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REVIEW

The role of miRNA in the direct and indirect effects of ionizing radiation

Jennifer S. Dickey · Franz J. Zemp · Olga A. Martin · Olga Kovalchuk

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Abstract This review focuses on a number of recent studies that have examined changes in microRNA (miRNA) expression profiles in response to ionizing radiation and other forms of oxidative stress. In both murine and human cells and tissues, a number of miRNAs display significant alterations in expression levels in response to both direct and indirect radiation exposure. In terms of direct irradiation, or exposure to agents that induce oxidative stress, miRNA array analyses indicate that a number of miRNAs are up- and down-regulated and, in particular, the let-7 family of miRNAs may well be critical in the cellular response to oxidative stress. In bystander cells that are not directly irradiated, but close to, or share media with directly irradiated cells or tissues, the miRNA expression profiles are also altered, but are somewhat distinct from the directly irradiated cells. Based on the results of these numerous studies, as well as our own data presented here, we conclude that miRNA regulation is a critical step in the cellular response to radiation and oxidative stress and that future studies should elucidate the mechanisms through which this altered regulation affects cell metabolism.

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J. S. Dickey (✉)
Laboratory of Biochemistry, Division of Therapeutic Proteins, CDER, FDA, 29 Lincoln Drive, Bldg 29A, Room 2B-24, Bethesda, MD 20892, USA
e-mail: Jennifer.dickey@fda.hhs.gov

F. J. Zemp · O. Kovalchuk
Department of Biological Sciences, University of Lethbridge, 4401 University Drive, Lethbridge, AB T1K 3M4, Canada

Present Address:
F. J. Zemp
Department of Oncology, Tom Baker Cancer Center, Southern Alberta Cancer Research Institute, 3330 Hospital Drive N.W., Calgary, AB T2N 4N1, Canada

O. A. Martin
Laboratory of Molecular Pharmacology, Center for Cancer Research, NCI, NIH, 9000 Rockville Pike, Bethesda, MD 20892, USA

Present Address:
O. A. Martin (✉)
Department of Radiation Oncology, Peter MacCallum Cancer Centre, St. Andrews Place, East Melbourne, VIC 8006, Australia
e-mail: Olga.martin@petermac.org

Introduction

The important roles that microRNAs (miRNAs) play in the cellular response to stress have become increasingly clear (Meltzer 2005). Additionally, several laboratories have now utilized miRNA microarray techniques to determine how miRNA expression profiles change upon exposure of cells to myriad forms of stress including ionizing radiation, oxidative stress, and various drugs (Cummins and Velculescu 2006; Pogribny et al. 2010; Simone et al. 2009). As these studies grow in number, it is interesting to compare the results of their analyses to determine whether any common patterns become apparent. Because laboratories frequently use their own model cell systems, stress inducers, and array systems, distinct differences in expression profiles are often obtained. However, if a single group or small number of miRNAs is found in common among distinct laboratory studies using different cell line systems, then this indicates that changes in the expression pattern of

these molecules may serve as a common cellular response to stress and may present a valuable therapeutic target.

Therefore, here, we present a review of miRNA microarray expression profiling studies in both rodent and human cellular systems in response to both direct and indirect stress exposure. For the human systems section, we present the results of our own independent miRNA microarray expression profiling study using two doses of ionizing radiation and three time points in a unique human 3-D tissue model system. We also review the results of similar studies and discuss the common results found (Cha et al. 2009a, b; Maes et al. 2008; Simone et al. 2009; Templin et al. 2011b; Wagner-Ecker et al. 2010; Weidhaas et al. 2007).

Finally, we review our recent examinations of the role that miRNA plays in indirect, or bystander, effects of radiation exposure. We have found that miRNA expression profiles change dramatically in response to both direct irradiation and bystander signaling, and the nature of the changes are fairly similar in human systems (Kovalchuk et al. 2010). Of the 12 miRNAs identified to be differentially expressed in bystander tissue, 8 were also identified in directly irradiated samples of the same tissue model and 4 of these are common ionizing radiation-responsive miRNAs. Thus, similar mechanisms may be at play in cellular response to both direct and bystander ionizing radiation.

