardi, C., De Pittà, C., Casara, S., Sales, G., Lanfranchi, G., Celotti L., and Mognato, M. (2012). Analysis of miRNA and mRNA expression profiles highlights alterations in ionizing radiation response of human lymphocytes under modeled microgravity. PLoS One **7,** e31293.

Grosso, S., Doyen, J., Parks, S., Bertero, T., Paye, A., Cardinaud, B., Gounon, P., Lacas-Gervais, S., Noël, A., and Pouysségur, J. (2013). MiR-210 promotes a hypoxic phenotype and increases radioresistance in human lung cancer cell lines. Cell Death Dis **4,** e544.

Gu, Y.L., and Zhang, Q.-H. (2013). Effects of microRNA-106 on proliferation of gastric cancer cell through regulating p21 and E2F5. Asian Pac J Cancer Prev **14,** 2839–2843.

Haley, B., Paunesku, T., Protic, M., and Woloschak, G.E. (2009). Response of heterogeneous ribonuclear proteins (hnRNP) to ionising radiation and their involvement in DNA damage repair. Int J Radiat Biol **85,** 643–655.

Halimi, M., Asghari, S.M., Sariri, R., Moslemi, D., and Parsian, H. (2012). Cellular response to ionizing radiation: a microRNA story. Int J Mol Cell Med **1,** 1–7.

Han, J., Lee, Y., Yeom, K.-H., Kim, Y.-K., Jin, H., and Kim, V.N. (2004). The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev **18,** 3016–3027.

Han, M., Yang, Z., Sayed, D., He, M., Gao, S., Lin, L., Yoon, S., and Abdellatif, M. (2012). GATA4 expression is primarily regulated via a miR-26b-dependent post-transcriptional mechanism during cardiac hypertrophy. Cardiovasc Res **93,** 645–654.

Han, W., and Yu, K. (2010). Ionizing Radiation, DNA double strand break and mutation. Adv Genet Res **4,** 13.

He, L., He, X., Lim, L.P., De Stanchina, E., Xuan, Z., Liang, Y., Xue, W., Zender, L., Magnus, J., and Ridzon, D. (2007). A microRNA component of the p53 tumour suppressor network. Nature **447,** 1130–1134.

Hermeking, H. (2009). The miR-34 family in cancer and apoptosis. Cell Death Differ **17,** 193–199.

Hu, H., Du, L., Nagabayashi, G., Seeger, R.C., and Gatti, R.A. (2010). ATM is down-regulated by N-Myc–regulated microRNA-421. Proc Natl Acad Sci U S A **107,** 1506–1511.

Hu, H., and Gatti, R.A. (2011). MicroRNAs: new players in the DNA damage response. J Mol Cell Biol **3,** 151–158.

Hu, J., Fang, Y., Cao, Y., Qin, R., and Chen, Q. (2014). miR-449a regulates proliferation and chemosensitivity to cisplatin by targeting cyclin D1 and BCL2 in SGC7901 cells. Dig Dis Sci 59, 336–345.

Huang, Y., Ishiko, T., Nakada, S., Utsugisawa, T., Kato, T., and Yuan, Z.-M. (1997). Role for E2F in DNA damage-induced entry of cells into S phase. Cancer Res **57,** 3640–3643.

Huertas, D., Sendra, R., and Muñoz, P. (2009). Chromatin dynamics coupled to DNA repair. Epigenetics **4,** 31–42.

Iizuka, D., Imaoka, T., Nishimura, M., Kawai, H., Suzuki, F., and Shimada, Y. (2012). Aberrant microRNA expression in radiation-induced rat mammary cancer: the potential role of miR-194 overexpression in cancer cell proliferation. Radiat Res **179,** 151–159.

Ilnytskyy, Y., Zemp, F.J., Koturbash, I., and Kovalchuk, O. (2008). Altered microRNA expression patterns in irradiated hematopoietic tissues suggest a sex-specific protective mechanism. Biochem Biophys Res Commun **377,** 41–45.

