**Prospects**

# Radiation-induced microRNA: Discovery, Functional Analysis, and Cancer Radiotherapy†

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**Abstract**

MicroRNAs are small non-protein coding RNA that play an important role in gene regulation. These RNA molecules function as post-transriptional regulators. MicroRNAs bind to complementary sequences on target messenger RNA transcripts, usually resulting in translational repression or target mRNA degradation and gene silencing. MicroRNA are abundantly present in all human cells, target approximately 60% of all genes, and are able to repress hundreds of targets each. Since their discovery in 1993 miRNA are emerging as important modulators in cellular pathways such as growth and proliferation, apoptosis, carcinogenesis, timing of cell-fate decision, and metabolic pathways. A large number of studies have examined the general and specific effects of miRNAs perturbation in radiation exposed cells. These studies include expression profiling of miRNA, functional analysis, the role of specific microRNAs in tumor radiosensitivity, and targeting miRNA for improved cancer radiotherapy. Other studies have explored the involvement of miRNA in radiobiological phenomenon like bystander effect. Emerging evidence is establishing that miRNA are involved in regulating radiation-induced cellular processes, can be exploited to improve cancer radiation therapy, and could serve as biomarkers of human radiation exposure.

## 1. Biological effects of Ionizing radiation

Ionizing radiation can affect living organisms by inducing cellular damage particularly to the DNA. The cellular response to ionizing radiation exposure is very complex and involves many pathways. Many studies have indicated that cell exposure to ionizing radiation induces various physiological responses including DNA damage processing, cell cycle arrest, differentiation, signal transduction, mutations, altered gene expression, genomic instability, and induction of carcinogenesis to cell death. The cellular response to ionizing radiation (IR) damage relies on simultaneous activation of a number of these pathways along with signaling networks. A large number of studies have examined radiation-induced biological effects ranging from DNA damage processing [Yang et al., 2006], altered gene expression [Chaudhry, 2006c; Chaudhry, 2008a], cell cycle perturbation [Chaudhry, 2007], bystander effect [Chaudhry, 2006a; Chaudhry and Omaruddin, 2011], DNA methylation alterations [Chaudhry and Omaruddin, 2012a], mitochondrial gene expression [Chaudhry and Omaruddin, 2012c], and microRNA (miRNA) modulation [Chaudhry et al., 2012a]. The inability to cope with radiation stress leads to many consequences including physiological dysfunction, abnormal growth and metabolism, altered expression profile of a large number of genes, cell death, and even cancer or other diseases. To deal with these potentially damaging effects from radiation damage, the cell is equipped with a multitude of pathways for DNA repair, cell cycle check points, damage tolerance systems and apoptosis. Improper functioning of damage response processes can result in transformation to malignant phenotype. Radiation acts as genotoxic stressor and induces cellular damage that activates pre-programmed repair pathways. Recent evidence suggests that miRNA act in these processes. Cells use preexisting pro-survival signaling pathways to evade the damaging and cytotoxic effects of radiation.

## 2. MicroRNA

Gene regulation occurs at the transcriptional and post-transcriptional levels. Recent studies have suggested that micro-RNA (miRNA) plays a significant role at the post-transcriptional gene regulation. It is becoming increasingly evident that miRNA regulate cellular machinery at multiple levels. These ~22-nt non-coding RNAs function as negative regulators of gene expression [Lhakhang and Chaudhry, 2012a]. The miRNAs function as global negative regulators of gene expression and have been associated with a multitude of biological processes. The dysfunction of the microRNAome has been linked to various diseases including cancer. 2578 mature miRNAs are found in the human genome (miRBase 20) and are responsible for diverse cellular processes including the control of developmental timing, cell proliferation, apoptosis and tumorigenesis. miRNAs can activate the cessation of the cell cycle and aging in case of DNA damage by stimulating the tumor suppressor target gene p53 directly and indirectly.

miRNAs are implicated in the pathogenesis and prognosis of human cancers. Recent studies have provided evidence to associate miRNA with many cellular processes, including carcinogenesis, timing of cell-fate decision, apoptosis, and metabolic pathways controlling a range of events. MiRNA have a critical effect on carcinogenesis through post-transcriptional modification of many genes and many specific miRNA are being explored to see if they are involved in certain cancers or how they can be affected or targeted to protect the cell. miRNAs can function as either oncogenes or tumor suppressor genes via regulation of cell proliferation and/or apoptosis. It is shown that the miRNAs have the capability to modulate responses to anti-cancer therapy. miRNAs were found to modulate cell death and proliferation after irradiation.

To gain insight into this complex response, global alterations in the expression of genes in irradiated cells have been examined. Relatively few miRNAs have been studied in detail and hence the biological relevance of majority remains to be uncovered. The molecular basis of gene regulation in cells exposed to ionizing radiation is not fully understood.

## 3. Quest for discovering radiation-responsive miRNA

Large scale expression profiling of miRNA after radiation exposure using a variety of methodologies, including real-time quantitative PCR, microarray technology, and next-generation deep sequencing have identified a number of IR-responsive miRNA.

### 3.1 Microarray Approach

Gene expression profiling is the measurement of the activity of thousands of genes at once, to create a global picture of cellular function. These profiles can distinguish between cells that react to a particular treatment. A number of studies have been done on expression profiling of miRNA after exposure to radiation [Simone et al., 2009]. Microarray technology measures the relative expression of target miRNA and has been applied to generate complete miRNA profiles in order to provide broad insight into gene expression and regulation. Microarray analysis indicated alterations in miRNA expression following exposure to ionizing radiation [Simone et al., 2009]. Pre-treatment with cysteine prevented radiation-induced alterations in miRNA expression, which suggests that miRNAs are responsive to oxidative stress. miRNA expression profile following 20 Gy and 40 Gy doses of IR in the human lung carcinoma cell line A549 identified several miRNAs including *miR-34a* with potential roles in apoptosis and cell cycle arrest. [Shin et al., 2009]. Microarray based analysis of ionizing radiation responsive miRNAs in 10 Gy irradiated p53 expressing IM9 human B lymphoblastic cells showed that *miR-34a* was up regulated [Cha et al., 2009]. miRNA expression profile of human peripheral blood lymphocytes (PBL) in normal gravity (1 g) and in modeled microgravity (MMG) after irradiation with 0.2 and 2Gy of γ-rays showed that let-7i*, miR-7, miR-7-1*, miR-27a, miR-144, miR-200a, miR-598, miR-650 are deregulated by the combined action of radiation and MMG [Girardi et al., 2012]. Interrogation of expression levels of 1,090 miRNA species in irradiated human embryonic stem cells showed changes following 1 Gy of X-ray exposures. Positive regulation of differentiation-, cell cycle-, ion transport- and endomembrane system-related processes were predicted to be negatively affected by miRNAome changes [Sokolov et al., 2012]. 1100 microRNAs were analyzed in 3 glioma and 3 squamous cell carcinoma cell lines following irradiation with 2 Gy. The expression levels of miR-1285, miR-24-1, miR-151-5p, let-7i involved in the regulation of cellular processes like apoptosis, proliferation, invasion, local immune response and radioresistance were found to be altered [Niemoeller et al., 2011]. miR-152, miR-410, miR-431, and miR-493 were up-regulated and miR-155, miR-20a, miR-25, and miR-15a were down-regulated in both IR induced prematurely senescent and replicatively senescent WI-38 cells. [Wang et al., 2011]. It was shown that miR-155 modulates IR-induced senescence by acting downstream of the p53 and p38 mitogen-activated protein kinase (MAPK) pathways and in part via regulating tumor protein 53-induced nuclear protein 1 (TP53INP1) expression. Down-regulation of miR-92b, miR-137, miR-660, and miR-656 and up-regulation of miR-558 and miR-662 was observed following 2 Gy doses of radiation. miR-579, miR-608, miR-548-3p, and miR-585 target genes involved in radioresponsive pathways, such as cell cycle checkpoint and apoptosis [Maes et al., 2008]. Differential expressions of miR-483-3p, miR-494, miR-2115*, miR-33b, miR-1246, miR-3202, miR-18a, miR-125b, miR-17*, and miR-886-3p was observed in cells exposed to radon gas [Cui et al., 2013]. Using high-throughput analysis 421 known miRNAs and 337 unknown sequences were identified after radiation treatment [Ding et al., 2011]. Exposure to 1 and 10 Gy of γ-irradiation on miRNA expression in normal human thyroid cells using a miRNA expression array identified 30 miRNAs that were unregulated or downregulated after irradiation were identified. [Nikiforova et al., 2011]. The miRNA status in endothelial cells after 2 Gy radiation treatment was measured using microarrays. The expression levels let-7g, miR-16, miR-20a, miR-21 and miR-29c were up-regulated, while miR-18a, miR-125a, miR-127, miR-148b, miR-189 and miR-503 were down-regulated [Wagner-Ecker et al., 2010]. The profiles of radiation-induced miRNA expression were completely different in CD34+ vs. human fetal osteoblast (hFOB) cells. Radiation treatment up-regulated miR-30b, miR-30c and miR-30d in CD34+ cells, whereas it inhibited miR-30c expression in hFOB cells [Li et al., 2012]. The screening of radiation-induced miRNAs in laryngeal squamous cell carcinoma cells with microarray technology identified 62 down-regulated and 8 up-regulated miRNA [Huang et al., 2013].