Materials and methods

The original data submitted in this study were obtained utilizing a human 3-D EpiAirway (Air-112) tissue system (MatTek Corporation, Ashland, MA). These artificial tissues were designed to maintain normal tissue architecture and preserve in vivo cell differentiation patterns. As a result, these tissues are mitotically and metabolically active and capable of releasing cytokines and forming gap junctions and closely resemble epithelial tissues of the respiratory tract (Boelsma et al. 2000). The tissues were cultured according to the manufacturer's specifications. The tissues were γ-irradiated with 0.2 and 2 Gy, or were sham irradiated. After irradiation, tissue samples were returned to 37°C and 5% CO_2 for various time points (30 min, 48 h, or 7 days post-irradiation). Two biological replicates were done for each experimental time point and irradiation dose. To obtain miRNA for expression profiling, tissues were suspended in TRIzol reagent (500 µl) and vortexed for 5–10 s to disperse cells from support membranes. RNA extraction was then carried out as per the manufacturer's instructions (TRIzol, Invitrogen, Carlsbad, CA). The miRNA microarray analyses were performed on the resulting samples by LC Sciences (Houston, TX).

In order to examine the commonality of miRNA expression level changes in response to ionizing radiation, recent papers that utilized miRNA microarrays were examined. miRNAs that were identified in those arrays as being significantly changed were noted leading to the identification of over 150 potential ionizing radiation-responsive miRNAs (see Supplemental Table 1) (Cha et al. 2009a, b; Maes et al. 2008; Simone et al. 2009; Templin et al. 2011b; Wagner-Ecker et al. 2010; Weidhaas et al. 2007). All further review and discussion arises from these identified miRNAs.

Human models

Results of Present Study

The miRNA expression profiles of human 3-D airway model tissues were compared to time-matched mock controls at 30 min, 48 h, and 7 days post-irradiation. MiRNAs were considered for comparison only if their average expression levels were over 1,000 arbitrary units of fluorescence (AUF). The reasons for this were three-fold. First, miRNAs with very low expression levels, <1,000 AUF, are analyzed with less sensitivity in our microarray methods. Second, downstream studies involving qRT-PCR methods are limited to miRNAs with expression patterns of >1,000 AUF. Finally, fold induction of miRNAs with low expression patterns seems less biologically significant than those of higher expression levels. For example, a 1.5-fold change of a miRNA expressed at 100 versus 1,000 AUF would coincide with a change of 50 and 500 units, respectively.

At 30-min post-irradiation (mpi), we found 46 and 39 significantly regulated miRNAs ($p < 0.05$) in the low- and high-dose irradiation treatments, namely, 0.2 and 2.0 Gy, respectively. At 48-h post-irradiation (hpi), this number fell to 27 and 37 significantly regulated miRNAs for the two doses. And finally, at 7 days post-irradiation (dpi), 35 and 48 miRNAs were significantly regulated. The list of significantly regulated miRNAs in this study can be found in Supplemental Table 2.

Interestingly, the 30 mpi and 48 hpi miRNA expression data show a great deal of similarity at the two doses; whereby 35 and 22 miRNAs are commonly regulated at the 30 mpi and 48 hpi time points, respectively. 24 miRNAs are commonly regulated at the 7 dpi time point (Fig. 1). Similarity is also found between the time points at different doses (Fig. 2). These results demonstrate that the miRNAome changes at these time points for each dose are more similar to each other than they are different. Further, the miRNAs that are commonly and similarly regulated between the time points provided us with a good starting point for the additional analysis of specific miRNAs and their families.