Imam, J., Buddavarapu, K., Lee-Chang, J., Ganapathy, S., Camosy, C., Chen, Y., and Rao, M. (2010). MicroRNA-185 suppresses tumor growth and progression by targeting the Six1 oncogene in human cancers. Oncogene **29,** 4971–4979.

Ishii, H., and Saito, T. (2006). Radiation-induced response of micro RNA expression in murine embryonic stem cells. Med Chem **2,** 555–563.

Ji, Y., He, Y., Liu, L., and Zhong, X. (2010). MiRNA-26b regulates the expression of cyclooxygenase-2 in desferrioxamine-treated CNE cells. FEBS Lett **584,** 961–967.

Jiang, P., Rao, E.Y., Meng, N., Zhao, Y., and Wang, J.J. (2010). MicroRNA-17-92 significantly enhances radioresistance in human mantle cell lymphoma cells. Radiat Oncol **5,** 100.

Johnson, C.D., Esquela-Kerscher, A., Stefani, G., Byrom, M., Kelnar, K., Ovcharenko, D., Wilson, M., Wang, X., Shelton, J., and Shingara, J. (2007). The let-7 microRNA represses cell proliferation pathways in human cells. Cancer Res **67,** 7713–7722.

Josson, S., Sung, S.Y., Lao, K., Chung, L.W., and Johnstone, P.A. (2008). Radiation modulation of microRNA in prostate cancer cell lines. Prostate **68,** 1599–1606.

Kato, M., Paranjape, T., Ullrich, R., Nallur, S., Gillespie, E., Keane, K., Esquela-Kerscher, A., Weidhaas, J., and Slack, F. (2009). The mir-34 microRNA is required for the DNA damage response *in vivo* in *C. elegans* and *in vitro* in human breast cancer cells. Oncogene **28,** 2419–2424.

Kawai, S., and Amano, A. (2012). BRCA1 regulates microRNA biogenesis via the DROSHA microprocessor complex. J Cell Biol **197,** 201–208.

Kent, O., Fox-Talbot, K., and Halushka, M. (2012). RREB1 repressed miR-143/145 modulates KRAS signaling through downregulation of multiple targets. Oncogene **32,** 2576–2585.

Kim, Y.K., and Kim, V.N. (2007). Processing of intronic microRNAs. EMBO J **26,** 775–783.

Kosaka, N., Iguchi, H., and Ochiya, T. (2010). Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci **101,** 2087–2092.

Lal, A., Navarro, F., Maher, C.A., Maliszewski, L.E., Yan, N., O'Day, E., Chowdhury, D., Dykxhoorn, D.M., Tsai, P., and Hofmann, O. (2009a). miR-24 Inhibits cell proliferation by targeting E2F2, MYC, and other cell-cycle genes via binding to ''seedless'' 3′ UTR microRNA recognition elements. Mol Cell **35,** 610–625.

Lal, A., Pan, Y., Navarro, F., Dykxhoorn, D.M., Moreau, L., Meire, E., Bentwich, Z., Lieberman, J., and Chowdhury, D. (2009b). miR-24–mediated downregulation of H2AX suppresses DNA repair in terminally differentiated blood cells. Nat Struct Mol Biol **16,** 492–498.

Lavin, M.F. (2008). Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. Nat Rev Mol Cell Biol **9,** 759–769.

Lee, J., and Paull, T. (2007). Activation and regulation of ATM kinase activity in response to DNA double-strand breaks. Oncogene **26,** 7741–7748.

Lee, K.M., Choi, E.J., and Kim, I.A. (2011). microRNA-7 increases radiosensitivity of human cancer cells with activated EGFR-associated signaling. Radiother Oncol **101,** 171–176.

Lee, Y., Kim, M., Han, J., Yeom, K.H., Lee, S., Baek, S.H., and Kim, V.N. (2004). MicroRNA genes are transcribed by RNA polymerase II. EMBO J **23,** 4051–4060.