### 3.2 Real-time quantitative PCR Approach

Many studies used the quantitative assessment of miRNA expression changes in irradiated cells by real-time PCR. The real-time RT–PCR scheme for miRNA quantification includes the hybridization of a stem-loop RT primer to the 3' end of miRNA and RT. The RT products are quantified by using conventional TaqMan PCR that includes a

miRNA specific forward primer, a reverse primer, and a dye-labeled TaqMan probe. The purpose of the tailed forward primer at 5' is to increase its melting temperature ($T_m$), depending on the sequence composition of miRNA molecules. The expression levels of several members of the let-7 family miRNA that functionally inhibit the mRNAs of Ras oncogenes were upregulated after ionizing radiation treatment in Jurkat cells but were downregulated in TK6 cells. The expressions of miRNA associated with MYC translocation were upregulated in both cell types [Chaudhry, 2009]. The expression patterns of many miRNA markedly differed within the same cell line after exposure to either 0.5 Gy or 2 Gy doses of X-rays. The expression of eight miRNA belonging to the let-7 family was upregulated in irradiated p53 positive TK6 cells but was downregulated in related p53 negative WTK1 cells [Chaudhry et al., 2010a]. The micro RNA, miR-15a and miR-16 were upregulated in 0.5 Gy-irradiated TK6 cells but were downregulated after a 2 Gy dose of X-rays. In contrast miR-15 and miR-16 were repressed in 0.5 Gy-exposed WTK1. The miR-21 was upregulated in 0.5 Gy-treated TK6 cells and its target genes programmed cell death factor 4 (hPDCD4) phosphatase and tensin homolog (hPTEN), and sprouty homolog 2 (hSPRY2) were downregulated in these cells. The miR-21 was downregulated in 2 Gy-irradiated TK6 cells, and all three of its target genes were upregulated in 2 Gy-exposed TK6 cells [Chaudhry et al., 2010a]. The relative expression level for let-7 family miRNAs indicated an up-regulation in irradiated M059K glioblastoma cells. In contrast, these miRNAs were down-regulated in irradiated M059J glioblastom cells deficient in DNA dependent protein kinase. The miR-17-3p, miR-17-5p, miR-19a, miR-19b, miR-142-3p, and miR-142-5p were upregulated in both M059K and M059J cells. The miR-15a, miR-16, miR-143, miR-155, and miR-21 were upregulated in M059K [Chaudhry et al., 2010b].

### 3.3 Next-Generation Deep Sequencing Approach

High-throughput methods such as next generation sequencing now allow simultaneous detection and analysis of thousands of miRNAs. The application of massively parallel sequencing (MPS) technology to catalogue miRNA that respond to IR treatment has provided a genome-wide view of the miRNAome disturbances. The genome-wide expression changes of miRNA transcriptome by massively parallel sequencing of human cells irradiated with X-rays indicated that miR-19b and miR-93 were induced and miR-222, miR-92a, and miR-941 were repressed after radiation treatment. miR-142-3p, miR-142-5p, miR-107, miR-106b, miR-191, miR-21, miR-26a, miR-182, miR-16, miR-146a, miR-22 and miR-30e exhibited two peaks of induction: one at 8 h and the other at 24 h post-irradiation. miR-378, miR-let-7a, miR-let-7g, miR-let-7f, miR-103b, miR-486-3p, miR-423-5p, miR-4448, miR-3607-5p, miR-20b, miR-130b, miR-155, miR-181, miR-30d and miR-378c were induced only at the 8-h time-point [Chaudhry et al., 2013]. In other studies the miRNA expression in testis, brain, and liver tissues of mice exposed to 2 Gy whole-body proton irradiation has been determined by next-generation deep sequencing [Khan et al., 2013].

## 4. Functional analysis of miRNA in irradiated cells

Through interplaying with the key components in radiation related signal transduction pathways, miRNA activate the expression of DNA damage response genes and cell cycle related genes, and play a critical role in the modulation of radiation response and radiosensitivity in tumor cells. The regulatory mechanisms and functions of miRNA in these radiation related signal transduction pathways have been reviewed [Zhao et al., 2013]. Alterations in miRNA expression patterns in response to ionizing radiation have been shown. Global suppression of miRNA expression was achieved through downregulation of Argonaut e-2 (AGO2) or DICER proteins using RNAi. The reductions in either DICER or AGO2 led to increased cell death after irradiation, indicating a prosurvival function of miRNAs. Furthermore, while cell cycle checkpoint activation and apoptosis were compromised, DNA double-strand break repair was not affected by the lack of miRNAs. The differential sensitivity of these pathways implies the independent activation of the two response pathways rather than a concerted DNA damage response [Kraemer et al., 2011].

Ionizing radiation stimulates miR-21 expression in different types of mammalian cells. miR-21 is an oncomir overexpressed in most human tumors and promotes malignant growth and progression. miR-21 regulates the formation of reactive oxygen species (ROS). Key targets of miR-21 in mediating this function are SOD3 and TNFα. miR-21 inhibit the metabolism of superoxide to hydrogen peroxide, produced either by endogenous activities or by exposure to ionizing radiation. Based on these observations it is suggested that miR-21 promotes tumorigenesis through its regulation of cellular ROS levels [Zhang et al., 2012]. Radiation stimulated miR-21 expression in mouse hippocampal cells and upregulates EGFR. The high expression of miR-21 in the brain is

associated with high expression of EGFR in irradiated mice. Radiation stimulates miR-21 through the EGFR/Stat3 pathway and miR-21 activates the EGFR pathway [Shi et al., 2012]. The tumor suppressor gene Big-h3 is down-regulated and miR-21 is up-regulated in radiation induced thymic lymphoma. miR-21 is induced by TGFβ, and has both positive and negative effects in regulating TGFβ signaling. [Liu et al., 2011a]. miR-21 was up-regulated in human or mouse hepatocytes after exposure to IR, as well as in liver tissues derived from whole body irradiated mice. IR-induced up-regulation of miR-21 depends on the up-regulation/activation of AP-1 and the ErbB/Stat3 pathway [Zhu et al., 2010].