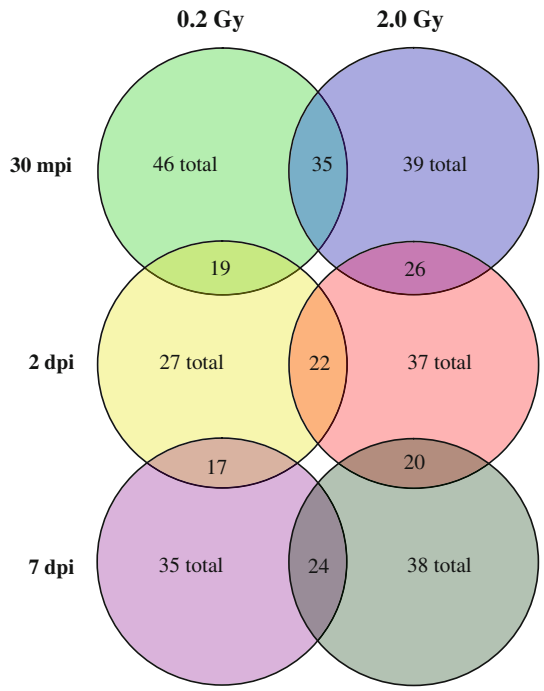

Fig. 1 Modified Venn diagram showing the similarity in miRNA expression patterns after 0.2 or 2.0 Gy γ-radiation. These miRNAs are grouped irrespective of whether up- or down-regulation of each miRNA occured. Total miRNA in each category is shown in the middle of the circle. *Mpi* minutes post-irradiation, *dpi* days post-irradaition

Direct effects of ionizing radiation

Since miRNA arrays have become available, several laboratories have utilized this tool to understand how miRNA expression changes are affected by pathological states, cellular stress, and other stimuli (Hummel et al. 2010; Simone et al. 2009). In terms of ionizing radiation exposure, a number of very informative array studies have been performed. However, the difficulty in interpreting these studies lies in the fact that different laboratories use distinct cell types, array platforms, dose strengths, and time courses. As shown above, there is variability in miRNA response depending on dose given and time examined. This complication is compounded if different cell types are being examined. Additionally, different sources of radiation that all have ionizing effects also may induce differential regulation in terms of miRNA expression. In order to investigate whether among all the different miRNA microarray studies, a common signature of miRNA can be found, we examined miRNA results published within the last 4 years that utilized ionizing radiation. As can be seen in Supplemental Fig. 1, studies have been performed in a wide range of doses (0.05–10 Gy) and in a number of cell types. For example, while one study examined lung cancer cells in culture (Weidhaas et al. 2007), another study

