Long and short non-coding RNA and radiation response: a review

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Once thought of as arising from "junk DNA," noncoding RNAs (ncRNAs) have emerged as key molecules in cellular processes and response to stress. From diseases such as cancer, coronary artery disease, and diabetes to the effects of ionizing radiation (IR), ncRNAs play important roles in disease progression and as biomarkers of damage. Noncoding RNAs regulate cellular processes by competitively binding DNA, mRNA, proteins, and other ncRNAs. Through these interactions, specific ncRNAs can modulate the radiosensitivity of cells and serve as diagnostic and prognostic biomarkers of radiation damage, whether from incidental exposure in radiotherapy or in accidental exposure scenarios. Analysis of RNA expression after radiation exposure has shown alterations not only in mRNAs, but also in ncRNAs (primarily miRNA, circRNA, and lncRNA), implying an important role in cellular stress response. Due to their abundance and stability in serum and other biofluids, ncRNAs also have great potential as minimally invasive biomarkers with advantages over current biodosimetry methods. Several studies have examined changes in ncRNA expression profiles in response to IR and other forms of oxidative stress. Furthermore, some studies have reported modulation of radiosensitivity by altering expression levels of these ncRNAs. This review discusses the roles of ncRNAs in the radiation response and evaluates prior research on ncRNAs as biomarkers of radiation damage. Future directions and applications of ncRNAs in radiation research are introduced, including the potential for a clinical ncRNA assay for assessing radiation damage and for the therapeutic use of RNA interference (RNAi). (Translational Research 2021; 233:162–179)

Abbreviations: ASO = anti-sense oligonucleotides; CAD = coronary artery disease; circRNA = circular RNA; DCA = dicentric chromosome assay; EMT = epithelial-to-mesenchymal transition; Gy = gray (unit); IR = ionizing radiation; lncRNA = long noncoding RNA; miRNA = microRNA; mRNA = messenger RNA; ncRNA = noncoding RNA; NSCLC = non-small cell lung cancer; PBMC = peripheral blood mononuclear cells; piRNA = piwi-interacting RNA; RNAi = RNA interference; ROS = reactive oxygen species; siRNA = small interfering RNA; shRNA = short hairpin RNA; snoRNA = small nucleolar RNA; snRNA = small nuclear RNA; TBI = total body irradiation; tRNA = transfer RNA; UTR = untranslated region

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Submitted for Publication November 9, 2020; received submitted February 2, 2021; accepted for publication February 7, 2021.

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1931-5244/$ - see front matter

https://doi.org/10.1016/j.trsl.2021.02.005

INTRODUCTION

Radiation injury, either due to radiation therapy or a mass casualty radiation disaster, will affect multiple cellular pathways and produce heterogeneous effects across the population. In the instance of radiation therapy, the goal of biodosimetry is to aid in prognosis and early treatment of normal tissue damage. In a mass casualty scenario, biodosimetry will allow for rapid triaging of patients to distinguish the injured from the 'worried well' and to utilize limited resources most effectively. Biodosimetry was born from the recognition that the physical dose of radiation may differ from the extent of biological perturbations and examines chromosomal, metabolomic, proteomic, molecular and other physiological changes.[1] Additionally, biodosimetry can be more quantifiable and verifiable than evaluation of clinical symptoms, which can have ranges in severity and onset.[2] Sproull and Camphausen recognized 7 fields of biodosimetry: cytogenics, electron paramagnetic resonance, proteomics, metabolomics, genomics, lymphocyte kinetics, and transcriptomics, among which transcriptomics are the least studied.[3] RNA biodosimetry markers offer several advantages over the current gold standard dicentric chromosome assay (DCA). The DCA method is limited by the reliance on culturing lymphocytes *ex vivo*, which requires several days to obtain a result and is labor intensive.[4] Thus, in a mass casualty scenario, only a fraction of the population could be tested. The delay in results may also decrease the efficacy of early medical countermeasures. Additionally, the DCA method requires the presence of sufficient lymphocytes in the blood, so patients exposed to very high doses (>10 Gy) and without actively dividing cells would be precluded.[4] Stable from degradation, readily abundant in bodily fluids and assayable via RT-PCR, ncRNAs could significantly shorten the time required for results over a cultured lymphocyte assay.

This review will briefly describe characteristics of noncoding RNA and the physiological and biological effects of radiation damage, followed by specific ncRNAs involved in radiation response and examples of ncRNA biomarkers. Future directions for the field, including druggable RNA targets, considerations for bringing RNA biomarkers to practice, and organ-specific RNA markers will be addressed.

Characteristics of ncRNAs. The completion of the ENCyclopedia of DNA Elements (ENCODE) project and the rapid advances in RNA-sequencing technology have revealed that 70% of the human genome is transcribed into RNA, with approximately 2.5% of that fraction translated into proteins through messenger RNA (mRNA).[5] These revelations have drawn attention to the vast amount of noncoding RNA (ncRNA) and led to efforts to ascertain their functions. It is now readily apparent that ncRNAs, including micro RNA (miRNA), piwi-interacting RNA (piRNA), long noncoding RNA (lncRNA), transfer RNA (tRNA), circular RNA (circRNA), small nucleolar RNA (snoRNA), and small nuclear RNA (snRNA), have important roles in mRNA processing, chromatin remodeling, gene silencing, protein synthesis, and transcriptional and translational regulation. The primary and most studied ncRNAs implicated in radiation research are lncRNA, miRNA, and circRNA; thus, this review focuses on the contributions of these three classes of RNA to the radiation response and their roles as diagnostic and prognostic biomarkers and therapeutic targets.

Long ncRNAs are characterized by transcript lengths of >200 nucleotides and lack of a clear protein coding region,[6] though recent studies indicate a limited potential to code for micropeptides in select lncRNAs.[7] With approximately twice as many lncRNAs as protein-coding genes in human and mouse genomes, many functions of lncRNA are not yet well understood.[6] Although more tissue-specific than mRNA, Derrien et al reported that 11% of lncRNAs are detected in all tissue types analyzed.[8] They also reported a generally lower expression level of lncRNAs compared to mRNAs. Cellular processes are influenced by lncRNAs through 4 main methods: imprinting, scaffolding for epigenetic and transcription factors, enhancer activation, and as molecular sponges.[6,9] Despite their vast roles, lncRNAs are poorly conserved between species by sequence.[6] However, lncRNAs may also be conserved by secondary structure, allowing different sequences to exert the same functions.[10] In circulation, lncRNAs have been reported in bodily fluids including serum, urine, saliva.[11] Figure 1 illustrates some of the ways lncRNAs interact with DNA, mRNA, proteins, and other ncRNAs to influence cellular processes. The significant involvement of lncRNAs in the innate immune response is reviewed by Hadjicharalambous and Lindsay.[12]

MicroRNAs are small single-stranded RNA transcripts of 21−25 nucleotides in length produced from hairpin loop precursors.[13] The primary method of miRNA action is through competitive partial binding with the 3' UTR of the target mRNA to inhibit translation and/or lead to mRNA degradation.[14] By binding complementary sequences, miRNAs have also been reported to interact with the 5' UTR, coding sequences, and gene promoters.[15] New miRNAs continue to be discovered, however there are several cases where the transcripts are highly conserved across species.[16-19] Specific to tissue and function the regulation of cellular processes by miRNA depends on the subcellular localization and the relative abundance of the miRNA and its target.[20] Additionally, miRNAs can be secreted into extracellular fluid and transported via exosomes or

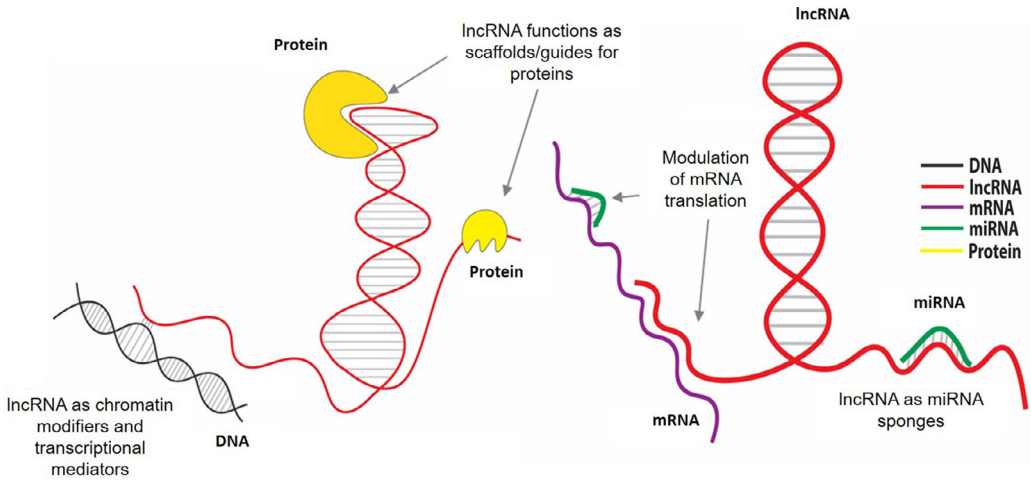

Fig. 1. Diagram representing the ways lncRNAs and miRNAs regulate cellular processes by binding mRNA, proteins, DNA, and other ncRNAs. lncRNA can pair with DNA to modify chromatin and transcription. lncRNAs can direct cellular processes by scaffolding/guiding for epigenetic or transcriptional factors. lncRNA and miRNA can both bind to homologous mRNA sequences to repress translation. Lastly, lncRNA can regulate miRNA by molecular sponging.

binding with proteins.[14] Previous studies have found detectable levels of miRNAs in serum, plasma, and urine and they are stable against degradation due to their small size and protection by exosomes.[21,22] In addition to showing the actions of lncRNA, Figure 1 also highlights how miRNAs may bind mRNA to modulate translation.