The participation of many miRNA in apoptosis and cell death pathways has been identified. The miR-185 is downregulated in response to ionizing radiation. Elevation of miR-185 sensitizes renal cell carcinoma cells to X-rays. ATM- and Rad3-related (ATR) kinase, a master conductor of cellular responses to DNA damage and DNA replication stresses, is a target of miR-185. miR-185 negatively regulates ATR expression at post-transcriptional level, enhances radiation-induced apoptosis, and inhibits proliferation by repressing ATR pathway [Wang et al., 2013a]. miR-27a is a direct regulator of ATM and its up-regulation promotes cell proliferation of irradiated cells [Di Francesco et al., 2013]. Moreover, increased expression of miR-27a affects DBS rejoining kinetics after irradiation [Di Francesco et al., 2013]. miR-193a-3p induced apoptosis in both glioma cell line U-251 and HeLa cells. miR-193a-3p induced the accumulation of intracellular reactive oxygen species (ROS) and DNA damage. The induction of both apoptosis and DNA damage by miR-193a-3p was blocked by antioxidant treatment, indicating the crucial role of ROS in the action of miR-193a-3p. miR-193a-3p transfection decreased the expression of target protein Mcl-1, an anti-apoptotic Bcl-2 family member. Down-regulation of Mcl-1 by siRNA transfection closely mimicked the outcome of miR-193a-3p transfection showing increased ROS, DNA damage, cytochrome c release, and apoptosis. Ectopic expression of Mcl-1 suppressed the pro-apoptotic action of miR-193a-3p, suggesting that Mcl-1 depletion is critical for miR-193a-3p induced apoptosis [Kwon et al., 2013]. miR-31 has been shown to inhibit cell migration, invasion, and metastasis. The enhanced expression of miR-31 inhibits the oncogenic NF-κB pathway, and supports the tumor-suppressive function of this miRNA. Protein kinase C epsilon (PKCε encoded by the PRKCE gene) as a direct target of miR-31 and the down-regulation of PKCε results in impaired NF-κB signaling, enhanced apoptosis, and increased sensitivity of breast cancer cells toward ionizing radiation. This sensitization is attributed to anti-cancer treatments to the PRKCE-mediated down-regulation of the anti-apoptotic factor BCL2. An inverse correlation between miR-31 and BCL2 expression exists, highlighting the functional relevance of the indirect down-regulation of BCL2 via direct targeting of PRKCE by miR-31 [Korner et al., 2013].

The let-7 family of miRNA regulates multiple cellular processes including cell division and DNA repair pathways. The let-7 family of miRNAs regulates expression of RAS oncogene and is down-regulated in many cancers. In fact, low levels of let-7 predict a poor outcome in lung cancer. let-7a and let-7b expression decreases following exposure to ionizing radiation. This decrease in expression is dependent on p53 and ATM. p53 binds to a region upstream of the let-7 gene following radiation exposure [Saleh et al., 2011]. miR-34 is regulated by the p53 protein and is induced by γ-radiation in HeLa cells, but not in MCF-7 cells. Radiomimetic agent bleomycin did not affect miR-34 expression in both cell types. miR-34 induction by genotoxic stress is cell-type specific [Mert et al., 2012]. miR-34a expression is regulated by the P53 gene and might be required for cell response to DNA damage. The basal and X-ray-induced expression levels of miR-34a in p53 positive cells were much higher than those in p53 negative cells. The miR-34a was also up-regulated to respond to X-ray exposure without a functional P53 gene [Chen et al., 2012]. In human umbilical vein endothelial cells miRNAs involved in vascular biology are modulated in response to radiation. A transcriptional induction of several members of the miRNA cluster 17-92 and miR-221/222 cluster was observed [Vincenti et al., 2011]. Downregulation of oncomiR-17-92 cluster was observed only in the p53 positive prostate cancer cells treated with single-dose radiation and fractionated radiation. An inverse correlation between miR-17-92 cluster and several targets including TP53INP1 in p53 signaling pathway was observed. Tumor suppressor miR-34a and let-7 miRNAs were upregulated by fractionated radiation in radiosensitive LNCaP (p53 positive) and PC3 (p53-null) cells indicating that radiation-induced miRNA expression may not be regulated by p53 alone [John-Aryankalayil et al., 2012].

Recent studies are uncovering the functions of many more radiation-induced miRNA. CD40 was identified as a miR-503 target with bioinformatics approach. Ionizing radiation altered the expression of miR-503 and its target

gene CD40. In 5 Gy irradiated U937 cells, the expression of miR-503 was up regulated while the expression of CD40 gene was down regulated. It was suggested that the regulation of immune responses to irradiation is mediated via miR-503 [Cheng et al., 2012]. Time-series changes in microRNA expression following γ-irradiation in lung cancer cells using microarray analysis indicated that the expression of miR-26b was down regulated, and its target mRNA ATF2 (activating transcription factor 2) was up regulated in γ-irradiated lung cancer cells. When c-Jun N-terminal kinase activity was inhibited, expression of miR-26b was induced following γ-irradiation. IR-induced up-regulation of ATF2 was coordinately enhanced by suppression of miR-26b in lung cancer cells, which may enhance the effect of IR in the MAPK signaling pathway [Arora et al., 2011b]. miR-30 has potential target sites in the REDD1 gene. pre-miR-30c transfection suppressed REDD1 expression in CD34+ cells and human fetal osteoblast (hFOB) cells and resulted in hFOB cell death. In contrast, inhibition of miR-30c expression significantly enhanced clonogenicity in CD34+ cells. miR-30c plays a key role in radiation-induced cell damage through regulation of REDD1 expression [Li et al., 2012]. Increase in the expression of tumor-suppressor miR-34a was paralleled by a decrease in the expression of its target oncogenes NOTCH1, MYC, E2F3 and cyclin D1. miR-7 targets the lymphoid-specific helicase LSH, a pivotal regulator of DNA methylation and genome stability. While miR-7 was down-regulated LSH was up-regulated. These cellular changes may constitute an attempt to counteract radiation-induced hypomethylation. Sex and tissue specificity of miRNA responses was observed [Ilnytskyy et al., 2008]. ARID4a (AT-rich interacting domain 4a, also known as RBP1) and CCL5 (C-C motif ligand 5) are targets for miR-302. Ionizing radiation decreased miR-302 levels and an increase in ARID4a and CCL5 mRNA levels. Overexpression of miR-302 suppressed ARID4a and CCL5 mRNA levels, and increased the percentage of S-phase cells. [Kumar et al., 2012]. A mechanistic connection between the radiation-induced stress response and the regulation of miRNA and radiation-induced cytotoxicity suggest that this process may be a molecular target for anticancer agents.

**5. microRNA impacting the cellular radiosensitivity**

Radiation therapy is a primary form of cytotoxic anticancer treatment. Tumor resistance to radiation is a substantial clinical problem. Tumor radiation response is critical in radiotherapy and a core determining factor of tumor radioresistance or radiosensitivity. Radiosensitivity can be regulated through several signal transduction pathways involving multiple biological functions. Multiple factors can influence tumor radiation response, including genetic and epigenetic background, microenvironment and blood flow status. Due to the intrinsic resistance of many tumors to radiotherapy, current methods to improve the survival of cancer patients largely depend on increasing tumor radiosensitivity. The mechanism(s) of radioresistance are still poorly understood, however, mounting evidence supports a role for miRNA in modulating key cellular pathways mediating response to radiation. miRNA regulate pro-survival pathways. However, there is little understanding of how cellular miRNA expression affects the response of a cancer to radiation therapy. Agents that successfully modify the radiation response in vivo are lacking. miRNA offer potential new avenues to complement the radiation-induced tumor cell killing.

Recent studies have demonstrated that miRNA can control tumor radiosensitivity by affecting DNA damage repair, cell cycle checkpoint, apoptosis, signal transduction pathways and tumor microenvironment [Zhao et al., 2012]. MiRNA are also capable of modulating tumor radiosensitivity by blocking the non-homologous end-joining repair and homologous recombination repair pathways and are involved in IR-induced PI3-K/Akt, NF-κB, MAPK and TGFβ signaling pathways [Zhao et al., 2012]. Many studies have attempted to identify miRNA that impact the cellular sensitivity. The overexpression or inhibition of let-7g, miR-189, and miR-20a markedly influenced clonogenic survival and cell proliferation. The radiosensitivity of endothelial cells was influenced by differential expression of miR-125a, -127, -189, and let-7g. While miR-125a and miR-189 had a radioprotective effect, miR-127 and let-7g enhanced radiosensitivity in these cells [Wagner-Ecker et al., 2010]. MiR-34a, a direct target of p53, has been shown to exert anti-proliferative effects and is induced by irradiation. miR-34a played a critical role in radio-sensitivity variations of different mice tissues by enhancing cell apoptosis and decreasing cell viability. The induction of miR-34a by radiation was p53 dependent and downstream target of miR-34a was the anti-apoptosis molecule Bcl-2 that led to radio-sensitivity [Liu et al., 2011b]. Additional studies focused on specific cancers and investigated the role of certain miRNA in enhancing the tumor radiosensitivity.