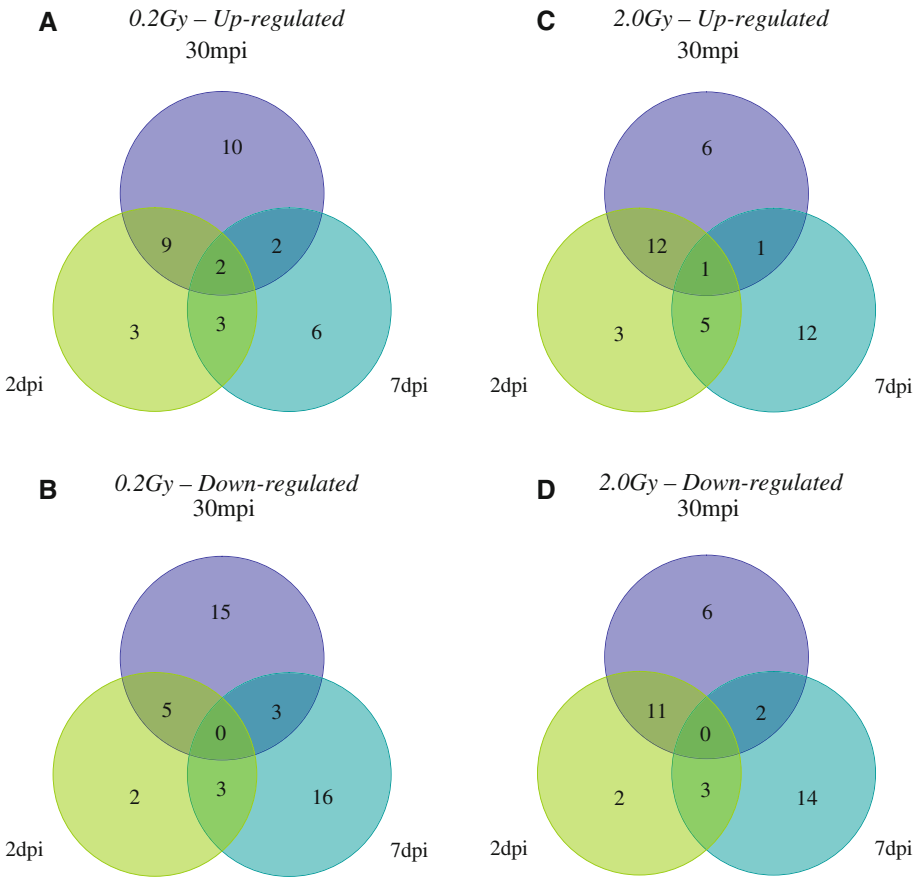

Fig. 2 Venn diagrams displaying the number of commonly regulated miRNAs after 0.2 Gy (**a** and **b**) or 2.0 Gy (**c** and **d**) γ-radiation. *Mpi* minutes post-irradiation, *dpi* days post-irradiation

utilized peripheral blood mononuclear cells (PBMCs) from a patient population exposed to radiation during cancer therapy (Templin et al. 2011b). Additionally, the time points used for analysis ranged from 30 mpi to 7 dpi (our present study). Given the wide disparity in study conditions, it is unsurprising that different populations of miRNAs were identified. What is perhaps more surprising is that a common group of miRNAs was frequently identified in many of these studies, despite differences in data acquisition and analysis.

From our examination, 24 miRNAs were identified that were significantly different after ionizing radiation exposure in at least 3 independent microarray studies from different laboratories (Table 1). These miRNAs are certainly not the only important miRNA to consider in ionizing radiation responses. However, their commonality across experimental platforms and doses indicates that they are probably key upstream regulators of the cellular response to ionizing radiation. For instance, miR-21 was identified in 6 separate laboratory miRNA microarray studies utilizing an array of doses, time points, and cell types. Thus, after extensive study by a number of laboratories into changes in miRNA expression profiles, a pattern is emerging of common miRNA regulators that may be key in understanding the mechanisms through which miRNA function affect cellular responses.

Like ionizing radiation, common signatures for miRNA responses to other cellular stresses may also be soon identified. Simone and colleagues examined oxidative genotoxic stress-inducing agents, such as ionizing radiation, hydrogen peroxide (H_2O_2), and etoposide to determine whether they cause similar biological consequences. The miRNA expression profile in primary human fibroblasts exposed to these agents was reported, and seven miRNA species were commonly altered by ionizing radiation, etoposide, and H_2O_2 exposure (Simone et al. 2009). Ionizing radiation causes severe DNA damage as a result of formation of intracellular free radicals; H_2O_2 also imposes free radical stress, and etoposide is a topoisomerase II poison that generates double-strand DNA breaks and also induces oxidative stress. Therefore, DNA damage and free radical formation reveal a common miRNA expression signature of exogenous genotoxic stress.

In other studies, primary human fibroblasts AG01522, primary human endothelial cells HDMEC, and the human lymphoblastoid cell line IM9 were irradiated with 0.25–10 Gy (Cha et al. 2009a; Wagner-Ecker et al. 2010). The expression profiles revealed significant irradiation-induced changes in miRNA levels; however, very few overlapped. This unexpected result can possibly be explained by the great sensitivity and flexibility of miRNA regulation, which may greatly depend on radiation dose and cell type. Most of the commonly deregulated miRNAs belong to the let-7 cluster, which negatively regulates Ras genes and, therefore, cell proliferation pathways in human cells (Simone et al. 2009). The other targeted pathways included apoptosis, DNA repair, glutathione metabolism, and cell detoxification.

In our own study, many of the let-7 family of miRNAs were significantly down-regulated after both the 0.2 and 2.0 Gy exposures (Table 2). Thirty minutes after radiation, all miRNAs of the let-7 family were significantly down-regulated at 0.2 Gy, while all but let-7g and let-7i are at 2.0 Gy. Interestingly, there is a more severe down-regulation at 0.2 than 2.0 Gy, with an average down-regulation of $\sim$2.5-and $\sim$1.5-fold, respectively. However, the down-regulation of this family of miRNAs is more persistent at 2.0 Gy. This higher dose resulted in changes that extended to 7 dpi in all, but two of the family members, while the 0.2 Gy group returned to around basal levels after 2 dpi.