Unlike typical linear RNA, circRNA is a covalently closed loop and lacks both a 5' cap and 3' poly A tail.[23] Geng et al recognized five main characteristics of circRNA: abundance in eukaryotic organisms, stability due to the covalently closed loop structure that is protected from exonucleases, high conservation between species, primary cytoplasmic localization, and high tissue-specificity.[23] There are five categories of circRNAs: exonic, intronic, antisense, intragenic, and intergenic.[24] Cellular processes can be modulated by circRNA through sequestering miRNA, regulating transcription by binding RNA Polymerase II, competing with linear splicing, and in select cases, by being translated into peptides.[24] It is unclear if circRNA retains its function when not circularized. With approximately a 2.5-fold longer half-life than linear RNA, circRNA are resistant to exonuclease degradation and abundant in urine, plasma, saliva and gastric fluid.[25]

Pathophysiology of radiation damage. Early determination of the extent of radiation injury through ncRNA biomarker detection could provide a means to treat this damage early and potentially prevent late radiation-induced disease. Radiation induces direct and indirect damage, with radiation-induced double strand breaks considered the most lethal.[26] Direct damage results from deposition of energy directly onto DNA molecules, whereas indirect damage stems from deposition of energy in other molecules, which then react with DNA.[27] The 4 steps of radiation damage to intracellular molecules are as follows: (1) Physical deposition of energy, (2) Generation of primary radicals in the target molecules, (3) Reaction of primary radicals on adjacent molecules, and (4) Production of chemically stable damage by unstable radicals.[26] Additionally, mitochondria that are irradiated produce reactive oxygen species (ROS), another driver of DNA damage.[28] Historically, hydroxyl radicals have been considered the key species in DNA damage, however recent research has determined the key species is a carbonate radical anion formed in the Fenton reaction.[29] This carbonate radical anion is more abundant at physiological conditions than the hydroxyl radical and generates 8-oxo-7,8-dihydroguanine at GGG sites in DNA, which signals for DNA repair to begin.[29]

Effects of radiation are a function of dose with a threshold, above which injury is imminent and below which most people, cells, tissues, or animals experience little to no acute effects.[30] Radiation response is also determined by type of radiation source, volume of tissue irradiated, and time after irradiation. A cell's specific radiosensitivity is in part determined by DNA replication, recombination, and repair.[31] Double strand breaks are repaired via homologous recombination (HR) or nonhomologous end joining (NHEJ).[31] DNA damage response to double strand breaks includes protein recruitment, modifications such as histone phosphorylation, and activation of signaling pathways such as p53-p21.[28] The role of noncoding RNAs in DNA damage repair have been thoroughly reviewed by Thapar.[32] Ionizing radiation can also induce senescence in

normal cells, resulting in persistent cell cycle arrest or mutation leading to cancer or cell death.[28]

Severity of systemic effects depends on the volume of the body irradiated, exposure of critical organs, and dose received. In humans, whole body doses over 2 Gy can yield clinical symptoms of acute radiation syndrome, though symptoms, severity, and onset vary in populations.[33] Organ systems dependent on continuous cell turnover like the hematopoietic, pulmonary and gastrointestinal systems are affected sooner and more severely since they depend on frequent cell-divisions from stem cells and/or mucous membranes with rapid turnover of epithelial cells, which are destroyed by radiation.[34,35] Systems with terminally differentiated cells like cardiovascular and nervous systems exhibit fewer acute effects unless damage is supralethal.[36]

Acute effects on the skin are largely erythema but may also include destruction of sebaceous glands, hair loss, and hyperpigmentation at higher doses. Chronic effects on skin and mucosa include fibrosis and decreased vascularity.[30] In the musculoskeletal system, radiation may cause hypoplasia of facial bones in children, whereas adult bones are much more resistant. Interestingly, necrosis of muscle requires extreme doses exceeding 500 Gy.[30] In the gastrointestinal system, radiation results in the breakdown of the mucosal layer, which may present clinically as abdominal pain, diarrhea, vomiting, and malnutrition.[36] The hematopoietic system suffers significantly from whole or partial body irradiation, with widespread cell death occurring in bone marrow progenitors exposed.[36] Irradiation also leads to lymphocyte depletion within the first 48 hours, resulting in alterations of whole blood RNA expression patterns.[36] From studies on radiotherapy patients treated for thoracic tumors, it is known that radiation induces acute pneumonitis and chronic pulmonary fibrosis in the late stage.[37] In the cardiovascular system, radiation can induce pericardial disease, fibrosis, coronary artery disease (CAD), valvular injury and cardiac conduction system injury.[38] Chronically, the accumulation of genetic abnormalities from radiation-induced DNA damage can result in the development of leukemia approximately 10 years after exposure or other solid tumors up to 30 years after exposure.[39]

Of immediate concern is the development of acute radiation syndrome, which encompasses the damage to the hematopoietic, gastrointestinal, and cerebrovascular, and cutaneous symptoms that appear shortly after exposure.[36] Although analysis of clinical symptoms may provide some information on dose of radiation received, symptoms may not manifest for several days or weeks after exposure and have a range of severity, complicated by the possibility of psychological impacts.[36] Development of ncRNAs as a biomarker could potentially allow clinicians to determine preventative measures to treat radiation damage in both cancer patients and civilians exposed to radiation, mitigating symptoms such as nausea, vomiting, diarrhea, fibrosis, and anemia. Early intervention may also confer a survival advantage.[36]

ncRNAs in radiation response. With widespread roles in disease progression and as biomarkers for disease, efforts began to focus on the role of ncRNAs in radiation response. Specifically, many studies were undertaken to discern how ncRNAs can modulate the radiosensitivity in normal and cancerous tissues *in vitro*. Although certain chemotherapeutics differentially modulate radiosensitivity of healthy versus cancerous cells,[40] ncRNAs may have the same effect but further research is needed. Several examples of ncRNAs involved with radiation response are given in Tables 1-3. Additionally, it is now clear that cells respond differentially to single-dose and multi-fraction radiation providing unique opportunities for radiation combined with molecular therapeutics.[41] This section presents ncRNAs which have been shown to impact radiosensitivity of cells.

lncRNAs in radiation response. Table 1 highlights several lncRNAs that affect radiation response in a variety of tissue models, as well as the targets of the lncRNA and mechanism of action. Figure 2 illustrates the action and the effects of three lncRNAs, *HOTAIR, TUG1*, and *GAS5*, which have multiple targets described in the literature. In breast cancer models, the lncRNA *HOTAIR* has been shown to increase radioresistance through targeting of miR-218 and miR-449b-5p.[42,43] Hu et al showed that *HOTAIR* is upregulated in breast cancer cell lines over normal tissue and increases in expression after 8 Gy irradiation. Furthermore, they reported that knockdown of HOTAIR prevents sponging of miR-218, which sensitizes cells.[42] Zhang et al reported that *HOTAIR* sponges miR-449b-5p, which facilitates downstream chaperone protein HSPA1A expression and thereby enhances radioresistance.[43] This provides a potential therapeutic target for the treatment of breast cancer.

The lncRNA *TUG1* has been shown to impact radiosensitivity in multiple models via 2 targets: HMGB1 and miR-139-5p. *TUG1* and HMGB1 expressions were elevated in a bladder cancer model which was further increased in response to radiotherapy.[44] *TUG1* silencing led to enhanced radiosensitivity by inhibiting cell proliferation and promoting apoptosis.[44] In a prostate cancer model, Xiu et al described an upregulation of *TUG1*, downregulation of target miR-139-5p, and an upregulation of downstream target SMCA1.[45] Again, knockdown of *TUG1* enhanced radiosensitivity by decreasing the expression of downstream target SMCA1. Together, these studies demonstrate the importance of *TUG1* in the radiation response and its potential as a molecular target for radiosensitization.