### 5.1. microRNA influencing the radiotherapy of hepatoma

miR-210 and miR-16 have been correlated with the radiosensitivity of hepatoma cells. MiR-210 was induced under hypoxia in different types of tumor cells and normal cells and functions as a regulator of a wide range of cellular responses to hypoxia. Knockdown of miR-210 in combination with radiotherapy is more effective in suppressing tumor growth and extending survival time and might be a means to enhance the effectiveness of radiotherapy to human hepatoma [Yang et al., 2013]. Hypoxia led to an increased hypoxia inducible factor-1α (HIF-1α) and miR-210 expression and cell arrest in the $G_0/G_1$ phase in hepatoma cell lines. miR-210 downregulation suppressed cell viability, induced cell arrest in the $G_0/G_1$ phase, increased apoptotic rate and enhanced radiosensitivity in hypoxic human hepatoma cells. Apoptosis-inducing factor, mitochondrion-associated, 3 (AIFM3) was identified as a direct target gene of miR-210. AIFM3 downregulation by siRNA attenuated radiation induced apoptosis in miR-210 downregulated hypoxic human hepatoma cells [Yang et al., 2012]. The multidrug resistant hepatocellular carcinoma cells, expressed higher levels of P-glycoprotein (Pgp) as well as miR-16, and lower level of Bcl-2. Pgp is an efflux pump and is associated with multidrug resistance. These cells were more radiation sensitive and showed more pronounced radiation-induced apoptotic cell death [Tsang et al., 2011].

### 5.2. microRNA influencing the radiotherapy of lung cancer

miRNAs can suppress resistance to radiation therapy and may be a viable tool to augment current lung cancer therapies The let-7 family of miRNAs exhibits altered expression in response to radiation. A radiosensitive state can be created when the select let-7 family of miRNAs is overexpressed in lung cancer cells, whereas decreasing their levels causes radioresistance [Weidhaas et al., 2007]. The potential of targeting Lin28-let7 miRNA regulatory network for overcoming the radioresistance of cancer cells having activated K-Ras signaling was evaluated. The overexpression of let-7a decreased expression of K-Ras and radiosensitized lung cancer cells. Inhibition of Lin28, a repressor of let-7, attenuated K-Ras expression and radiosensitized the cells. The Lin28-let7 regulatory network may be a potential therapeutic target for overcoming the radioresistance of human cancers having activated K-Ras signaling [Oh et al., 2010]. The activation of nuclear factor-kappa B1 (NFkB1) in cancer cells confer resistance to ionizing radiation (IR). The expression of miR-9 was inversely correlated with that of NFκB1. Overexpression of miR-9 down-regulated the level of NFκB1 in lung cancer cells, and the surviving fraction of γ-irradiated cells was decreased. let-7g also suppressed the expression of NFκB1. The expression of miR-9 and let-7g could enhance the efficiency of radiotherapy for lung cancer treatment through the inhibition of NFκB1 [Arora et al., 2011a].

Down-regulation of miRNA-21 inhibited proliferation and cell cycle progress of A549 lung cancer cells and sensitized cells to radiation. Decreased miRNA-21 expression promoted the apoptosis of A549 cells induced by irradiation [Xiao-Chun et al., 2013]. anti-miR-21 inhibits growth, migration and invasion, and reverse radioresistance of non-small cell lung cancer (NSCLC) cells, while miR-21 mimics increase growth, promote migration and invasion, and enhance radioresistance of lung cancer cells. miR-21 mimics inhibit expression of PTEN mRNA in A549 lung cancer cells, while anti-miR-21 increase expression of PTEN mRNA and protein in A549 cells. The overexpression of PTEN mimic the same effects of anti-miR-21 in NSCLC cells, and siRNA-mediated downregulation of PTEN rescue the effects on NSCLC cells induced by anti-miR-21 [Liu et al., 2013c].

miR-155 regulates genes involved in immunity and cancer-related pathways. miR-155 is overexpressed in lung cancer, which correlates with poor patient prognosis. Increased levels of miR-155 radioprotects lung cancer cells, while inhibition of miR-155 radiosensitizes these cells. Inhibition of miR-155 may have important therapeutic potential as a means to radiosensitize hypoxic lung cancer cells [Babar et al., 2011]. Ectopic expression of miR-101 sensitizes lung cancer cells to radiation by targeting ATM and DNA-PK catalytic subunit (DNA-PKcs) to inhibit DNA repair, as the endogenous miR-101 levels are generally low in tumors [Chen et al., 2011]. Hypoxia-inducible factor 1 (HIF-1) induction of miR-210 stabilizes HIF-1 through a positive regulatory loop. By stabilizing HIF-1 in normoxia, miR-210 may protect lung cancer cells from radiation. The miR-210-expressing cells showed stabilization of HIF-1 associated with mitochondrial defects and a glycolytic phenotype. Cells expressing miR-210 in normoxia had the same level of radioresistance as control cells in hypoxia. Under hypoxia, pmiR-210 cells showed a low mortality rate owing to a decrease in apoptosis, with an ability to grow even at 10□Gy. miR-210 expression promotes a more efficient DSB repair. This advantage could be used by tumor cells in conditions where reoxygenation has occurred and suggests that strategies targeting miR-210 could enhance tumor radiosensitization

[Grosso et al., 2013]. miR-449a was down-regulated in lung adenocarcinoma cells after irradiation. Overexpression of miR-449a effectively increased radiation-induced DNA damage and apoptosis, altered the cell cycle and led to sensitization of lung adenocarcinoma cells to irradiation [Liu et al., 2013b].

The modulation of miRNA biogenesis machinery is not sufficient to increase the radiosensitivity of lung tumors. The main regulators of miRNA biogenesis are the ribonucleases Drosha, Dicer and Ago2. Drosha and Dicer were expressed at higher levels in radioresistant but not in sensitive lung cancer cell lines. However, down-regulation of either Dicer or Drosha had no effect on the sensitivity of cells to irradiation. Elimination of components of the RNA-induced silencing complex Ago2 and Tudor staphylococcal nuclease also did not sensitize cells [Surova et al., 2012].

**5.3. microRNA influencing the radiotherapy of glioblastoma**

A recent review highlights that miRNAs have altered expression profiles in gliomas [Chistiakov and Chekhonin, 2012]. miRNA-17 regulates the expression of ATG7 and modulates the autophagy process, improving the sensitivity to radiation treatments in human glioblastoma cells. miR-17 modulation in glioblastoma cells resulted in sensitization to radiation [Comincini et al., 2013]. Investigation of the miRNAs in the modulation of radiosensitivity in the glioblastoma multiform U87MG cell line revealed that *miR-181a*, was down regulated and it's over expression sensitized malignant glioma cells to radiation treatment. *miR-181a* modulated radiosensitivity by targeting Bcl-2 [Chen et al., 2010]. *miR-181a* could be a target for enhancing the effect of radiation treatment on malignant glioma cells. The oncongenic miR-21 promotes cancer cell resistance to apoptotic signals. The silencing of miR-21 in U87MG glioblastoma (GBM) cells resulted in a marked reduction in its expression and the external beam radiation therapy improved the sensitivity to radiation treatment [Griveau et al., 2013]. miR-211, predicted to target MMP-9, is suppressed in glioblastoma multiforme. miR-211 suppression in GBM involves aberrant methylation-mediated epigenetic silencing of the miR-211 promoter. An inverse correlation between miR-211 expression and MMP-9 protein levels was observed. The shRNA specific for MMP-9 (pM) promoted miR-211 expression via demethylation of miR-211 promoter-associated CpG islands. miR-211 overexpression and pM treatments led to the activation of the intrinsic mitochondrial/Caspase-9/3-mediated apoptotic pathway in glioma cells. The inhibitory effect of miR-211 on glioma cell invasion and migration is via suppression of MMP-9. miR-211 in combination with ionizing radiation (IR) induces apoptosis. Thus either rescuing miR-211 expression or downregulation of MMP-9 may have a new therapeutic application for GBM [Asuthkar et al., 2012]. miRNAs involved in regulating the expression of genes inducing apoptosis and other specific genes have been proposed for use, in order to induce the apoptosis of radioresistant glioblastoma cells. The miR-34a expression levels in cells after irradiation at 30 and 60 Gy were 0.17- and 18.7-times the BCL2 and caspase-9 expression levels, respectively. The high miR-34a expression level in the cells after irradiation at 60 Gy reduced the p53 expression level. This study suggests that apoptosis might be promoted by regulating the action of miRNAs, even in cells that have acquired radioresistance [Sasaki et al., 2012].