Overall, these study results, along with the results of other studies using human cellular systems, indicate that the let-7 family of miRNAs along with miR-21 and the other species identified in Table 1 may be commonly regulated in response to ionizing radiation and should be investigated in regard to other forms of oxidative cellular stress (Johnson et al. 2005; Weidhaas et al. 2007). Thus, this family of miRNAs may be extremely interesting to track during the onset of carcinogenesis, as many cancers are known to exist at a higher level of oxidative stress than normal cells.

Indirect effects of ionizing radiation

The central dogma in radiation biology stating that IR effects are restricted to directly targeted cells was altered in 1992, when the radiation-induced bystander effect (RIBE) was first described by Nagasawa and Little (Nagasawa and Little 1992). After α-particle irradiation of only 1% of cells in a dish, 30% exhibited increased sister chromatid exchange, substantiating the existence of communication between damaged and intact cells. Since then, RIBE has been intensely examined. Following numerous studies confirming the existence of the RIBE by various biological endpoint assays, mechanistic insights have emerged. Several molecules have been shown to play important roles in different bystander systems in vitro and in vivo. These various cytokines, and reactive oxygen and nitrogen species are also involved in general stress responses, intercellular communication, and inflammation (Prise and O'Sullivan 2009). Thus, RIBE signaling may be not specific for radiation exposure.

We and others reported that in addition to ionizing radiation, many forms of cellular stress are capable of inducing bystander-like responses (Dickey et al. 2009; Sokolov et al. 2007). These include exogenous factors such

Table 1 Common ionizing radiation-responsive miRNAs

miRNA	Response	Cell type	Reference
Let-7a	Decrease	Primary and cancer	Cha et al. (2009b), Simone et al. (2009), Weidhaas et al. (2007), present
Let-7b	Decrease	Primary and cancer	Simone et al. (2009), Weidhaas et al. (2007), present
Let-7c	Decrease	Primary and cancer	Cha et al. (2009b), Weidhaas et al. (2007), present
Let-7d	Increase/decrease	Primary and cancer	Simone et al. (2009), Weidhaas et al. (2007), present
Let-7e	Increase/decrease	Primary and cancer	Cha et al. (2009b), Simone et al. (2009), Weidhaas et al. (2007), present
Let-7f	Increase/decrease	Primary and cancer	Cha et al. (2009b), Templin et al. (2011a, b), Weidhaas et al. (2007), present
Let-7g	Increase/decrease	Primary and cancer	Cha et al. (2009b), Simone et al. (2009), Templin et al. (2011a, b), Weidhaas et al. (2007), present
Let-7i	Increase/decrease	Primary and cancer	Simone et al. (2009), Weidhaas et al. (2007), present
miR-15-b	Increase/decrease	Primary and cancer	Cha et al. (2009b), Simone et al. (2009), present
miR-16	Increase/decrease	Primary and cancer	Cha et al. (2009a, b), Templin et al. (2011a, b), Wagner-Ecker et al. (2010), present
miR-18a	Decrease	Primary and cancer	Cha et al. (2009a, b), Maes et al. (2008), Wagner-Ecker et al. (2010)
miR-20a	Increase/decrease	Primary and cancer	Cha et al. (2009a, b), Templin et al. (2011a, b), Wagner-Ecker et al. (2010), present
miR-21	Increase/decrease	Primary and cancer	Cha et al. (2009a, b), Simone et al. (2009), Templin et al. (2011a, b), Wagner-Ecker et al. (2010), present
miR-24	Increase/decrease	Primary and cancer	Cha et al. (2009b), Simone et al. (2009), Templin et al. (2011a, b), present
miR-26b	Increase/decrease	Primary/patient only	Simone et al. (2009), Templin et al. (2011a, b), present
miR-29a	Increase/decrease	Primary and cancer	Cha et al. (2009b), Templin et al. (2011a, b), present
miR-29c	Increase/decrease	Primary and cancer	Cha et al. (2009b), Templin et al. (2011a, b), Wagner-Ecker et al. (2010), present
miR-103	Increase/decrease	Primary and cancer	Cha et al. (2009b), Templin et al. (2011a, b), present
miR-106a	Increase/decrease	Primary and cancer	Cha et al. (2009a, b), Templin et al. (2011a, b), Weidhaas et al. (2007)
miR-106b	Increase/decrease	Primary and cancer	Cha et al. (2009a, b), Templin et al. (2011a, b), Weidhaas et al. (2007)
miR-148b	Increase/decrease	Primary/patient only	Templin et al. (2011a, b), Wagner-Ecker et al. (2010), Weidhaas et al. (2007)
miR-222	Increase/decrease	Primary/patient only	Simone et al. (2009), Templin et al. (2011a, b), present
miR-376a	Increase/decrease	Primary and cancer	Cha et al. (2009a, b), Maes et al. (2008), Templin et al. (2011a, b)
miR-663	Increase/decrease	Primary and cancer	Cha et al. (2009b), Maes et al. (2008), Simone et al. (2009), present