Li, B., Shi, X.B., Nori, D., Chao, C.K., Chen, A.M., Valicenti, R., and de Vere White, R. (2011). Down-regulation of microRNA 106b is involved in p21-mediated cell cycle arrest in response to radiation in prostate cancer cells. Prostate **71,** 567–574.

Li, J., Kong, X., Zhang, J., Luo, Q., Li, X., and Fang, L. (2013). MiRNA-26b inhibits proliferation by targeting PTGS2 in breast cancer. Cancer Cell Int **13,** 7.

Lin, F., Li, R., Xiang Pan, Z., Zhou, B., Guang Wang, X., Shan Ma, X., Han, J., Shen, M., and Lin Liu, H. (2012). miR-26b promotes granulosa cell apoptosis by targeting ATM during follicular atresia in porcine ovary. PLoS One **7,** e38640.

Lin, Y.-X., Yu, F., Gao, N., Sheng, J.-P., Qiu, J.-Z., and Hu, B.-C. (2011). microRNA-143 protects cells from DNA damage-induced killing by downregulating FHIT expression. Cancer Biother Radiopharm **26,** 365–372.

Liu, C., Li, B., Cheng, Y., Lin, J., Hao, J., Zhang, S., Mitchel, R., Sun, D., Ni, J., and Zhao, L. (2011a). MiR-21 plays an important role in radiation induced carcinogenesis in BALB/c mice by directly targeting the tumor suppressor gene Big-h3. Int J Biol Sci **7,** 347.

Liu, C., Zhou, C., Gao, F., Cai, S., Zhang, C., Zhao, L., Zhao, F., Cao, F., Lin, J., and Yang, Y. (2011b). MiR-34a in age and tissue related radio-sensitivity and serum miR-34a as a novel indicator of radiation injury. Int J Biol Sci **7,** 221.

Liu, J., Lu, K.-H., Liu, Z.-L., Sun, M., De, W., and Wang, Z.-X. (2012a). MicroRNA-100 is a potential molecular marker of non-small cell lung cancer and functions as a tumor suppressor by targeting polo-like kinase 1. BMC Cancer **12,** 519.

Liu, L., Yu, X., Guo, X., Tian, Z., Su, M., Long, Y., Huang, C., Zhou, F., Liu, M., and Wu, X. (2012b). miR-143 is downregulated in cervical cancer and promotes apoptosis and inhibits tumor formation by targeting Bcl-2. Mol Med Report **5,** 753–760.

Liu, Y.-J., Lin, Y.-F., Chen, Y.-F., Luo, E.-C., Sher, Y.-P., Tsai, M.-H., Chuang, E.Y., and Lai, L.-C. (2013). MicroRNA-449a enhances radiosensitivity in CL1-0 lung adenocarcinoma cells. PLoS One **8,** e62383.

Lize, M., Pilarski, S., and Dobbelstein, M. (2009). E2F1-inducible microRNA 449a/b suppresses cell proliferation and promotes apoptosis. Cell Death Differ **17,** 452–458.

Lund, E., Güttinger, S., Calado, A., Dahlberg, J.E., and Kutay, U. (2004). Nuclear export of microRNA precursors. Science **303,** 95–98.

MacRae, I.J., Zhou, K., Li, F., Repic, A., Brooks, A.N., Cande, W.Z., Adams, P.D., and Doudna, J.A. (2006). Structural basis for double-stranded RNA processing by Dicer. Science **311,** 195–198.

Maes, O.C., An, J., Sarojini, H., Wu, H., and Wang, E. (2008). Changes in microRNA expression patterns in human fibroblasts after low-LET radiation. J Cell Biochem **105,** 824–834.

Maki, Y., Asano, H., Toyooka, S., Soh, J., Kubo, T., Katsui, K., Ueno, T., Shien, K., Muraoka, T., and Tanaka, N. (2012). MicroRNA miR-34b/c enhances cellular radiosensitivity of malignant pleural mesothelioma cells. Anticancer Res **32,** 4871–4875.