**5.4. microRNA influencing the radiotherapy of gastric cancer**

*miR-221* and *miR-222* induce cell growth and cell cycle progression via direct targeting of p27 and p57 in various human malignancies. *MiR-221* and *miR-222* were found to regulate radiosensitivity, cell growth, and invasion of human gastric cancer cells, via direct modulation of PTEN expression. The inhibition of *miR-221* and *miR-222* might form a therapeutic strategy for human gastric cancer [Chun-Zhi et al., 2010]. Elevated miR-375 level was found in recurring gastric cancer. It is suggested that miR-375 may be a regulator of p53 gene. Exogenous expression of miR-375 promoted the growth of gastric cancer cells and down-regulated p53 expression. The expression of miR-375 desensitized cells to ionizing radiation. miR-375 abrogated the cell cycle arrest and apoptosis after DNA damage. These results demonstrate that miR-375 targets p53 to regulate the response to ionizing radiation treatment [Liu et al., 2013a].

**5.5. microRNA influencing the radiotherapy of lymphoma**

Over expression of *miR-17-92* increased the cell survival, proliferation and decreased cell death of lymphoma cells after 0, 2, and 4 Gy suggesting its role to increase the radioresistance [Jiang et al., 2010]. The *miR-17-92* could be targeted for improving radiotherapy. miR-148b was up-regulated in response to 2 Gy dose of radiation treatment in B cell lymphoma cell. miR-148b increased the radiosensitivity by promoting radiation-induced apoptosis [Wu et

al., 2012]. The overexpression of miR-34b reduced cell survival after radiation treatment and a reduction in B-cell CLL/lymphoma 2 (BCL2) expression [Balca-Silva et al., 2012]. The miR-17-92 cluster also known as oncomir-1, consists of seven miRNAs that are transcribed as a polycistronic unit. Over-expression of miRNA-17-92 has been observed in lymphomas and other solid tumors. Over-expression of miRNA-17-92 increased cell survival, proliferation and decreased cell death of mentle cell lymphoma (MCL) after radiation treatment. Phosphatase and tension homolog (PTEN) and PHLPP2 were down-regulated and pAkt activity was enhanced in miRNA-17-92 over-expressing cells after irradiation suggesting that over-expression of miRNA-17-92 cluster increases the radioresistance of human MCL cells and offers a novel target molecule for improving the radiotherapy of MCL [Jiang et al., 2010].

**5.6. microRNA influencing the radiotherapy of prostate cancer**

A small number of studies have examined the functional role of miRNAs in response to prostate cancer treatment as reviewed [O'Kelly et al., 2012]. In response to the radiation *miR-106b* showed altered expression in prostate cancer cells. Cells transfected with precursor *miR-106b* suppressed radiation induced p21 activation, overrode the $G_2$/M arrest, and diminished growth inhibition. This points to a potential therapeutic target in certain prostate cancer cells whose radiation resistance is due to elevated level of *miR-106b* [Li et al., 2011]. Overexpressed miR-521 sensitized prostate cancer cells to radiation treatment and ectopic inhibition of miR-521 resulted in radiation resistance. miR-521 modulates radiation sensitivity by its predicted target protein, Cockayne syndrome protein A (CSA). CSA is a DNA repair protein, and its levels correlated inversely with the levels of miR-521. Radiation treatment downregulated the levels of miR-521 and upregulated CSA protein. Ectopic inhibition of miR-521 resulted in increased CSA protein levels. Therefore by altering the levels of CSA protein, miR-521 sensitized prostate cancer cells to radiation treatment. Thus miR-521 can be a potential target for enhancing the effect of radiation treatment on prostate cancer cells [Josson et al., 2008].

**5.7. microRNA influencing the radiotherapy of esophageal cancer**

Inhibition of *miR-21* increased radiosensitivity of esophageal cancer cells, and this effect was through the activation of PTEN. Inhibition of *miR-21* may form a novel therapeutic strategy to increase the radiosensitivity of esophageal cancer [Huang et al., 2012]. miR-31 is downregulated in radioresistant oesophageal adenocarcinoma cells in response to radiation and altered the expression of 13 genes involved in DNA repair. Over expression of miR-31 re-sensitized radioresistant cells to radiation [Lynam-Lennon et al., 2012]. The effect of tumor suppressor miRNA-22 expression on radiosensitivity of esophageal squamous cell carcinoma cells (ESCC) revealed a positive correlation between miRNA-22 expression and the survival of patients who received radiotherapy. Increased expression of miRNA-22 sensitized ESCC cells to γ-ray radiation and promoted the apoptosis induced by γ-ray radiation. Increased expression level of miRNA-22 had effects on Rad51 expression after irradiation. These results demonstrate that decreased miRNA-22 expression correlates with increased radiotherapy resistance of ESCC, and that this effect is mediated by the Rad51 pathway [Wang et al., 2013d].

**5.8. microRNA influencing the radiotherapy of breast cancer**

The expression level of Lin28 is associated with resistance to radiation treatment. The cancer cells with high levels of Lin28 are more resistant to radiation than cells with low-level Lin28 expression. Exogenous Lin28 siRNA increases sensitivity to radiation. Stable expression of Lin28 in breast cancer cells attenuate the sensitivity to radiation treatment and inhibited radiation-induced apoptosis. Caspases, H2A.X and Let-7 miRNA were the molecular targets of Lin28. Stable expression of Lin28 and treatment with radiation induced H2AX expression, while inhibited p21 and γ-H2A.X. Overexpression of Let-7 enhanced the sensitivities to radiation in breast cancer cells. Lin28 might be one mechanism underlying radiation resistance, and Lin28 could be a potential target for overcoming radiation resistance in breast cancer [Wang et al., 2013b]. Low levels of miR-200c expression correlate with radiotolerance in breast cancer cells. miR-200c overexpression increased radiosensitivity in breast cancer cells by inhibiting cell proliferation, and by increasing apoptosis and DNA double-strand breaks. miR-200c directly targeted TANK-binding kinase 1 (TBK1). However, overexpression of TBK1 partially rescued miR-200c mediated apoptosis induced by ionizing radiation. Thus, miR-200c can be a potential target for enhancing the effect of radiation treatment on breast cancer cells [Lin et al., 2013]. Transfection of miR-199a-5p mimic suppresses IR-induced autophagy in MCF7 breast cancer cells, and up-regulates basal and IR-induced autophagy in MDA-MB-231 breast cancer cells. Autophagy is a self-degrading process that is triggered by diverse stimuli including

ionizing radiation. DRAM1 and Beclin1 are target genes for miR-199a-5p. Overexpression of miR-199a-5p inhibits DRAM1 and Beclin1 expression in MCF7 cells, while it enhances expression of these genes in MDA-MB-231 cells. miR-199a-5p sensitizes MDA-MB-231 cells to irradiation [Yi et al., 2013].