These 24 miRNAs were found to be significantly deregulated by ionizing radiation in at least 3 independent microarray studies from different laboratories

as ultraviolet (UV) exposure, photolytic DNA damage, irritants such as SDS, physical cell disruption, and endogenous stresses such as tumorigenesis and senescence (Coppe et al. 2010; Dickey et al. 2009; Dickey et al. 2011a). Recently, we also found that tumors growing in vivo affect neighboring and distant tissues of a host, inducing DNA damage (Redon et al. 2010) similar to radiation-induced abscopal bystander-like effects

Table 2 Regulation of let-family of miRNAs red text—$p < 0.05$; orange text—$p < 0.10$; black text—$p > 0.10$

	0.2 Gray			2.0 Gray		
miRNA	30min	2 days	7 days	30min	2 days	7 days
hsa-let-7a	-1.97	-1.17	-1.17	-1.34	-1.40	-1.56
hsa-let-7b	-2.17	-1.12	-1.12	-1.72	-1.49	-1.07
hsa-let-7c	-2.36	-1.15	-1.09	-1.57	-1.57	-1.51
hsa-let-7d	-2.77	-1.16	1.01	-1.53	-1.52	-1.63
hsa-let-7e	-4.47	-1.14	1.74	-1.67	-1.90	-1.67
hsa-let-7f	-2.62	-1.16	-1.04	-1.33	-1.31	-1.72
hsa-let-7g	-2.47	-1.15	1.19	-1.01	1.03	-1.44
hsa-let-7i	-1.53	-1.05	1.22	-1.01	1.30	1.23

The let-7 family of miRNAs was significantly deregulated by ionizing radiation in our present study

(Koturbash et al. 2006). This DNA damage is oxidative in nature and is mediated by pro-inflammatory cytokines and macrophages.

It would be logical to assume that miRNA expression profiles in bystander cells or tissues would resemble the genotoxic miRNA expression signature. In fact, small RNAs are good candidates for propagating bystander signaling. They mediate fundamental cellular processes including cell–cell communication, they are small, relatively stable, gap junction transmissible, and can travel long distances (Meltzer 2005). We analyzed miRNAome changes in bystander human 3-D airway tissue models at different time points after post-α-particle microbeam irradiation of a thin cell layer in the middle of the tissues (Kovalchuk et al. 2010). We found that among others, miR-20a, miR-29a and c, and miR-16 were up-regulated in bystander tissues. These miRNAs control genes participating in major RIBE endpoints, de-regulation of cell cycle, apoptosis, and DNA hypomethylation (Koturbash et al. 2007), and, therefore, may mediate these RIBE consequences.

Direct comparison of α-particle-induced bystander and directly irradiated 3-D tissues technically is not possible, because of the low α-particle range of tissue penetration and irradiation methodology limitations (Kovalchuk et al. 2010). Of the miRNAs identified in Table 1 that are commonly irradiation responsive, 4 were also identified in 3D bystander tissues. When comparison was performed only on the miRNAs identified in the same cellular system (3D human tissue models in culture), 8 were the same of the 12 identified in the current experiments. These results indicate that there may be a similar miRNA response in both directly irradiated and bystander cells. However, investigations on the effects of direct and indirect exposure to ionizing radiation on miRNA expression are just beginning.