Table 1. Roles of specific lncRNAs in the regulation of radiosensitivity and radioresistance. The specific lncRNA, target molecule, model, and its role in radiation response are described. *Italics* = lncRNA whose expression increases radioresistance; underlined = lncRNA whose expression increases radiosensitivity.

lncRNA	Target	Model	Notes	Reference
HOTAIR	miR-218	Breast cancer	HOTAIR is upregulated in BC cells, increases in expression after IR, knockdown sensitizes cells to IR by freeing miR-218	(42)
	miR-449b-5p	Breast cancer	HOTAIR enhances radioresistance by sponging miR-449b-5p, facilitating HSPA1A expression	(43)
TUG1	HMGB1	Bladder cancer	Knockdown of TUG1 enhances radiosensitivity by suppressing HMGB1	(44)
	miR-139-5p/SMC1A Axis	Prostate cancer	Knockdown of TUG1 enhanced radiosensitivity via miR-139-5p	(45)
GAS5	miR-106b/IER3 axis	Cervical cancer	Overexpression of GAS5 enhanced radiosensitivity of CC cells via up-regulating IER3 through miR-106b	(46)
	miR-205-5p and Wnt/β-catenin	Ovary granulosa tumor	GAS5 regulates apoptosis after IR by sponging miR-205-5p	(47)
WWC2-AS1	miR-16	Intestinal fibrosis	WWC2-AS1 regulates FGF2 expression by sponging miR-16, expression was increased after IR and contributes to fibrosis progression	(49)
linc-SPRY3-2/3/4	IGF2BP3	NSCLC	Radiosensitive male NSCLC cell lines demonstrated a dose-dependent induction of linc-SPRY3-2/3/4 following irradiation	(50)
NEAT1	miR-193b-3p/CCND1 axis	Cervical cancer	NEAT1 competitively binds miR-193b-3p to up-regulate the expression of cyclin D1	(51)
	NQO1	Triple negative breast cancer	NEAT1 is a positive regulator of NQO1, inhibition of NEAT1 caused increased radiosensitivity	(52)
ANCR	PTEN	Nasopharyngeal carcinoma	ANCR promotes NPC growth and radiation resistance through an epigenetic regulation of PTEN expression	(53)
ANRIL	miR-125a	Nasopharyngeal carcinoma	Downregulation of ANRIL enhances radiosensitivity by sponging miR-125a	(54)
H19	miR-193a-3p axis/PSEN1	Hepatocellular carcinoma	H19 sponges miR-193a-3p, which modifies radiosensitivity by targeting PSEN1	(55)
CCAT1	miR-148b	Breast cancer	Downregulation of CCAT1 enhances radiosensitivity by sponging miR-148b	(56)
LINC00963	miR-324-3p	Breast cancer	LINC00963 sponges miR-324-3p, enhancing ACK1 expression and radioresistance	(57)
PVT1	miR-195	NSCLC	Knockdown of PVT1 enhances radiosensitivity by freeing miR-195	(59)
UCA1	Akt pathway	Prostate cancer	UCA1 depletion inhibited cell growth and induces radiosensitivity	(60)
NKILA	NF-κB	Laryngeal cancer	Knockdown of NKILA results in radioresistance	(61)
lncGRS-1		Glioma	Knockdown of lncGRS-1 inhibits growth and proliferation and sensitizes cells to radiation	(62)

GAS5 has also been shown to enhance radiosensitivity in multiple models via 2 primary targets: miR-106b and miR-205-5p. In a model of cervical cancer, *GAS5* was shown to affect radiosensitivity by targeting miR-106b, which in turn is then unable to inhibit immediate-early response gene 3 (*IER3*), a suppressor of cervical cancer.[46] As a result, the overexpression of *GAS5* enhanced radiosensitivity. It is important to note that Gao et al found low levels of *GAS5* in cervical cancer cells,[46] and thus therapies to upregulate *GAS5* can be utilized as a radiosensitizer to increase treatment efficacy. Similarly, *GAS5* was found to modulate radiation response by targeting miR-205-5p, which in turn targets the Wnt/β-catenin pathway and regulates apoptosis.[47] *GAS5* was also shown to regulate p21, a well-known cell cycle regulator, in stomach cancer models.[48]

In a model of radiation-induced intestinal fibrosis, the lncRNA *WWC2-AS1* was shown to facilitate FGF2 expression by sponging miR-16.[49] Additionally, the expression of *WWC2-AS1* was upregulated after IR. By silencing *WWC2-AS1*, Zhou et al demonstrated inhibition of FGF2 associated proliferation, migration, invasion and fibrosis, raising the potential for *WWC2-AS1* silencers as treatment for radiation-induced intestinal fibrosis.[49]

In a study of male Y-chromosome related lncRNAs and radiation resistance, Brownmiller et al found that radiosensitive non-small cell lung cancer (NSCLC) lines differentially upregulated *linc-SPRY3-2/3/4* in a dose-dependent fashion.[50] On the contrary, radioresistant NSCLC lines did not demonstrate this induction and rather displayed a loss of Y chromosome. Loss of *linc-SPRY3-2/3/4* was negatively correlated with survival and thus *linc-SPRY3-2/3/4* may serve as a marker of radiotherapy efficacy.[50]

Han et al demonstrated the role of *NEAT1* in promoting radioresistance in a cervical cancer model.[51] *NEAT1* upregulation was found to upregulate cyclin D1 via sponging miR-193b-3p, which promotes radioresistance.[51] Recently, Lin et al described the role of *NEAT1* in regulating radioresistance by targeting NAD(P)H:quinone oxidoreductase 1 (NQO1).[52] They showed the positive regulation of NQO1 by *NEAT1* at the translational level in radioresistant triple negative breast cancer cells. By inhibiting *NEAT1*, they were able to trigger oxidative stress and increase the radiosensitivity of the cells,[52] giving a potential treatment strategy for resistant cancer phenotypes.

Table 2. Roles of specific miRNAs in the regulation of radiosensitivity and radioresistance. The specific miRNA, target molecule, model, and its role in radiation response are described. *Italics* = miRNA whose expression increases radioresistance; underlined = miRNA whose expression increases radiosensitivity.

miRNA	Target	Model	Notes	Reference
let-7	*Ras*	Lung cancer *in vitro*	Overexpression of Let-7 family miRNAs leads to radiosensitivity	(63)
	E2F2	LNCaP and PC3	let-7 miRNAs were upregulated by fractionated radiation in radiosensitive cell lines	(64)
miR-21	Apoptosis pathway	A549 cells	Downregulation sensitized cells to radiation	(65)
	PTEN	Mouse RIPF	miR-21 targets PTEN/Akt pathway to mediate IR-induced EMT	(66)
	hPDCD4, hPTEN, hSPRY2, and hTPM1	Human skin fibroblasts	miR-21 upregulated after radiation and regulates targets	(67)
miR-214	PRDX-6	Rat skin	Radiation induces miR-214 which suppresses PRDX-6 resulting in skin injury	(68)
miR-221	PTEN	CRC cells	miR-221 targets PTEN; increased PTEN lead to radiation sensitivity	(69)
miR-521	CSA	PC cells	Inhibition of miR-521 confers radioresistance	(72)
miR-222/miR-221	PTEN	Rad. Resistant tumor cells	miR-222 targets PTEN/Akt pathway to mediate radiosensitivity	(70)
miR-95	SGPP1	PC3 cells	Overexpression of miR-95 increased tumor growth and radiation resistance	(73)
miR-181a	Bcl-2	Glioma U87MG cells	Overexpression of miR-181a downregulated Bcl-2 and sensitized cells to radiation	(74)
miR-34a	TP53INP1	LNCaP and PC3 cells	Upregulated in response to fractionated radiation in radiosensitive cell lines	(64)
miR-17	MDM2	Glioblastoma	miR-17 was upregulated after irradiation and is a direct target of p53	(76)

Table 3. Roles of specific circRNAs in the regulation of radiosensitivity and radioresistance. The specific lncRNA, target molecule, model, and its role in radiation response are described. *Italics* = circRNA whose expression increases radioresistance.

circRNA	Target	Model	Notes	Reference
circRNA_100367	miR-217/Wnt3 pathway	Esophageal squamous cell carcinomas	Silencing circRNA_100367 inhibited the proliferation and migration by regulating miR-217/Wnt3 pathway, enhancing radiosensitivity	(78)
circPITX1	miR-329-3p/NEK2 axis	Glioma	CircPITX1 knockdown repressed viability, glycolysis and colony formation, promoted radiosensitivity	(79)
circTUBD1	miR-146a-5p/TRL4	Human hepatic stellate cells	Silencing circTUBD1 prevents inhibition of miR-146a-5p, which then targets TRL4 to increase apoptosis	(80)

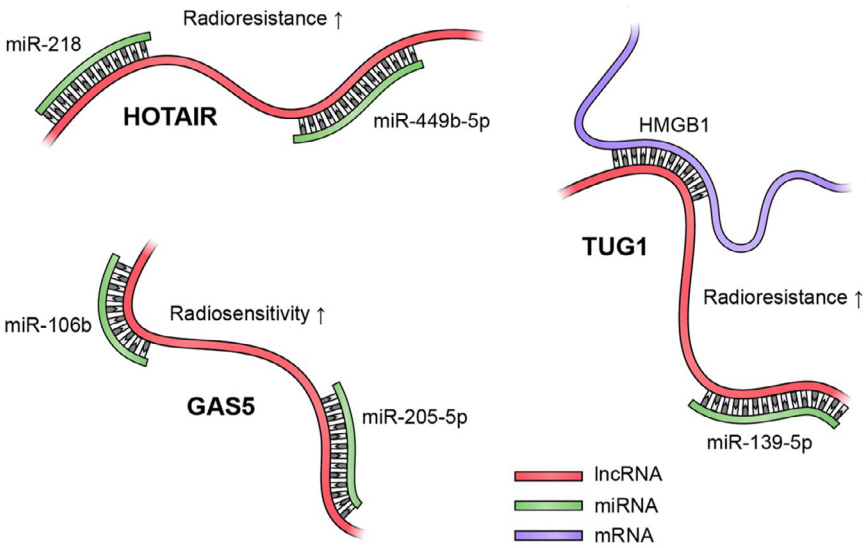

Fig. 2. Schematic depicting the method of 3 selected lncRNAs, *HOTAIR, TUG1*, and *GAS5*. *HOTAIR* has been shown to sponge miR-218[42] and miR-449b-5p[43] to increase radioresistance. *TUG1* has been shown to sponge miR-139-5p[44] and target the mRNA *HMGB1*[45] to increase radioresistance. *GAS5* has been shown to sponge miR-106b[46] and miR-205-5p.[47]