### 5.9. microRNA influencing the radiotherapy of nasopharyngeal cancer

The *miR-205* contributes to radioresistance of NPC by directly targeting PTEN. Following radiation treatment, *miR-205* is elevated in nasopharyngeal carcinoma cells (NPS) and suppresses PTEN protein expression, followed by activation of AKT, and reduction of apoptosis. Knocking down of *miR-205* compromises the inhibition of PTEN and increases apoptosis [Qu et al., 2012]. *miR-205* may serve as targets for successful radiotherapy of NPC. Low expression of miR-29c was positively associated with therapeutic resistance in 159 NPC cases. Ectopic restoration of miR-29c enhanced the sensitivity of NPC cells to IR treatment by promoting apoptosis. miR-29c repressed the expression of anti-apoptotic factors, Mcl-1 and Bcl-2 in NPC tissues and cell lines. miR-29c might serve as a potential therapeutic sensitizer in NPC treatment [Zhang et al., 2013]. The expression of miRNA-324-3p was decreased in nasopharyngeal carcinoma cells. WNT2B is predicted to be the target of miRNA-324-3p. The aberrant expression of miRNA-324-3p following irradiation result in radiosensitivity alterations and changes of WNT2B signaling pathway. Down-regulation of miRNA-324-3p and up-regulation of WNT2B are correlated with advanced clinical stages of NPC and this inverse expression pattern is also observed in NPC tissues before and after irradiation. miRNA-324-3p contributes to the radioresistance of NPC by regulating the WNT2B signaling pathway. miRNA-324-3p is a potential biomarkers for radioresistance and could serve as target for reversing radioresistance in NPC [Li et al., 2013].

### 5.10. microRNA influencing the radiotherapy of mesothelioma

Radiation alters the expression of miR-34a in malignant mesothelioma cells [Ghawanmeh et al., 2011]. The miR-34b/c radiosensitized malignant pleural mesothelioma (MPM) cells. γH2AX foci assay showed that DNA double-strand break repair was delayed in miR-34b/c transfectants. The proportion of sub-$G_1$ phase cells was increased in miR-34b/c transfectants after irradiation. miR-34b/c inhibited expression of cyclin-D1, cyclin-dependent kinase 4/6, B-cell lymphoma-2 (BCL-2), increased cleaved poly (ADP-ribose) polymerase (cPARP), and cleaved caspase-3 after irradiation. miR-34b/c might be a useful therapeutic molecule to enhance radiotherapy of MPM [Maki et al., 2012].

### 5.11. microRNA influencing the radiotherapy of oral squamous cell carcinoma

miR-125b regulates intercellular adhesion molecule-2 (ICAM2) mRNA expression. The expression of miR-125b is downregulated in oral squamous cell carcinoma (OSCC) cells. The miR-125b-transfected cells showed a decreased proliferation rate, enhanced radiosensitivity to X-ray irradiation and diminished ICAM2 mRNA expression. Thus, controlling the expression or activity of miR-125b might contribute to suppressing proliferation and overcoming radioresistance in OSCC [Shiiba et al., 2013].

### 5.12. microRNA influencing the radiotherapy of pancreatic cancer

Ionizing radiation promoted mTOR expression and activation in pancreatic cancer cells through reducing miR-99b expression, which negatively regulated mTOR. mTOR inhibitor synergistically promoted radiation induced cell growth inhibition and apoptosis [Wei et al., 2013]. Radioresistant pancreatic cancer cells had reduced levels of the miR-23B and increased autophagy, compared with cells that were not radioresistant. Overexpression of miR-23B sensitized pancreatic cancer cells to radiation. The target of miR-23B, ATG12, was overexpressed in radioresistant cells; levels of ATG12 protein correlated with the occurrence of autophagy. In pancreatic cancer cells, reduced levels of the miR-23B increase levels of ATG12 and autophagy to promote radioresistance [Wang et al., 2013c].

### 5.13. miRNA influencing the radiosensitivity through DNA damage response

Radiotherapy kills tumor cells by inducing DNA double strand breaks (DSB). However, the efficient repair in tumor cells frequently prevents successful treatment. Identifying new sensitizers is an essential step towards successful radiotherapy. miRNA could be used to target DNA repair genes and thus sensitize tumors to radiation, providing a new way for improving tumor radiotherapy. The role of miRNA in tumor radiosensitivity is defining novel targets to improve radiotherapy. Identifying the miRNAs to target DNA double strand breaks repair genes

has been suggested a new way for sensitizing tumors to ionizing radiation. Two genes in the DSB repair pathway DNA-PKcs and ATM were searched for their regulating miRNAs. *MiR-101* was found to efficiently target DNA-PKcs and ATM. Up-regulating *miR-101* efficiently reduced the DNA-PKcs and ATM protein levels in tumor cells and sensitized the tumor cells to radiation [Yan et al., 2010]. miR-31 is downregulated in radioresistant oesophageal adenocarcinoma cells in response to radiation and altered the expression of genes involved in DNA repair. Over expression of miR-31 re-sensitized radioresistant cells to radiation [Lynam-Lennon et al., 2012]. An artificial miRNA engineered to target 3'-untranslated regions of XRCC2 (a homologous recombination repair factor) or XRCC4 (a nonhomologous end-joining factor) was able to achieve radiosensitization [Zheng et al., 2012]. Designed artificial microRNAs (amiRs) efficiently target XRCC4 (an essential factor for nonhomologous end-joining (NHEJ) repair) or XRCC2 (an essential factor for homologous recombination repair (HRR) and sensitize human tumor cells to X-rays [Zheng et al., 2013]. Ectopic expression of miR-101 sensitizes lung cancer cells to radiation by targeting ATM and DNA-PK catalytic subunit (DNA-PKcs) to inhibit DNA repair, as the endogenous miR-101 levels are generally low in tumors [Chen et al., 2011]. Overexpression of miR-7 down-regulated DNA-dependent protein kinases (DNA-PKcs) [Lee et al., 2011]. Expression of the miR-99 family of miRNAs are induced following radiation and correlates with radiation sensitivity by targeting the SWI/SNF chromatin remodeling factor SNF2H/SMARCA5, a component of the ACF1 complex. The induction of the miR-99 family following radiation prevents an increase in SNF2H expression and reduces the recruitment of BRCA1 to the sites of DNA damage, reducing the efficiency of repair [Mueller et al., 2013]. Overexpressed miR-521 sensitized prostate cancer cells to radiation treatment and ectopic inhibition of miR-521 resulted in radiation resistance. miR-521 modulates radiation sensitivity by its predicted target protein, Cockayne syndrome protein A (CSA). CSA is a DNA repair protein, and its levels correlated inversely with the levels of miR-521. Radiation treatment downregulated the levels of miR-521 and upregulated CSA protein. Ectopic inhibition of miR-521 resulted in increased CSA protein levels. Therefore by altering the levels of CSA protein, miR-521 sensitized prostate cancer cells to radiation treatment. Thus miR-521 can be a potential target for enhancing the effect of radiation treatment on prostate cancer cells [Josson et al., 2008]. Low levels of miR-200c expression correlated with radiotolerance in breast cancer cells. miR-200c overexpression increased radiosensitivity in breast cancer cells by inhibiting cell proliferation, and by increasing apoptosis and DNA double-strand breaks. miR-200c directly targeted TANK-binding kinase 1 (TBK1). However, overexpression of TBK1 partially rescued miR-200c mediated apoptosis induced by ionizing radiation. In summary, miR-200c can be a potential target for enhancing the effect of radiation treatment on breast cancer cells [Lin et al., 2013].