Marcet, B., Chevalier, B., Luxardi, G., Coraux, C., Zaragosi, L.-E., Cibois, M., Robbe-Sermesant, K., Jolly, T., Cardinaud, B., and Moreilhon, C. (2011). Control of vertebrate multiciliogenesis by miR-449 through direct repression of the Delta/Notch pathway. Nat Cell Biol **13,** 693–699.

Marsit, C.J., Eddy, K., and Kelsey, K.T. (2006). MicroRNA responses to cellular stress. Cancer Res **66,** 10843–10848.

Martin, N.T., Nakamura, K., Davies, R., Nahas, S.A., Brown, C., Tunuguntla, R., Gatti, R.A., and Hu, H. (2013). ATM–dependent MiR-335 targets CtIP and modulates the DNA damage response. PLoS Genet **9,** e1003505.

Matsuoka, S., Ballif, B.A., Smogorzewska, A., McDonald, E.R., Hurov, K.E., Luo, J., Bakalarski, C.E., Zhao, Z., Solimini, N., and Lerenthal, Y. (2007). ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science **316,** 1160–1166.

Metheetrairut, C., and Slack, F.J. (2013). MicroRNAs in the ionizing radiation response and in radiotherapy. Curr Opin Genet Dev **23,** 12–19.

Mueller, A., Sun, D., and Dutta, A. (2012). The miR-99 family regulates the DNA damage response through its target SNF2H. Oncogene **32,** 1164–1172.

Ng, W.L., Yan, D., Zhang, X., Mo, Y.-Y., and Wang, Y. (2010). Over-expression of miR-100 is responsible for the low-expression of ATM in the human glioma cell line: M059J. DNA Repair **9,** 1170–1175.

Niemoeller, O.M., Niyazi, M., Corradini, S., Zehentmayr, F., Li, M., Lauber, K., and Belka, C. (2011). MicroRNA expression profiles in human cancer cells after ionizing radiation. Radiat Oncol **6,** 29.

Nikiforova, M.N., Gandhi, M., Kelly, L., and Nikiforov, Y.E. (2011). MicroRNA dysregulation in human thyroid cells following exposure to ionizing radiation. Thyroid **21,** 261–266.

Noonan, E., Place, R., Pookot, D., Basak, S., Whitson, J., Hirata, H., Giardina, C., and Dahiya, R. (2009). miR-449a targets HDAC-1 and induces growth arrest in prostate cancer. Oncogene **28,** 1714–1724.

Oh, J.-S., Kim, J.-J., Byun, J.-Y., and Kim, I.-A. (2010). Lin28-let7 modulates radiosensitivity of human cancer cells with activation of K-Ras. Int J Radiat Oncol Biol Phys **76,** 5–8.

Ory, B., and Ellisen, L.W. (2011). A microRNA-dependent circuit controlling p63/p73 homeostasis: p53 family cross-talk meets therapeutic opportunity. Oncotarget **2,** 259.

Palumbo, T., Faucz, F., Azevedo, M., Xekouki, P., Iliopoulos, D., and Stratakis, C. (2012). Functional screen analysis reveals miR-26b and miR-128 as central regulators of pituitary somatomammotrophic tumor growth through activation of the PTEN–AKT pathway. Oncogene **32,** 1651–1659.

Pichiorri, F., Suh, S.-S., Rocci, A., De Luca, L., Taccioli, C., Santhanam, R., Zhou, W., Benson, D.M., Jr., Hofmainster, C., and Alder, H. (2010). Downregulation of p53-inducible microRNAs 192, 194, and 215 Impairs the p53/MDM2 autoregulatory loop in multiple myeloma development. Cancer Cell **18,** 367–381.

Pothof, J., Verkaik, N.S., van Ijcken, W., Wiemer, E.A., Ta, V.T., van der Horst, G.T., Jaspers, N.G., van Gent, D.C., Hoeijmakers, J.H., and Persengiev, S.P. (2009). MicroRNA-mediated gene silencing modulates the UV-induced DNA-damage response. EMBO J **28,** 2090–2099.