As discussed above, it is clear that miRNA expression is altered in both directly irradiated and in bystander tissue. A logical question would, therefore, be whether miRNA plays a direct role in propagating the bystander effect in response to IR. As the mechanisms through which the RIBE propagates remain not fully understood, and miRNAs are small and easily diffusible, it would not be unreasonable to think that miRNAs themselves could directly serve as bystander signaling molecules. Therefore, we recently undertook an examination of bystander effects in Dicer knock-down cell lines. Dicer knock-down cells have significantly reduced levels of mature miRNAs, as has been shown previously (Cummins et al. 2006). When we examined the bystander effect to ionizing radiation in these Dicer knock-down cells versus their normal controls, there was no difference in bystander effect induction level or time (Dickey et al. 2011a, b). Therefore, we can conclude that while miRNAs are significantly regulated by both direct and indirect cellular stresses, such as ionizing radiation, they are not themselves the primary bystander signaling molecules.

Rodent models

Direct effects of ionizing radiation

Studies on the effects of murine whole-body ionizing radiation exposure on microRNA levels have been conducted using various tissues and organs. In some of the first experiments, ionizing radiation exposure was shown to significantly affect the miRNAome of hematopoietic tissues, spleen, and thymus in a sex-specific manner (Ilnytskyy et al. 2008). Among the regulated miRNAs, the most prominent changes were seen in expression of miR-34a and miR-7. These miRNAs may be involved in important protective mechanisms counteracting radiation cytotoxicity (Ji et al. 2008; Tahara et al. 2010). Ionizing radiation exposure led to a significant increase in the expression of the tumor-suppressor miRNA, miR-34a, paralleled by a decrease in the expression of its target oncogenes NOTCH1, MYC, E2F3, and cyclin D1. MiR-7 was shown to target the lymphoid-specific helicase LSH, a pivotal regulator of DNA methylation and genome stability. While miR-7 was significantly down-regulated, LSH was significantly up-regulated in response to radiation exposure. Taken together, these miRNAome changes may constitute a cellular protective effect and an attempt to counteract radiation-induced

hypomethylation (Ilnytskyy et al. 2008). More recent work has also examined microRNAome changes in whole mouse blood as a potential biomarker for radiation exposure (Templin et al. 2011a). This work indicates that perhaps a common miRNA signature of radiation damage could be developed for future diagnostic use.

In addition to miRNA analysis in hematopoietic tissues, analysis of miRNA expression in the testes of whole-body irradiated mice revealed that a large number of miRNAs were significantly changed following radiation exposure, and that, the majority of altered miRNAs were up-regulated (Tamminga et al. 2008). MiR-709 was highly expressed in both control and irradiated testes, but a significant up-regulation in miR-709 levels was observed in the irradiated group. This miRNA is controlled through the DNA damage response ATR/Rfx1 pathway and regulates the expression of Brother of the Regulator of Imprinted Sites (BORIS), a testes-specific gene that directs epigenetic reprogramming and DNA methylation during male germ cell differentiation. Ionizing radiation-induced changes in miR-709 levels were associated with altered levels of BORIS and DNA methylation in exposed murine testes. Overall, it was shown that the DNA damage-induced and ATR/Rfx1-mediated increase of miR-709 expression in exposed testes may be a protective mechanism that effectively decreases the cellular level of BORIS to prevent massive aberrant erasure of DNA methylation after radiation exposure (Tamminga et al. 2008).