**5.14. miRNA influencing the radiosensitivity through signaling pathways**

Functional studies are defining specific miRNA that interplay with signaling pathways and manipulate tumor response to radiation therapy. These studies were extensively reviewed recently [Zhao et al., 2012]. The expression of *miR-221* and *miR-222* is elevated in radioresistant tumor cell lines. The PTEN gene was identified as a target of *miR-221/-222* and knocking down of *miR-221/-222* by antisense oligonucleotides up-regulated PTEN expression. Up-regulated PTEN expression suppressed AKT activity and increased radiation-induced apoptosis, resulting in enhancement of radiosensitivity in tumor cells suggesting that *miR-221/-222* control radiation sensitivity by regulating the PTEN/AKT pathway [Zhang et al., 2011]. The epidermal growth factor receptor (EGFR) is frequently overexpressed in a wide range of solid tumors. miRNA-7 was targeted for overcoming radio-resistance of cancer cells with activated EGFR-associated signaling. Ectopic overexpression of miR-7 attenuated EGFR and Akt expression and radiosensitized squamous cell carcinoma of the larynx, breast cancer cells, lung carcinoma cells, and malignant glioma cells. In contrast, antisense-mediated inhibition of mature miR-7 expression led to the up-regulation of EGFR and its downstream effectors, and increased radio-resistance. Overexpression of miR-7 down-regulated DNA-dependent protein kinases (DNA-PKcs) [Lee et al., 2011]. miR-7 may be a useful therapeutic target for overcoming the radio-resistance of human cancers with activated EGFR-PI3K-AKT signaling.

**5.15. miRNA influencing the radiosensitivity through their biogenesis pathway**

The modulation of miRNA biogenesis machinery is not sufficient to increase the radiosensitivity of lung tumors. The main regulators of miRNA biogenesis are the ribonucleases Drosha, Dicer and Ago2. Drosha and Dicer were expressed at higher levels in radioresistant but not in sensitive lung cancer cell lines. However, down-regulation of either Dicer or Drosha had no effect on the sensitivity of cells to irradiation. Elimination of components of the RNA-induced silencing complex Ago2 and Tudor staphylococcal nuclease also did not sensitize cells [Surova et

al., 2012]. miR-3928 activates ATR pathway by targeting Dicer. After exposure to X-rays, miR-3928 expression was increased, meanwhile Dicer increased gradually. miR-3928 directly binds to the 3'-untranslated region of Dicer mRNA. Consequently, Dicer expression was suppressed and the maturation of other miRNAs including miR-185, miR-300, and miR-663, was inhibited. Overexpression of miR-3928 induced DNA damage, activated ATR, and phosphorylated Chk1 accompanied by G1 arrest. Taken together, these findings replenished ATR/Chk1 pathway by revealing a novel miRNA regulatory network in response to radiation treatment, in which miR-3928 plays an important role in regulating the expression of Dicer [Chang et al., 2012].

In summary, the regulatory effect of miRNA in radiosensitivity can be enhanced when interacting with various key molecules, including H2AX, BRCA1, ATM, DNA-PK, RAD51, Chk1, Cdc25A, p53, PLK1, HIF-1 and VEGF, which are involved in a variety of radiation responsive processes [Zhao et al., 2012]. Some of miRNAs whose expression is markedly up-regulated in tumors are likely to have a pro-oncogenic role through supporting growth, proliferation, migration, and survival of cancer cells. In contrast, a population of miRNA possessing anti-tumor effects is suppressed in certain cancers. These miRNAs harbor therapeutic significance as potent agents in future targeted radiation therapy to sensitize tumor cells to cytotoxic effects of radiation exposure.

## 6. Role of miRNA in modern radiation biology

### 6.1. Involvement of miRNA in radiation-induced Bystander Effect

The irradiated cells communicate with unirradiated cells and induce changes in them through a phenomenon termed as the bystander effect. Unexposed cells exhibit molecular symptoms of stress exposure when adjacent or nearby cells are traversed by ionizing radiation (IR). The nature of the bystander signal and how it impacts unirradiated cells remains to be discovered. Examination of molecular changes in bystander cells due to signals from irradiated cells could lead to the identification of the pathways underlying the bystander effect [Chaudhry, 2006b]. To gain insight into the molecular pathways operating in bystander cells, the miRNA transcriptional changes have been monitored. The molecular analysis revealed that the let-7 family of miRNAs was upregulated in irradiated cells but most of these miRNAs remained repressed in bystander cells. The miR-17-3p, miR-19b, and miR-18a were upregulated in irradiated cells but were repressed in the bystander cells. The miR-17-5p, miR-142-3p, miR-142-5p, and miR-19a were induced only for a short time in bystander cells. the miR-143 and miR-145 expression was induced in bystander cells [Chaudhry and Omaruddin, 2012b]. Using a bystander mouse model, where the animal's head is exposed, while the body is completely protected by a medical grade shield, it was shown that radiation exposure triggers sex specific de-regulation of the miRNAome in the non-exposed spleen. The altered miRNA levels were paralleled by specific changes in the levels of the miRNA processing enzyme Dicer and components of the RNA induced silencing complex [Koturbash et al., 2008]. Microarray analysis of miRNA changes in three-dimensional artificial tissue after α-particle microbeam irradiation indicated deregulation of miRNA expression in bystander tissues. *c-MYC* mediated upregulation of the *miR-17* family was associated with decreased levels of *E2F1* and *RB1*, suggesting a switch to a proliferative state in bystander tissues, while priming these cells for impending death signals. Upregulation of the *miR-29* family resulted in decreased levels of its targets *DNMT3a* and *MCL1*, consequently affecting DNA methylation and apoptosis. Altered expression of *miR-16* led to changes in expression of *BCL2*, suggesting modulation of apoptosis [Kovalchuk et al., 2010].

### 6.2. Changes in miRNA after low dose irradiation

Human health risks of exposure to occupational low dose ionizing radiation have not been understood. The study of miRNA alterations in low dose radiation-treated cells provides a new avenue to better understand these effects. Human cells exposed to acute or chronic low doses of 10 cGy or a moderate dose of 400 cGy of $^{137}$Cs γ-rays showed dose, dose rate and time dependent differences in the relative expression of several miRNA. The expression patterns of many miRNA differed after exposure to either chronic or acute 10 cGy. The expression of miRNA let-7e and the c-MYC miRNA cluster were upregulated after 10 cGy chronic dose but were downregulated after 3 h of acute 10 cGy. The miR-21 was upregulated in chronic or acute low dose and moderate dose treated cells and its target genes hPDCD4, hPTEN, hSPRY2, and hTPM1 were found to be downregulated [Chaudhry et al., 2012b]. Radiation-induced expression changes on miRNA level in coronary artery endothelial cells after 200 mGy radiation dose identified that miR-21 and miR-146b were deregulated. A negative correlation was observed between miR-21 levels and the predicted target proteins, desmoglein 1, phosphoglucomutase and Myb

protein. This study shows that a low-dose exposure has an impact on miRNA expression that is directly related to protein expression alterations [Barjaktarovic et al., 2013]. The changes in miRNA levels after low dose radiation exposure and its effect to neoplastic process in cell could be relevant to secondary cancers. It is shown that there are differences between the level changes of miRNA in low dose fields which are overlooked because of not resulting with acute or chronic side effect. Radiation oncologists must be careful when using these techniques in childhood cancers and the patient group which have long overall survival period. Demonstrating the pathway which is influenced by miRNA changes can provide us the opportunity to focus on the exact cancer type which has to be followed. So that we can detect the secondary cancers earlier and can treat them with long expected survival period.