Rand, T.A., Petersen, S., Du, F., and Wang, X. (2005). Argonaute2 cleaves the anti-guide strand of siRNA during RISC activation. Cell **123,** 621–629.

Saleh, A.D., Savage, J.E., Cao, L., Soule, B.P., Ly, D., DeGraff, W., Harris, C.C., Mitchell, J.B., and Simone, N.L. (2011). Cellular stress induced alterations in microRNA let-7a and let-7b expression are dependent on p53. PLoS One **6,** e24429.

Sawey, M.J., Hood, A.T., Burns, F., and Garte, S.J. (1987). Activation of c-myc and cK-ras oncogenes in primary rat tumors induced by ionizing radiation. Mol Cell Biol **7,** 932–935.

Shi, L., Jiang, D., Sun, G., Wan, Y., Zhang, S., Zeng, Y., Pan, T., and Wang, Z. (2012). miR-335 promotes cell proliferation by directly targeting Rb1 in meningiomas. J Neurooncol **110,** 155–162.

Shin, K.-H., Bae, S.D., Hong, H.S., Kim, R.H., Kang, M.K., and Park, N.-H. (2011). miR-181a shows tumor suppressive effect against oral squamous cell carcinoma cells by downregulating K-ras. Biochem Biophys Res Commun **404,** 896–902.

Shin, S., Cha, H.J., Lee, E.-M., Lee, S.-J., Seo, S.-K., Jin, H.-O., Park, I.-C., Jin, Y.-W., and An, S. (2009). Alteration of miRNA profiles by ionizing radiation in A549 human non-small cell lung cancer cells. Int J Oncol **35,** 81–86.

Simone, N.L., Soule, B.P., Ly, D., Saleh, A.D., Savage, J.E., DeGraff, W., Cook, J., Harris, C.C., Gius, D., and Mitchell, J.B. (2009). Ionizing radiation-induced oxidative stress alters miRNA expression. PLoS One **4,** e6377.

Song, B., Wang, Y., Kudo, K., Gavin, E.J., Xi, Y., and Ju, J. (2008). miR-192 regulates dihydrofolate reductase and cellular proliferation through the p53-microRNA circuit. Clin Cancer Res **14,** 8080–8086.

Song, L., Lin, C., Wu, Z., Gong, H., Zeng, Y., Wu, J., Li, M., and Li, J. (2011). miR-18a impairs DNA damage response through downregulation of ataxia telangiectasia mutated (ATM) kinase. PLoS One **6,** e25454.

Stiewe, T. (2007). The p53 family in differentiation and tumorigenesis. Nat Rev Cancer **7,** 165–167.

Su, X., Chakravarti, D., Cho, M.S., Liu, L., Gi, Y.J., Lin, Y.-L., Leung, M.L., El-Naggar, A., Creighton, C.J., and Suraokar, M.B. (2010). TAp63 suppresses metastasis through coordinate regulation of Dicer and miRNAs. Nature **467,** 986–990.

Sun, C.-Y., She, X.-M., Qin, Y., Chu, Z.-B., Chen, L., Ai, L.-S., Zhang, L., and Hu, Y. (2013). miR-15a and miR-16 affect the angiogenesis of multiple myeloma by targeting VEGF. Carcinogenesis **34,** 426–435.

Suzuki, H.I., Yamagata, K., Sugimoto, K., Iwamoto, T., Kato, S., and Miyazono, K. (2009). Modulation of microRNA processing by p53. Nature **460,** 529–533.

Tamminga, J., Kathiria, P., Koturbash, I., and Kovalchuk, O. (2008). DNA damage-induced upregulation of miR-709 in the germline downregulates BORIS to counteract aberrant DNA hypomethylation. Cell Cycle **7,** 3731–3736.

Tan, G., Niu, J., Shi, Y., Ouyang, H., and Wu, Z.-H. (2012). NF-κB-dependent microRNA-125b up-regulation promotes cell survival by targeting p38α upon ultraviolet radiation. J Biol Chem **287,** 33036–33047.