Ma et al demonstrated the role of *ANCR* in radioresistance of nasopharyngeal carcinoma (NPC).[53] *ANCR* was shown to be upregulated in NPC cells and by inhibiting *ANCR* with siRNA, they promoted radioresistance through epigenetic regulation of *ANCR*'s target, PTEN.[53] In a model of human NPC, downregulation of the lncRNA *ANRIL* was shown to enhance radiosensitivity by targeting miR-125a.[54] Ma et al reported on the lncRNA *H19*/miR-139a-3p/PSEN1 axis, which modifies radiosensitivity in liver cancer.[55] By silencing PSEN1, part of the gamma secretase complex, and/or using miR-139a-3p mimics, they revealed the role of PSEN1 expression in enhancing radiosensitivity.[55] In another study, *CCAT1* was shown to sponge miR-148b in a breast cancer model, and the downregulation of *CCAT1* was linked to enhanced radiosensitivity.[56]

Further examples exist of lncRNAs affecting radiation response by targeting a miRNA, which in turn targets a protein or mRNA. In a recent study, Zhang et al reported the role of *LINC00963* in breast cancer radioresistance.[57] By sponging miR-324-3p, *LINC00963* indirectly enhances nonreceptor tyrosine kinase ACK1 expression and therefore radioresistance.[57] ACK1 has been shown to be a tumor initiator and involved with tumor progression.[58] Similarly, Wu et al reported that the lncRNA *PVT1* targets miR-195, and the knockdown of *PVT1* enhances radiosensitivity of NSCLC cells by freeing miR-195.[59] The downstream target of miR-195 and mechanism of modulation remains to be seen.[59] In a study of prostate cancer cells, Ghiam et al demonstrated that the silencing or depletion of lncRNA *UCA1* inhibited cell growth and enhanced radiosensitivity by limiting the activation of the prosurvival Akt pathway.[60] In a model of laryngeal cancer, the knockdown of *NKILA* resulted in increased radioresistance by targeting NF-κB.[61] In a glioma model, Liu et al reported that the knockdown of *lncGRS-1* inhibited growth and proliferation and increased radiosensitivity.[62] The target or mechanism of *lncGRS-1* is unclear.

miRNAs in radiation response. Table 2 displays miRNA that have been shown to differentially respond to radiation or modulate radioresistance. The miRNA targets of lncRNA described in the previous section are also involved in radiation response for their role as targets, but this section will focus on literature centered on miRNA modulation. The miRNAs let-7 and miR-21 have been implicated in the radiation response in multiple studies. Weidhaas et al showed that *RAS* is the downstream target of let-7, and radiation induced a decrease in let-7 expression in an A549 lung cancer *in vitro* model with activated *RAS*.[63] As a result, let-7 was unable to target *RAS* and the radioresistance of the cells increased.[63] Alternatively, our group has shown an induction of let-7 in LNCaP and PC3 prostate cancer cells. Ingenuity Pathway Analysis (IPA, Qiagen) predicted E2F2 as one downstream target of let-7.[64] With let-7 upregulated after fractionated radiation, radiosensitivity of the cells increased via the predicted E2F2 pathway.[64]

It has also been reported that miR-21 is involved with radiation response in multiple studies. In A549 cells, a model for lung cancer, Wang et al showed that miR-21 was differentially upregulated after ionizing radiation.[65] This upregulation led to increased inhibition of the apoptosis pathway, which promoted radioresistance. Alternatively, they showed that downregulation of miR-21 sensitized cells to radiation.[65] In 2 separate studies, miR-21 was shown to be upregulated after radiation exposure, and that PTEN is a downstream target of miR-21.[66,67] Upon irradiation with a single dose of 20 Gy, acute or chronic low dose of 10 cGy, and a moderate dose of 400 cGy, miR-21 expression inhibited PTEN and increased ionizing radiation-induced epithelial-to-mesenchymal transition (EMT).[66,67] Although not directly related to radiosensitivity, the induction of EMT represents a response to radiation.

Several other miRNAs, including miR-214, miR-221, miR-521, miR-222, and miR-34a, have also been shown to impact radiation response. In a model of radiation-induced skin injury in rats, Zhang et al reported the induction of miR-214, which targets the peroxiredoxin PRDX-6, thereby inducing skin injury.[68] Another inhibitor of PTEN, miR-221 is also implicated in the radiation response of tissues. In colorectal cancer cells, Xue et al showed that miR-221 targeted PTEN, and silencing miR-221 via anti-miR-221 increased PTEN expression and lead to radiosensitivity.[69] Similarly, miR-222 was also found to target PTEN to mediate radiosensitivity in radiation-resistant tumor cells.[70] The miR-221/222/PTEN axis represents a potential molecular target for sensitization to radiation. Although PTEN has been shown as a target of multiple miRNAs in radiation response, how these miRNAs interact with each other is unclear. The regulation of PTEN by ncRNAs is reviewed by Li et al.[71] Figure 3 illustrates the regulation of PTEN by miR-21, miR-221, and miR-222 following radiation and the relevant response.

In a study of radiation resistance in prostate cancer cells, miR-521 was found to target DNA repair protein CSA, and the inhibition of miR-521 led to radioresistance.[72] Similarly, in PC3 prostate cancer cells, overexpression of miR-95 was found to correlate with increased tumor size and lead to enhanced radioresistance by targeting the sphingolipid phosphatase SGPP1.[73] Chen et al also recognized the impact of miR-181a on radiosensitivity.[74] In glioma cells, they found that overexpression of miR-181a lead to increased radiosensitivity by targeting Bcl-2, an apoptosis regulator.[74] In our group, we have begun to describe the role of miR-34a in radiation response,

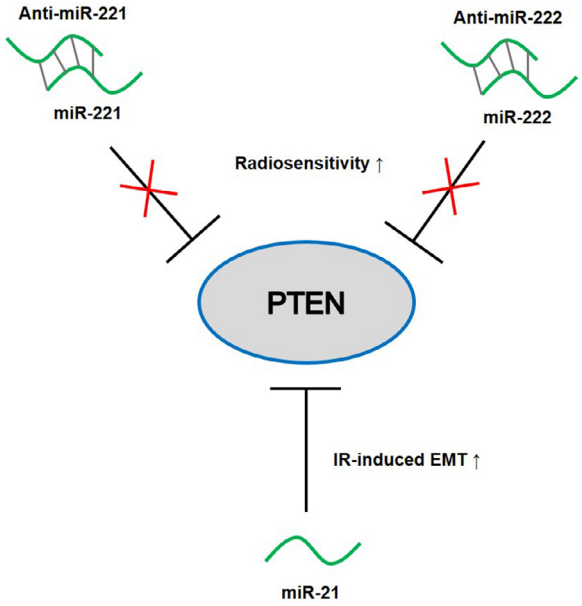

Fig. 3. Pathways of miRNA regulation of PTEN. miR-21, miR-221, and miR-222 target PTEN to decrease its expression. By utilizing anti-miR-221 and anti-miR-222, the expression of miR-221 and miR-222 was decreased, preventing the inhibition of PTEN and resulting in increased radiosensitivity.[69,70] Another study has shown that ionizing radiation induces miR-21 to target PTEN, which leads to radiation-induced epithelial to mesenchymal transition (EMT),[66] representing another response to radiation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

recognizing that it is upregulated in response to fractionated radiation in radiosensitive LNCaP and PC3 cell lines.[64]

It has been reported that miR-17 is a direct target of p53 in response to cellular stress.[75] Additionally, evidence exists that the miR-17 family is upregulated following irradiation in glioblastoma cells.[76] In another study, miR-17 was shown to repress the p53 regulator MDM2, which resulted in decreased cellular proliferation.[77] Given its role in the p53 pathway and role in cellular proliferation, the miR-17 family is an example of an attractive target for modifying the radiation response of cells.

circRNAs in radiation response. Out of the 3 primary classes of ncRNA this review focuses on, circRNAs have been the least studied. Table 3 summarizes the 3 reported examples of circRNAs in radiation response and Figure 4 illustrates the relevant pathways. In one case, the Wnt3 pathway is a known target of miR-217.[78] Liu et al demonstrated in an esophageal squamous cell cancer model that circRNA_100367 can sponge miR-217 to increase radioresistance by promoting Wnt3 expression.[78] Alternatively, silencing circRNA_100367 inhibited cellular proliferation and therefore increased radiosensitivity.[78] This provides evidence for the potential to treat tumors by molecular sensitization using circRNAs as a target or as a marker to delineate a target.