### 6.3. miRNA as biomarkers of radiation exposure

Biomarkers of ionizing radiation exposure are useful in medical diagnostic imaging, occupational exposures, and radiation accidents. Biomarkers that can rapidly identify severely-irradiated individuals requiring prompt medical treatment in mass-casualty incidents are urgently needed [Chaudhry, 2008b]. Plasma miRNA expression signatures are shown to distinguish mice that received total body irradiation doses of 0.5 Gy, 2 Gy, and 10 Gy and were suggested to be predictive of different levels of radiation exposure [Cui et al., 2011]. Another study found that miRNA signatures induced by ionizing radiation in mouse blood are radiation type- and dose-specific [Templin et al., 2011a]. miRNA expression signatures derived from mouse blood are exploited to serve as biomarkers for exposure to radiation [Templin et al., 2012]. The levels of over 600 miRNAs in serum from mice irradiated at a range of 1 to 12 Gy at 24 and 48 hr time points were monitored to develop biomarkers of radiation exposure. miR-34a is induced in many organs by radiation of both young and adult mice. miR-34a is stable in serum after IR and could serve as an indicator of radiation injury [Liu et al., 2011b]. In patients who receive total body radiation as preparative regimen for bone marrow transplantation, miRNA-150, abundant in lymphocytes, exhibited a dose and time dependent decrease in serum, and was suggested as a marker indicative of lymphocyte depletion and bone marrow damage [Jacob et al., 2013]. To develop miRNA signatures that can be used as biomarkers for radiation exposure, blood from 8 radiotherapy patients was collected immediately before and 4 hours after total body irradiation with 1.25 Gy x-rays. This study suggests that miRNA expression signatures can be used as biomarkers of radiation exposure [Templin et al., 2011b].

## 7. Conclusion

Ionizing radiation changes miRNA levels in a variety of normal and malignant human cells and exerts biological effects on cell growth and clonogenicity as validated in functional assays. The accumulating information suggests that the miRNAs which are differentially expressed after radiation modulate the intrinsic radiosensitivity of cells. This indicates that miRNAs are part of the innate response mechanism of cellular response to radiation. miRNAs may act as regulators of specific cellular responses, immediately down-regulated so as to stimulate DNA repair mechanisms, followed by up-regulation involved in suppressing apoptosis for cell survival.

The modulation of miRNA has been observed in a variety of cell types exposed to ionizing radiation (IR) [Chaudhry, 2008a]. To further understand miRNA role in IR-induced stress pathways, a set of common miRNAs modulated in various irradiated cell lines were catalogued and a list of their predicted target genes was generated [Lhakhang and Chaudhry, 2012b]. Advanced bioinformatics on the miRNA expression data confirmed previously identified IR stress pathways such as cell cycle, p53 pathway, TGF-beta pathway, ubiquitin-mediated proteolysis, focal adhesion pathway, MAPK signaling, thyroid cancer pathway, adherens junction, insulin signaling pathway, oocyte meiosis, regulation of actin cytoskeleton, and renal cell carcinoma pathway [Lhakhang and Chaudhry, 2012b]. Additionally, based on bioinformatics analysis, novel targeted pathways such as aldosterone-regulated sodium reabsorption, long-term potentiation, and neutrotrophin signaling pathways were predicted to be associated with IR response. This approach to utilize miRNA interactome in irradiated cells provides a platform for comprehensive modeling of the cellular stress response to IR exposure [Lhakhang and Chaudhry, 2012b].

The accumulating knowledge gained through many studies on miRNA responses in irradiated cells is leading us to understand the mechanism of radiation-induced biological effects. We are beginning our understanding on miRNAs role and involvement in certain cancers, and how we can use this information to affect or target cells for more successful treatment. The cell is affected by radiation exposure and responds by activation of many

processes. By exploring how the miRNA affect cellular pathways in specific cells we can learn to specifically alter the sensitivity to radiation. Taken together, a large body of data suggests that miRNA might be a potential therapeutic target and specific inhibition of certain miRNA expression in combination with radiotherapy might be expected to exert strong anti-tumor effect on human cancer cells. Future studies will enable us to find miRNA biomarkers for human radiation exposure and to better understand the radiobiology of the cell.

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**Table 1:** miRNA impacting cancer radiotherapy

| Cancer | miRNA Alteration | Outcome/Pathways affected | Target Genes |
|---|---|---|---|
| Hepatoma | miR-210 ↑ | Suppressed cell viability<br>Cell arrest in the $G_0/G_1$<br>Increased apoptosis<br>Enhanced radiosensitivity | AIFM3 |
| | miR-16 ↑ | Pronounced apoptosis | BCL-2 |
| Lung | Let-7g ↑ | Increased radiosensitivity | NFkB1 |
| | miR-9 ↑ | Increased cell death | NFkB1 |
| | miR-21 ↑ | Inhibited proliferation<br>Cell cycle arrest<br>Radiation sensitization<br>Apoptosis | PTEN |
| | miR-155 ↑ | Radioprotection | |
| | miR-101 ↑ | Radiation sensitization<br>DNA repair inhibition | ATM<br>DNA-PKcs |
| | miR-210 ↑ | HIF-1 stabilization<br>Mitochondrial defects<br>Glycolytic phenotype<br>Efficient DSB repair | |
| | miR-449a ↑ | Increased DNA damage<br>Apoptosis<br>Cell cycle arrest<br>Radiation sensitization | |
| Glioblastoma | miRNA-17 | Autophagy process<br>Radiation sensitization | ATG7 |
| | miR-181a ↑ | Radiation sensitization | BCL-2 |
| | miR-21 ↑ | Improved radiosensitivity | |
| | miR-211 ↑ | Invasion and migration<br>Activation of Caspase-9/3<br>Apoptosis | MMP-9 |
| | miR-34a ↑ | Reduced p53 expression<br>Apoptosis | BCL2<br>Caspase-9 |
| Gastric cancer | miR-221 and miR-222 | Radiosensitivity<br>Cell growth and invasion | p27 and p57<br>PTEN |
| | miR-375 ↑ | Growth promotion<br>Down-regulated p53<br>Desensitization<br>Abrogated the cell cycle arrest<br>Apoptosis | p53 |
| Lymphoma | miR-17-92 ↑ | Increased cell survival and proliferation<br>Decreased cell death | PTEN<br>PHLPP2<br>AKT |
| | miR-148b ↑ | Increased radiosensitivity<br>Apoptosis | |
| | miR-34b ↑ | Reduced cell survival | BCL2 |
| Prostate cancer | miR-106b | Overrode $G_2$/M arrest<br>Diminished growth inhibition | p21 |
| | miR-521 ↑ | Radiation sensitization | CSA |
| Esophageal cancer | miR-21↑ | Increased radiosensitivity | PTEN |

| | | | |
|---|---|---|---|
| | miR-31 ⍰ | Re-sensitization of radioresistant cells | DNA repair genes |
| | miRNA-22 ⍰ | Radiation sensitization<br>Apoptosis | RAD51 |
| Breast cancer | Let-7 ⍰ | Enhanced radiosensitivity | |
| | miR-200c ⍰ | Increased radiosensitivity<br>Increased apoptosis<br>Increased DSB | TBK1 |
| | miR-199a-5p | Radiation sensitization | DRAM1<br>BECLIN1 |
| Nasopharyngeal cancer | miR-205 | Activation of AKT<br>Reduction of apoptosis | PTEN |
| | miR-29c ⍰ | Enhanced radiosensitivity<br>Apoptosis | MCL-1<br>BCL-2 |
| | miRNA-324-3p | | WNT2B |
| Mesothelioma | miR-34b/c | Radiation sensitization<br>Delayed DSB repair<br>$G_1$ arrest | Cyclin-D1<br>Cdk 4/6<br>BCL-2<br>cPARP<br>Caspase-3 |
| Oral squamous cell carcinoma | miR-125b ⍰ | Decreased proliferation<br>Enhanced radiosensitivity | ICAM2 |
| Pancreatic cancer | miR-99b ⍰ | | mTOR |
| | miR-23b ⍰ | Radiation sensitization | ATG12 |

**Abbreviations:** AIFM3 (Apoptosis-inducing factor, mitochondrion-associated, 3), ATM (Mutated in Ataxia Telangiectasia), BCL2 (B-cell CLL/lymphoma 2), CSA (Cockayne syndrome protein A), Cdk 4/6 (Cyclin-dependent kinase 4/6), cPARP (cleaved poly (ADP-ribose) polymerase), DNA-PKcs (DNA-dependent protein kinase catalytic subunit), DSB (DNA double-strand breaks), HIF-1 (Hypoxia-inducible factor 1), ICAM2 (Intercellular adhesion molecule-2), NFkB1 (nuclear factor-kappa B1), PTEN (Phosphatase and tension homolog), TBK1 (TANK-binding kinase 1).