Tarasov, V., Jung, P., Verdoodt, B., Lodygin, D., Epanchintsev, A., Menssen, A., Meister, G., and Hermeking, H. (2007). Differential regulation of microRNAs by p53 Revealed by massively parallel sequencing. Cell Cycle **6,** 1586–1593.

Templin, T., Amundson, S.A., Brenner, D.J., and Smilenov, L.B. (2011a). Whole mouse blood microRNA as biomarkers for exposure to gamma-rays and (56)Fe ion. Int J Radiat Biol **87,** 653–662.

Templin, T., Paul, S., Amundson, S.A., Young, E.F., Barker, C.A., Wolden, S.L., and Smilenov, L.B. (2011b). Radiation-induced micro-RNA expression changes in peripheral blood cells of radiotherapy patients. Int J Radiat Oncol Biol Phys **80,** 549–557.

Tucci, P., Agostini, M., Grespi, F., Markert, E.K., Terrinoni, A., Vousden, K.H., Muller, P.A., Dötsch, V., Kehrloesser, S., and Sayan, B.S. (2012). Loss of p63 and its microRNA-205 target results in enhanced cell migration and metastasis in prostate cancer. Proc Natl Acad Sci U S A **109,** 15312–15317.

Tusher, V.G., Tibshirani, R., and Chu, G. (2001). Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A **98,** 5116–5121.

Vincenti, S., Brillante, N., Lanza, V., Bozzoni, I., Presutti, C., Chiani, F., Etna, M.P., and Negri, R. (2011). HUVEC respond to radiation by inducing the expression of pro-angiogenic microRNAs. Radiat Res **175,** 535–546.

Wagner-Ecker, M., Schwager, C., Wirkner, U., Abdollahi, A., and Huber, P.E. (2010). MicroRNA expression after ionizing radiation in human endothelial cells. Radiat Oncol **5,** 25.

Wang, J., He, J., Su, F., Ding, N., Hu, W., Yao, B., Wang, W., and Zhou, G. (2013). Repression of ATR pathway by miR-

185 enhances radiation-induced apoptosis and proliferation inhibition. Cell Death Dis **4,** e699.

Wang, X.C., Du, L.Q., Tian, L.L., Wu, H.L., Jiang, X.Y., Zhang, H., Li, D.G., Wang, Y.Y., Wu, H.Y., She, Y., Liu, Q.F., Fan, F.Y., and Meng, A.M. (2011a). Expression and function of miRNA in postoperative radiotherapy sensitive and resistant patients of non-small cell lung cancer. Lung Cancer **72,** 92–99.

Wang, Y., Huang, J.-W., Li, M., Cavenee, W.K., Mitchell, P.S., Zhou, X., Tewari, M., Furnari, F.B., and Taniguchi, T. (2011b). MicroRNA-138 modulates DNA damage response by repressing histone H2AX expression. Mol Cancer Res **9,** 1100–1111.

Wang, Y., Yu, Y., Tsuyada, A., Ren, X., Wu, X., Stubblefield, K., Rankin-Gee, E.K., and Wang, S.E. (2010). Transforming growth factor-β regulates the sphere-initiating stem cell-like feature in breast cancer through miRNA-181 and ATM. Oncogene **30,** 1470–1480.

Wei, W., Ba, Z., Gao, M., Wu, Y., Ma, Y., Amiard, S., White, C.I., Rendtlew, Danielsen, J.M., Yang, Y.-G., and Qi, Y. (2012). A role for small RNAs in DNA double-strand break repair. Cell **149,** 101–112.

Weidhaas, J.B., Babar, I., Nallur, S.M., Trang, P., Roush, S., Boehm, M., Gillespie, E., and Slack, F.J. (2007). MicroRNAs as potential agents to alter resistance to cytotoxic anticancer therapy. Cancer Res **67,** 11111–11116.

Yan, D., Ng, W.L., Zhang, X., Wang, P., Zhang, Z., Mo, Y.-Y., Mao, H., Hao, C., Olson, J.J., and Curran, W.J. (2010). Targeting DNA-PKcs and ATM with miR-101 sensitizes tumors to radiation. PLoS One **5,** e11397.