Similarly, circPITX1 was found to target miR-329-3p, which in turn targets a kinase involved in mitosis, Nek2.[79] In a glioma model, Guan et al demonstrated that by silencing circPITX1, its target miR-329-3p inhibited Nek2, resulting in repressed viability, glycolysis and colony formation and thus increased radiosensitivity.[79] Recently, Niu et al described the role of circTUBD1 in radiation-induced liver disease. circTUBD1 normally regulates apoptosis and proinflammatory cytokines by targeting miR-146a-5p, which in turn targets TRL4, a transmembrane protein.[80] In a human hepatic stellate cell model, Niu et al silenced circTUBD1, which promoted miR-146a-5p and therefore decreased TRL4 expression.[80] This resulted in increased apoptosis and proinflammatory cytokines. Niu et al also demonstrated the upregulation of circTUBD1 after irradiation,[80] which provides rationale for targeting circTUBD1 with a silencer during radiation treatment to improve efficacy. Although the understanding of the roles of circRNAs in radiation response is in its infancy, the complex regulatory pathways involving miRNAs, proteins, and other ncRNAs represent a bright future for possible therapeutics.

ncRNAs as biomarkers of radiation damage. Several ncRNAs have been proposed as diagnostic and prognostic biomarkers of radiation damage to normal tissue, whether in murine, *in vivo*, or nonhuman primate models. Radiation damage can stem from accidental or intentional mass exposure, incidental damage to adjacent tissues in radiotherapy, or industrial sources. In radiotherapy, biomarkers may be organ-specific and used to determine the success of radiotherapy treatment. In emergency scenarios, biomarkers may be used to diagnose the extent of body exposure, dose received, and prognosis. The expression patterns of mRNA after radiation exposure have been extensively studied compared to ncRNA and have been excellently reviewed by Lacombe et al.[81] This section highlights ncRNA molecules that are differentially expressed in normal tissue after irradiation, which can then be correlated with dose and mortality to potentially diagnose radiation damage in a scenario where actual dose received is unknown.

lncRNA biomarkers. With significant involvement in the regulation of radiation response via sponging of miRNA and interacting with proteins, it is expected that lncRNAs could have roles as diagnostic and prognostic biomarkers of damage. The upregulation of *Trp53cor1, TUG1, Dino, Meg3, Morrbid, Tp53tg1, FAS-AS1, PARTICLE* and *PAPPA-AS1* and the downregulation of *Tmevpg1* have been observed as described below. Three of these markers, *Trp53cor1, TUG1*, and *MEG3*, have

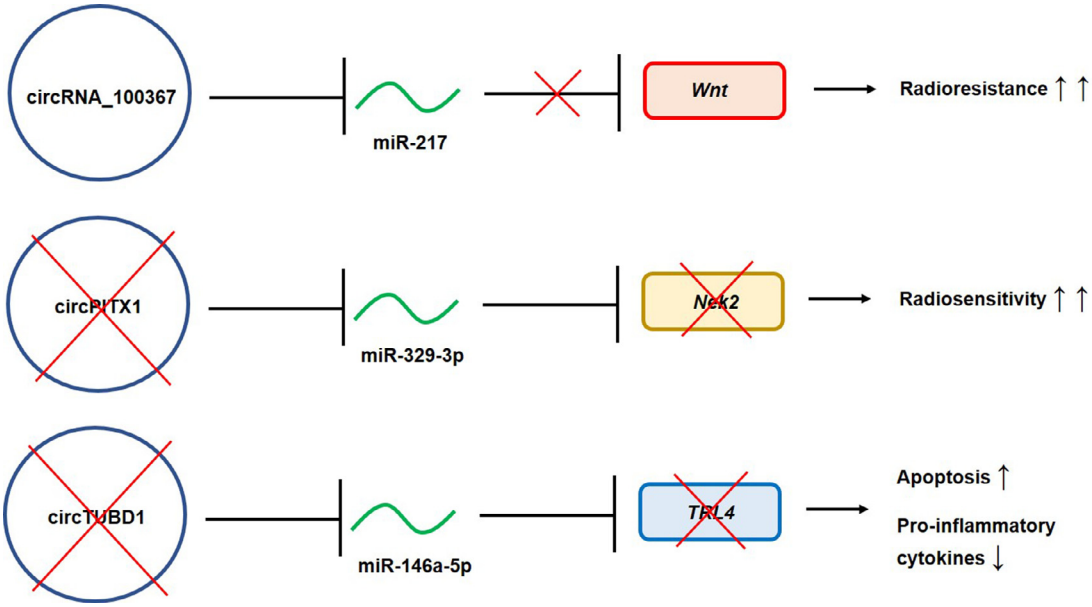

Fig. 4. Schematic depicting the pathways of 3 circRNAs, circRNA_100367, circPITX1, circTUBD1, in modulating radiation response. circRNA_100367 sponges miR-217, which prevents miR-217 from targeting the Wnt pathway resulting in increased radioresistance.[78] By silencing circPITX1, its target miR-329-3p can target Nek2 and results in increased radiosensitivity.[79] By silencing circTUBD1, which is upregulated after irradiation, its target miR-146a-5p can target TRL4, which leads to increased apoptosis and decreased proinflammatory cytokines.[80] (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

been validated in multiple studies involving different models of radiation exposure.

The upregulation of *Trp53cor1, TUG1*, and *MEG3* has been reported by Beer et al[82] as well as our own group.[83] In a model of human peripheral blood mononuclear cells *ex vivo,* Beer measured lncRNA expression using microarray analysis 20 hours after exposure to a single dose ranging from 0.9-60 Gy. They reported significant upregulation of *TRP53COR1* at high doses of 15 and 60 Gy.[82] Similarly, in a whole-body irradiated mouse model, we showed dose-dependent upregulation of *Trp53cor1* at 48 hours postirradiation of doses of 1, 2, 4, 8, and 12 Gy.[83] These results were further validated in mouse embryonic fibroblasts and yielded similar results. Interestingly, we showed concomitant induction of *Dino* and the mRNA *Cdkn1a*, which are both located on chromosome 17, giving evidence of the induction pathways of p53-linked DNA damage.[83]

The same two studies also showed the upregulation of *TUG1* and *MEG3*. In the *ex vivo* human PBMCs, Beer et al showed a significant upregulation of *TUG1* at 15 and 60 Gy,[82] while Aryankalayil showed a dose-dependent increase in *Tug1* expression in whole-body irradiated mice.[83] Additionally, *TUG1* was previously found to be upregulated in bladder cancer tissue and further upregulated in the cancerous cell lines after exposure to radiation.[44] In both the PBMC and whole-body mouse models, *MEG3* was shown to be upregulated at high doses of >30 Gy and 12 Gy, respectively.[82,83] Induction at these large single doses of *ex vivo* blood irradiation indicates active radiation response. Further data and investigation are required from *in vivo* studies to understand the potential for *MEG3* to serve as a marker of very high doses of radiation, which can assist in an emergency scenario to identify individuals who are unlikely to improve with treatment.

Our lab also showed induction of lncRNA targets of p53 *Dino* and *Pvt1,* as well as other lncRNAs *Morrbid* and *Tmevpg1*. *Dino* yielded significant dose-dependent upregulation while *Pvt1* and *Morrbid* were upregulated across all time-points and doses, but not in a uniform or dose-dependent fashion,[83] representing possible markers for differentiating exposed vs. unexposed patients. Additionally, *Tmevpg1* was dose-dependently downregulated in response to radiation in this same mouse model, which represents the only significant dose-dependent downregulation of a lncRNA in the literature.[83]

In a human cultured T lymphocyte model exposed to 2 Gy single-dose irradiation, Kabacik et al observed the weak upregulation of *TP53TG1* at time-points within the first 24 hours, peaking at a 1.5-fold upregulation normalized to the HPRT1 reference gene.[84] In the same study, the authors also observed an up to 5-

fold upregulation of *FAS-AS1* within the first 24 hours of radiation exposure.[84] This study also examined protein-coding gene and miRNA changes as well.