The effect of X-ray irradiation on microRNA expression in the hippocampus, frontal cortex, and cerebellum of male and female mice was also examined (Ilnytskyy et al. 2009; Koturbash et al. 2010a). In the course of the study, experimental animals received whole-body exposure to 1 Gy. The initial and persistent radiation-induced responses in the murine hippocampus, frontal cortex, and cerebellum were analyzed at 6 and 96 hpi. Analysis revealed a number of tissue-, time-, and sex-specific responses, as well as an important interplay between miRNAs and their targets. Nineteen miRNAs were altered in female murine hippocampal tissues after exposure to 1 Gy ionizing radiation. This corresponded to only a single down-regulated miRNA, miR-125a at 6 hpi, with thirteen up-regulated miRNAs. At 96 hpi, 5 miRNAs were down-regulated and 13 miRNAs were up-regulated in the female hippocampus. In the male hippocampus, radiation-induced changes were less pronounced. Specifically, 3 miRNAs were down-regulated and 4 were up-regulated at 6 hpi, with only 2 miRNAs down-regulated at 96 hpi. Interestingly, only 2 miRNAs, miR-34c, and miR-488* were altered in both male and female hippocampus; however, their radiation-induced expression patterns were different as these miRNAs were up-regulated in females and down-regulated in males. Twenty-six miRNAs were involved in cerebellar responses to ionizing radiation: 16 in females and 10 in males. Yet, the most pronounced changes in response to radiation were observed in the frontal cortex. In total, 38 miRNAs were found to be deregulated in females and 13 in males. Most interestingly, miRNAs of the miR-29 family, miR-29a and c, were found exclusively down-regulated in the frontal cortex tissue of exposed female mice at 6 and 96 hpi. This family of miRNAs has been shown to be involved in several very important processes, including the establishment of DNA methylation patterns by regulating the expression of de novo DNA methyltransferases DNMT3a and 3b (Fabbri et al. 2007). Interestingly, Western blotting analysis revealed increased levels of DNMT3a in the female frontal cortex of female mice at 96 hpi. This correlation suggests that increased DNMT3a levels may be due to radiation-induced down-regulation of the miR-29 family of miRNA in the frontal cortex tissue of female mice. No regulation of DNMT3a was seen in male frontal cortex tissue, where miR-29 expression levels did not change. Increased levels of DNMT3a were shown to impact the levels of global DNA methylation. Therefore, miR-29-mediated increase of DNMT3a in the female frontal cortex might be a protective mechanism aiming to restore and stabilize the levels of global genomic methylation after exposure to ionizing radiation.

Overall, whole-body in vivo radiation exposure of mice significantly affected the miRNAome expression of different tissues and organs (Ilnytskyy et al. 2009). Ionizing radiation-induced miRNAome alterations are detected as early as several hours after exposure (Ilnytskyy et al. 2008, 2009; Koturbash et al. 2008), and persists for days, weeks (Ilnytskyy et al. 2009; Koturbash et al. 2008; Tamminga et al. 2008), and even months (Koturbash et al. 2007) after irradiation.

Taken together, these data suggest that miRNA changes may have a protective effect after radiation exposure, yet more studies are needed to dissect the roles of miRNAs in whole-body radiation responses and the ionizing radiation-mediated regulation of miRNA expression. The exact roles of miRNAs in radiation-induced carcinogenesis also need to be delineated (Koturbash et al. 2010b). Thus, similar to studies in humans, more information may be needed to identify a common miRNA response that is not tissue specific.

Indirect effects of radiation

Localized X-ray irradiation induces persistent miRNAome changes in distant bystander tissues in the rodent in spleen tissue 7 months after localized cranial irradiation. These experiments demonstrated that miR-194 was up-regulated in the bystander rat spleen. Furthermore, miR-194 was the only miRNA that is statistically significantly up-regulated in bystander rat spleen tissue 24 h and 7 months after exposure. It is also strongly up-regulated in the spleen and plasma of rats

subjected to the whole-body irradiation. Overall, miR-194 seems to play some role in the maintenance of the long-term response in the mouse (Koturbash et al. 2007).

Using a miRNA microarray platform, miRNAome patterns have been profiled in skin and spleen tissues of mice subjected to sham treatment, whole body or head exposure. Radiation exposure led to significant tissue-specific changes in the microRNA expression profiles in bystander skin and spleen (Ilnytskyy et al. 2009). MiR-194 was significantly altered in bystander spleen, while miR-148a was changed in bystander skin (Ilnytskyy et al. 2009).

Conclusions

As discussed above, miRNA is significantly regulated by both direct and indirect radiation exposure in both human and rodent models. Though these important signaling molecules do not appear to play a direct role in propagating stress signaling to neighboring cells and tissues, miRNAs play important roles in the cellular response to radiation-induced damage. In addition to radiation effects, it seems clear that other forms of cellular stress that generate reactive oxygen species also induce changes in miRNA expression levels. Many of these changes, particularly within the let-7 family of miRNAs, are commonly induced by radiation in humans and rodents. The apparent congruency of the ionizing radiation responses seen in human and animal models supports the notion that miRNAs are important regulators of direct and indirect ionizing radiation responses. Future research will uncover in more detail the role that these miRNA play in cellular defense from oxidative stress and how these new mechanisms can be exploited for new therapeutic approaches.

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