Yan, H.-L., Xue, G., Mei, Q., Wang, Y.-Z., Ding, F.-X., Liu, M.-F., Lu, M.-H., Tang, Y., Yu, H.-Y., and Sun, S.-H. (2009). Repression of the miR-17-92 cluster by p53 has an important function in hypoxia-induced apoptosis. EMBO J **28,** 2719–2732.

Yang, W., Sun, T., Cao, J., Liu, F., Tian, Y., and Zhu, W. (2012). Downregulation of miR-210 expression inhibits proliferation, induces apoptosis and enhances radiosensitivity in hypoxic human hepatoma cells *in vitro*. Exp Cell Res **318,** 944–954.

Yi, C., Wang, Q., Wang, L., Huang, Y., Li, L., Liu, L., Zhou, X., Xie, G., Kang, T., and Wang, H. (2012). MiR-663, a microRNA targeting p21WAF1/CIP1, promotes the proliferation and tumorigenesis of nasopharyngeal carcinoma. Oncogene **31,** 4421–4433.

Yi, R., Qin, Y., Macara, I.G., and Cullen, B.R. (2003). Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev **17,** 3011–3016.

Yoda, M., Kawamata, T., Paroo, Z., Ye, X., Iwasaki, S., Liu, Q., and Tomari, Y. (2010). ATP-dependent human RISC assembly pathways. Nat Struct Mol Biol **17,** 17–23.

Zenz, T., Mohr, J., Eldering, E., Kater, A.P., Bühler, A., Kienle, D., Winkler, D., Dürig, J., van Oers, M.H., and Mertens, D. (2009). miR-34a as part of the resistance network in chronic lymphocytic leukemia. Blood **113,** 3801–3808.

Zhang, C.-Z., Zhang, J.-X., Zhang, A.-L., Shi, Z.-D., Han, L., Jia, Z.-F., Yang, W.-D., Wang, G.-X., Jiang, T., and You, Y.-P. (2010a). MiR-221 and miR-222 target PUMA to induce cell survival in glioblastoma. Mol Cancer **9,** 229.

Zhang, J., Sun, Q., Zhang, Z., Ge, S., Han, Z., and Chen, W. (2013). Loss of microRNA-143/145 disturbs cellular growth and apoptosis of human epithelial cancers by impairing the MDM2-p53 feedback loop. Oncogene **32,** 61–69.

Zhang, J.-G., Wang, J.-J., Zhao, F., Liu, Q., Jiang, K., and Yang, G.-H. (2010b). MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC). Clin Chim Acta **411,** 846–852.

Zhang, X., and Lu, X. (2011). Posttranscriptional regulation of miRNAs in the DNA damage response. RNA Biol **8,** 960–963.

Zhang, X., Wan, G., Berger, F.G., He, X., and Lu, X. (2011). The ATM kinase induces microRNA biogenesis in the DNA damage response. Mol Cell **41,** 371–383.

Zhang, X., Wan, G., Mlotshwa, S., Vance, V., Berger, F.G., Chen, H., and Lu, X. (2010c). Oncogenic Wip1 phosphatase is inhibited by miR-16 in the DNA damage signaling pathway. Cancer Res **70,** 7176–7186.

Zhao, L., Bode, A.M., Cao, Y., and Dong, Z. (2012). Regulatory mechanisms and clinical perspectives of miRNA in tumor radiosensitivity. Carcinogenesis **33,** 2220–2227.

Zhao, L., Lu, X., and Cao, Y. (2013). MicroRNA and Signal Transduction Pathways in Tumor Radiation Response. Cell Signal **25,** 1625–1634.

Zhou, X., Ren, Y., Moore, L., Mei, M., You, Y., Xu, P., Wang, B., Wang, G., Jia, Z., and Pu, P. (2010). Downregulation of miR-21 inhibits EGFR pathway and suppresses the growth of human glioblastoma cells independent of PTEN status. Lab Invest **90,** 144–155.

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