In cultured human epidermal cells, Kim et al reported several lncRNAs up- and downregulated in response to UVB irradiation, with 20 up- and 21 downregulated also in nonmelanoma skin cancers.[85] As other studies focused on gamma rays, x-rays, or other high energy sources, this study demonstrated the ability of lower energy waves to induce lncRNA expression changes. Gene ontology associations predicted regulation of gene transcription was upregulated while tumorigenesis was downregulated.[85] In a mouse model, Gao et al reported the differential expression of lncRNAs in thymocytes at low doses (TBI, 0.075 Gy) vs high doses (TBI, 4 Gy).[86] In the high dose group, 36 lncRNAs were upregulated while 17 were downregulated. By comparison, only one lncRNA (Gm7816) was downregulated in the low dose group and no upregulated lncRNAs were statistically significant.[86]

Furthermore, 2 additional lncRNAs have been reported as upregulated in response to ionizing radiation. In various cell lines, O'Leary et al found transient upregulation of the lncRNA *PARTICLE* in response to low-dose irradiation.[87] In human PBMC cells from healthy human donors, Macaeva et al showed that the lncRNA *PAPPA-AS1* is among the best genes to differentiate between 0, 0.1, and 1.0 Gy irradiation.[88] The role of lncRNAs as biomarkers of radiation damage represents a potential avenue for further exploration with their complex regulatory roles and so many transcripts yet to be fully annotated.

miRNA biomarkers. miRNAs have received significantly more attention as potential biomarkers of radiation damage than their lncRNA counterparts. Several miRNAs have shown consistent regulation patterns (either upregulated or downregulated) in multiple studies, including let-7g, miR-29c, miR-20a, miR-21, miR-16, miR-17, miR-126, miR-34a-5p, miR-34b-3p, and miR-21 as consistently upregulated and miR-150 in at least four studies as consistently downregulated. A summary of the relevant studies and significant findings is presented in Table 4.

In one of the earliest studies of miRNA response to radiation, Wagner-Ecker et al reported an upregulation of let-7g, miR-16, miR-20a, miR-21 and miR-29c and a downregulation of miR-18a, miR-125a, miR-127, miR-148b, miR-189 and miR-503 at 6 hours post-2 Gy irradiation in human dermal microvascular endothelial cells.[89] In two other earlier studies by the same group, the response of miRNA in mice exposed to γ-rays or ^{56}Fe ion radiation and in peripheral blood cells of radiotherapy patients were analyzed. In the murine model study, total-body irradiated mice showed different expression patterns from γ-rays or ^{56}Fe ions when comparing equitoxic doses, indicating molecular response to radiation is source-dependent.[90] Dose-dependent upregulation patterns at 6 hours post-γ-irradiation were observed for miR-135a, miR-147, miR-680, and miR-685, while miR-150 was the only miRNA differentially expressed in both sources of radiation.[90] In the radiotherapy patient model (patients in remission 1 or 2 for mantle cell lymphoma, acute myelogenous leukemia, or acute lymphoblastic leukemia), peripheral blood samples were collected from patients preparing for stem cell transplantation undergoing 1.25 Gy total-body irradiation 4 hours after treatment. Microarray analysis of 8 patient's samples compared to their control samples prior to radiation treatment revealed statistically significant upregulation of miR-21, let-7g, miR-29c, miR-20a, miR-16, and miR-17, plus 20 others significantly upregulated in every patient.[91]

Further early studies of mouse serum miRNA expression revealed the significant downregulation of miR-150 within 48 hours of total-body irradiation in a dose-dependent fashion.[92] Jacob et al showed the efficacy of miR-150 to diagnose radiation exposure, noting that miR-150 is abundant in lymphocytes, which are depleted by radiation and bone marrow damage.[92] In another study using C57BL/6J male mice exposed to TBI, Acharya et al demonstrated the upregulation of miR-30a-3p and miR-30c-5p and the downregulation of miR-187-3p, miR-194-5p, and miR-27a-3p.[93] Additionally, by measuring expression levels of these markers, they were able to distinguish between mice exposed to lethal (8 Gy) and sublethal (6.5 Gy) doses at 24 hours postexposure.[93]

In a study of organ-specific biomarkers of radiation damage, Lu et al examined liver samples of mice 14 days after 4 Gy TBI irradiation and found 18 miRNAs significantly upregulated and 9 miRNAs significantly downregulated, including miR-34a-5p, miR-34b-3p both upregulated.[94] This study provides evidence for the unique expression patterns and pathways that different organs utilize to respond to radiation. In a study utilizing large animal models, Menon et al were able to differentiate between lethal (6.5 Gy) and sublethal (1 and 3 Gy) doses in nonhuman primates exposed to TBI within the first 7 days. Consistent with previous biomarker and lymphocyte depletion studies, miR-150-5p was reported to decrease after irradiation.[95] They reported a dose-dependent increase of miR-574-5p at 24 hours, with returns to baseline levels by day 3, offering a potential early marker of exposure. Additionally, an increase in miR-126, miR-144, miR-21, miR-1-3p and miR-206 at days 3 and/or 7 was also observed.[95]

Table 4. List of miRNAs reported to be up- or downregulated categorized by study. The miRNAs and their respective regulation pattern, the model, and dose and timing notes are included.

Upregulated	Downregulated	Model	Dose/Timing Notes	Reference
let-7g, miR-16, miR-20a, miR-21 and miR-29c	miR-18a, miR-125a, miR-127, miR-148b, miR-189 and miR-503	Human dermal microvascular endothelial cells	RNA collected 6 hours after 2 Gy irradiation	(89)
miR-135a, miR-147, miR-680, miR-685		C57Bl/6 mice, TBI	miRNA are dose and radiation-type dependent, those miRNAs showed dose dependent expression	(90)
miR-21, let-7g, miR-29c, miR-20a, miR-21, miR-16, miR-17, plus 20 others significant upregulated		Human blood, TBI	Blood collected 4h after TBI of 1.25 Gy for patients in remission 1 or 2,	(91)
miR-200b and miR-762	miR-150	CBA/J and C57BL/6 male mice, TBI	At 24h and 48h post-exposure, serum miRNA have dose-dependent changes; miR-150 levels can indicate lymphocyte depletion	(92)
miR-30a-3p and miR-30c-5p	miR-187-3p, miR-194-5p, and miR-27a-3p	C57BL/6J male mice, TBI	These miRNAs could differentiate between lethal (8 Gy) and sublethal (6.5 Gy) dose at 24h	(93)
miR-34a-5p, miR-34b-3p, plus 16 other significant upregulated	9 miRNAs downregulated	Kunming mice, liver samples, TBI	Liver samples of mice 14 days after 4 Gy irradiation revealed 18 miRNAs upregulated and 9 miRNAs downregulated	(94)
miR-574-5p (at 24h); miR-126, miR-144, miR-21, miR-1-3p and miR-206 (Day 3 and/or 7)	miR-150-5p	Non-human primates, TBI	Differentiated between lethal (6.5 Gy) and sub-lethal (1 and 3 Gy) doses	(95)
miR-133a/b, miR-375, miR-30a, miR-126	miR-215, miR-150	Non-human primates, midline dose	Single dose 5.8, 6.5, or 7.2 Gy, can differentiate unexposed vs exposed and predict radiation induced mortality	(96)
miR-204-5p, miR-92a-3p and miR-31-5p		Healthy donor plasma *ex vivo*	Significantly increased in plasma exosome-like vesicles exposed to 2 Gy SD after 24 h	(97)
miR-223-3p, let-7b-5p, miR-574-5p	miR-140-5p, let-7f-5p, miR-150-5p, miR-17 family	C57BL/6 female mice, TBI	Maximum number of miRNAs were altered at 24-h and 48-h time-points post-irradiation, miR-17 family was repressed	(98)
miR-17, miR-128, miR-15b		Baboons, TBI and PBI	At doses of 2.5 and 5.0 Gy, these miRNAs were able to discriminate between TBI and PBI	(99)
20 miRNAs upregulated	7 miRNAs downregulated	Sprague-Dawley (SD) rats, SD esophageal radiation	20 miRNAs were upregulated and 7 miRNAs were downregulated after irradiation of 5 or 20 Gy	(100)
miR-34a-5p (Day 5, both), miR-34b-3p (Day 2, C57BL/6 only), miR-802-5p (Day 2, C57BL/2 only)	miR-199a-5p (Day 5, C57Bl/6 only), miR-199b-5p (Day 2, C3H), miR-150-5p (Day 2 and 5, C3H), miR-122-5p (Day 5, C57BL/2 only)	C3H (13.92 Gy) and C57BL/6 (13.99 Gy) mice, whole thorax irradiation	miR-34a-5p, -100-5p, and -150-5p predicted survival in C3H mice; miR-34b-3p, -96-5p, and -802-5p predicted survival in C57BL/6 mice	(101)
	miR-150-5p	C57BL/6 mice, TBI	Normalized to miR-23a-3p, dose reconstruction of ±0.5 Gy within first 7d	(102)
hsa-miR-185-5p, hsa-miR-107, hsa-miR-126-3p, hsa-miR-144-3p, hsa-miR-17-5p, hsa-miR-185-5p, hsa-miR-20b-5p, and hsa-miR-5194	hsa-miR-3180, hsa-miR-4730, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-223-3p, and hsa-miR-451a	Human blood, *ex vivo*	24 hours after exposure, miRNAs could distinguish 0.5, 1, and 5 Gy irradiation from control, while no miRNAs were able to significantly differentiate 2.5 Gy vs 0 Gy	(103)

Fendler et al also used nonhuman primates in a biomarker study, utilizing mid-line doses of 5.8, 6.5 and 7.2 Gy.[96] They found that three miRNAs (miR-133b, miR-215, and miR-375) can accurately identify NHPs exposed to radiation versus unexposed NHPs 24 hours after exposure.[96] Additionally, two miRNAs (miR-30a and miR-126) were able to accurately predict mortality. All observed markers were upregulated except miR-215.[96] They also observed a downregulation of miR-150, consistent with previous studies. In a human *ex vivo* plasma model from healthy donors, Yentrapalli reported a significant upregulation at 24 hours of miR-204-5p, miR-92a-3p and miR-31-5p in exosome-like vesicles exposed to a single dose of 2 Gy.[97]

In a study published by our group in 2018, we reported the upregulation of miR-223-3p, let-7b-5p, and miR-574-5p and the downregulation of miR-140-5p, let-7f-5p, miR-150-5p, and the miR-17 family.[98] Using female C57BL/6 mice exposed to 2-15 Gy TBI, we observed a maximum number of miRNAs altered within the first 48 hours.[98] Additionally, an inverse correlation between miR-17 family members and their targets was observed and validated in nonhuman primates.[98] Ostheim et al demonstrated the ability of miR-17, miR-128, and miR-15b to diagnose TBI or partial body-irradiation in a baboon model.[99] By observing the upregulation of these three miRNAs in response to 2.5 Gy and 5 Gy TBI and PBI, these markers were highly diagnostic of percentage of body exposed.[99] Luo et al studied single dose esophageal radiation in Sprague-Dawley rats, reporting 20 miRNAs upregulated and 7 miRNAs downregulated not observed in the other studies presented.[100]

In the plasma of C3H (13.92 Gy) and C57BL/6 mice exposed to whole thorax irradiation, Rogers et al demonstrated the upregulation of miR-34a-5p (day 5, both strains), miR-34b-3p (day 2, C57BL/6 only), and miR-802-5p (day 2, C57BL/2 only) and the downregulation of miR-199a-5p (day 5, C57Bl/6 only), miR-199b-5p (day 2, C3H only), miR-150-5p (days 2 and 5, C3H only), and miR-122-5p (day 5, C57BL/2 only).[101] Additionally, they showed that miR-34a-5p, miR-100-5p, and miR-150-5p forecasted survival in C3H mice while miR-34b-3p, miR-96-5p, and miR-802-5p forecasted survival in C57BL/6 mice.[101] Although the miRNAs differed between strains, KEGG analysis predicted MAPK signaling, regulation of actin cytoskeleton, and fatty acid metabolism were altered in both strains. This study demonstrated important differences between models used and considerations for translation

into human models. In a recent study, Yadav et al also demonstrated the ability of the downregulation of miR-150-5p to reconstruct dose within ±0.5 Gy in the first 7 days by normalizing to miR-23a-3p levels. This study utilized blood from C57BL/6 mice exposed to a range of doses.[102] In a model of human blood exposed *ex vivo* to doses of 0.5, 1, 2.5 and 5 Gy ^{60}Co irradiation, Lee et al described changes in miRNA expression that could be used to distinguish each dose from control samples.[103] However, *in vivo* animal studies must be performed to develop clinical biomarkers.

circRNA biomarkers. As the least studied of the three primary types of ncRNAs, the study of circRNAs as biomarkers of radiation damage to normal tissue is in its infancy. Zhang et al exposed female BALB/c mice to 7 Gy TBI and monitored the expression levels of circRNA in mouse bone marrow stromal cells, reporting the significant downregulation of mmu_circRNA_008488, mmu_circRNA_000551 and mmu_circRNA_005365 and the significant upregulation of mmu_circRNA_011235 and mmu_circRNA_016901, upon validation in further experiments.[104] The role of circRNAs as biomarkers represents a promising future area of study.

FUTURE DIRECTIONS

Druggable ncRNA targets. Attempts to increase radiation sensitivity by exploiting ncRNA targets have only been performed in cancer cell models. For example, Huang et al developed a small interfering RNA (siRNA) to target HIF-1α in PC3 prostate cancer cells and found the siRNA group had elevated radiosensitivity.[105] Similarly, Wang et al developed a short hairpin RNA (shRNA) that targeted HDAC1 mRNA in thyroid cancer cells to increase radiosensitivity.[106] Future research should focus on inducing or suppressing ncRNAs to increase radioresistance in normal tissue, as well as the development of ncRNA radiosensitizers for clinical radiotherapy applications. The role of ncRNA as radiosensitizers is a future aim for exploration. It is unclear how these may be administered; however, research should focus on cancer cell targeted therapies to mitigate potential harm to normal tissue by systemic modulation. With the complex ncRNA pathways described throughout this review, there are ample opportunities for ncRNAs to be explored to modulate radiosensitivity. Additionally, the connection of lncRNAs, immune response, and cancer is beginning to be explored, which represents another potential modality for ncRNAs as therapies.[107] The Atlas of Non-Coding RNA in Cancer (TANRIC, https://www.tanric.org), provides an interactive platform of ncRNAs in cancer of biomedical synthesis, which could be explored for druggable targets to affect radiosensitivity. Alone, siRNA and anti-sense oligonucleotides (ASOs) may be limited due to specificity to each patient and multiple mutations in tumors that may continue to evolve but offers a precision medicine approach combined with additional modalities such as radiation.

Interventional silencers for miRNAs have only recently begun to be studied in early clinical trials and are excellently reviewed by Hanna et al.[108] The miRNA targeted include miR-34, miR-92, miR-16, miR-122, miR-29, miR-21, and miR-155 and aim to improve liver cancer, lymphoma, melanoma, wound healing, heart failure, lung cancer, mesothelioma, hepatitis C, scar formation, Alport syndrome and T-cell lymphoma.[108] Synthetic *in vitro* transcribed lncRNAs (SINEUPs) also have potential as RNA therapies to target mRNA to upregulate their target.[109] In a recent study, these were employed to upregulate EGFP and Sox9 mRNA.[109] Further exploration will yield more mRNA targets that can be exploited to modulate radiation response. Although circRNAs have not been studied clinically as a targeted therapeutic, they offer the advantage of multiple miRNA binding sites per loop, making them ideal for inhibiting oncogenic miRNAs.[110] Further studies should focus on deploying naturally occurring circRNAs or designing synthetic circRNAs to target downstream miRNAs involved with the radiation response.

Interfering RNAs (RNAi) have been employed in other diseases and genetic conditions as a potential treatment or cure, with over 30 investigated in clinical trials and 8 of those advancing to Phase III trials.[111] Of these therapeutics, the targeted conditions range from hypercholesterolemia, cardiovascular disease, and hepatitis B to various solid and non-solid tumors.[111] RNAi technology does not suffer from the permanent genome editing challenges of emerging technologies like CRISPR-Cas9; like other drugs, it will eventually break down and be excreted.[112] This breakdown brings its own challenges, and RNAi molecules in the bloodstream face degradation from nucleases, clearance by the kidneys, difficulty crossing cell membranes, and potential triggering of immune response.[111] Even if RNAi molecules can successfully infiltrate the target cells/tissue, they also have the possibility to affect off-target molecules with sequence homology and trigger unintended consequences.[111] Delivery systems such as lipid nanoparticles, Dynamic PolyConjugates and linkage to target antibodies are among the most promising candidates to protect RNAi molecules.[111] These could potentially be adapted as prophylactic radiosensitizers prior to radiotherapy or radiation mitigators in response to a mass exposure.

RNA markers for biodosimetry – bench to clinic. RNA biomarkers of radiation damage must be verifiable,

detectable, sensitive, specific, and have strong correlations with certain characteristics (eg, dose, mortality, exposure vs. no exposure). Biomarkers of radiation damage face a significant challenge of translating results into humans because it is unethical (or not feasible) to gather clinical human data besides patients already undergoing radiotherapy, as Templin et al did.[91] One potential method our group is investigating is a novel approach to assessing human normal tissue biomarkers by employing a human organ-on-a-chip model, which allows for the coculture of two or more cells types that can exchange signals and fluids as real tissues do to emulate human physiology *in vitro*. The challenges, potential, future, and limitations of organ-on-a-chip models have been reviewed by Maschmeyer et al.[113]

Given the complexity of pathways involved with radiation response, biodosimetry may include several specific markers across multiple types of noncoding or coding RNA. For example, our group has previously demonstrated the concomitant induction of *Trp53cor1, Cdknla* and *Dino* after radiation in mice, which stems from the p53's DNA damage response affecting close-proximity genes on chromosome 17.[83] Additionally, the low baseline expression of *Trp53cor1* makes it an excellent candidate marker to distinguish significantly exposed vs unexposed. Once triaged into those two categories, additional markers could further distinguish extent of exposure. Equally important, a set of biomarkers must have some normalizing control marker since it would not be feasible to obtain baseline RNA expression values for all victims *before* a nuclear incident occurs. We have previously used mRNA *Rplp0* as a control[83]; Yadav et al normalized miRNA levels to miR-23a-3p.[102] An effective RNA biomarker signature may draw upon the strengths of miRNA, lncRNA, circRNA, and mRNA to improve diagnostic and prognostic capabilities.

A decision analysis approach is being considered that might be better able to guide medical providers in an emergency scenario as acute radiation syndrome symptoms develop. To efficiently test the population, technology will need to be developed to provide rapid and accurate results. Traditional diagnostic methods like PCR or microarray could prove useful but may struggle in a mass exposure scenario with a large influx of tests required at once and limited trained laboratory personnel and equipment, as happened during the COVID-19 pandemic.[114] For this reason, lab-on-a-chip devices are being considered (eg, DxTerity) Similar devices have previously been reported for diagnosis of other diseases including coronaviruses,[115] tuberculosis,[116] and breast cancer.[117] Yadav et al reported their radiation-induced miRNA expression results using a single drop of mouse blood,[102] which provides a promising starting point toward a minimally invasive microfluidic device that can accurately characterize radiation dose, prognosis, and mortality.

As with all biomarkers, the ability of ncRNA to determine biological damage and necessary medical response must be understood within context. Recently, we published a review summarizing the differences in radiation-induced adaptive responses based on fractionation of doses and highlighted the intended workflow of using RNA biomarkers for detection of radiation damage.[41] Reliance on a single marker can misdiagnose dose or time of exposure as expression levels of ncRNAs are dynamic and blood or serum samples merely represent a snapshot of patient's current transcriptional state.[41] In a nuclear weapon incident or radiotherapy, time of event and subsequent exposure may be well known; however, time of exposure may be less clear in occupational exposures or in the case of radioactive material exposures, so developing time-dependent markers as well as dose-dependent markers is critical. Equally important, biomarkers must be independent of confounding diseases: studies highlighted above described the up- or downregulation of many ncRNAs in cancerous tissues, and biomarkers must make this important distinction to accurately treat the condition. Because of this, it becomes even more critical to rely on a multi-marker signature which would not be limited by confounding expression. For example, Coleman et al demonstrated how a single biomarker, mmu-let-7e-3p, could be upregulated at certain timepoints and downregulated at others for the same dose,[41] rendering it unable to diagnose damage alone. Figure 5 is an expansion of a previously published figure[118] outlining the process by which medical responders will triage patients in a nuclear scenario and provides an illustration of how molecular biomarkers and ncRNAs can be utilized to triage patients and guide medical treatment.

Studies on the effects of different physiological conditions on ncRNA expression profiles after irradiation are also warranted to fully understand radiation damage markers. In a recent study, Fu et al reported on the effects of radiation and microgravity on the ncRNA and mRNA expression profiles of human lymphoblastoid cells,[119] simulating conditions astronauts experience in space where radiation absorbed is greater than on Earth. After exposure to 2 Gy γ-irradiation and incubation for 24h in simulated microgravity conditions, they reported that microgravity and irradiation additively affect RNA expression patterns. Further research on how additional factors including diet, age, and presence of other diseases impact radiation response of ncRNA biomarkers is warranted.

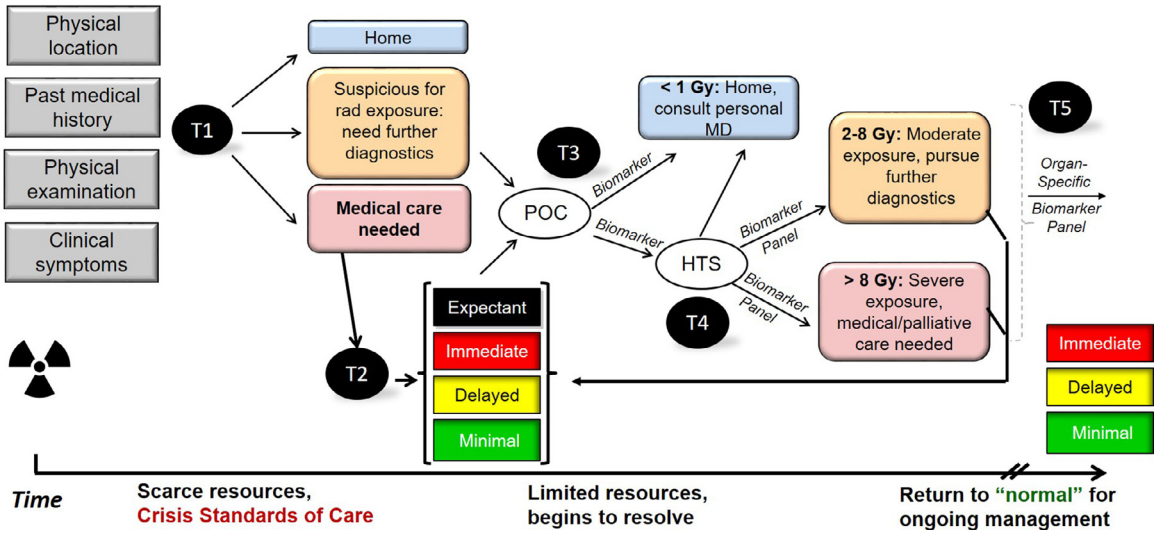

Fig. 5. Application of molecular biomarkers into the medical triage model for healthcare providers, adapted from Sullivan et al.[118] Initially (T1), Crisis Standards of Care will dominate, and healthcare providers must determine which patients require medical care (T2) and are eligible for limited point-of-care (POC) diagnostics (T3). Here, for example, the absence or presence of certain ncRNAs can efficiently determine nonexposed, worried-well from patients eligible for high throughput screening (HTS, T4). In HTS, further RNAs can more accurately predict dose and guide medical providers with decision making. Eventually, as resources recover, organ specific ncRNA markers (T5) can be assayed to predict and mitigate immediate, delayed or minimal injury. This allows medical providers to tailor treatment to an individual's specific injuries. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Additionally, ncRNA biomarkers may suffer from further limitations. Most biomarker studies rely on calculating the fold change of expression, which requires a sample taken prior to exposure. It would not be feasible to collect a baseline sample for each member of the population prior to an unpredictable exposure; however, this may be possible for radiotherapy patients to monitor exposure. Therefore, ncRNA biomarkers must rely on normalization to control markers that are unchanged by radiation. In other cases, the baseline expression of potential markers is undetectable and therefore only relative expression changes can be obtained, rather than fold change. For example, our group showed *Dino* was undetected in control samples, but present after exposure to radiation.[83]

Organ-specific ncRNAs. As mentioned above, ncRNAs are more tissue-specific than mRNA, although studies comparing expression differences between organs require further work. Many of the studies above focused on blood or serum ncRNAs, however some have focused on specific organs irradiated. By finding organ-specific markers of irradiation, medical providers can personalize radiation mitigation treatments to the damaged organs or organ systems. In the radiotherapy clinic, this data can allow radiation oncologists to determine inadvertently damaged tissues and administer radioprotectants or deploy shielding in subsequent treatments. Tissue-specific injury information could also provide early indications of long-term radiation effects, such as lung fibrosis or cardiovascular disease. Early detection can improve patient's quality of life and can reduce the medical cost substantially by reducing the advanced invasive correction methods at late-stage disease, before clinical symptoms arise. Our group has recently published findings that compare mRNA gene expression profiles of heart, lung, and liver in total body irradiated minipigs.[120] Currently, we are also examining ncRNA profiles in mouse hearts and in liver-on-a-chip and lung-on-a-chip models to determine organ-specific changes after irradiation.

CONCLUSIONS

Noncoding RNAs, including lncRNA, miRNA, and circRNA, have complex regulatory roles in response to radiation damage, stemming from their involvement in the DNA damage response. ncRNAs can influence cellular processes by targeting DNA, proteins, mRNA, or other ncRNA. Many miRNAs and lncRNAs involvement in radiation response and their targets have been reported, as well as specific miRNA and lncRNA biomarkers for damage to normal tissue. circRNAs have shown promising roles in the regulation of radiation response and as biomarkers, but further research is

necessary to better understand their vast and complex roles. Future work should also focus on developing a ncRNA biomarker signature which will incorporate multiple types of ncRNA for validation. New studies should focus on maximizing the translational strength of models such as utilizing organ-on-a-chip technology over cell lines or nonhuman primates over small animals when possible. The challenges and limitations of mouse or cell line models must be carefully understood before conclusions are drawn. Additionally, the effect of different types of radiation including x-ray, gamma ray, and FLASH should be investigated to understand the impacts on radiation response. The use of ncRNA, whether natural or synthetic, to selectively target molecules to increase or decrease radiosensitivity is worth pursuing to improve efficacy of radiotherapy. Noncoding RNAs involved with radiation response discovered in future studies may be important for future treatments or as biomarkers but may also define proteins or pathways involved.

ACKNOWLEDGMENT

This study was supported by the NIH Intramural Research Program, National Cancer Institute, Center for Cancer Research, National Institute of Allergy and Infectious Diseases (IAA# NRC-13028) and Biomedical Advance Research and Development Authority (IAA# XRC 16002). The authors would like to acknowledge Dr. Eric Bernhard for critical comments on the manuscript and Dr. Adeola Y. Makinde and Dr. Iris Eke for their help with illustrations. We also acknowledge Dr. Kevin Camphausen, Branch Chief, Radiation Oncology Branch, CCR, NCI for institutional support.

Conflicts of interest: The authors have read the journal's policy on disclosure of potential conflicts of interest and have none to declare. All authors have read the journal's authorship statement. The manuscript was reviewed and approved by all named authors.

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