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## Emergence of miR-34a in radiation therapy

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### Abstract

Expressions of many microRNAs (miRNAs) in response to ionizing radiation (IR) have already been investigated and some of them seem to play an important role in the tumor radioresistance, normal tissue radiotoxicity or as predictive biomarkers to radiation. MiR-34a is an emerging miRNA in recent radiobiology studies. Here, we review this miR-34 family member by detailing its different roles in radiation response and we will discuss about the role that it can play in radiation treatment. Thus, we will show that IR regulates miR-34a by increasing its expression. We will also highlight different biological processes involved in cellular response to IR and regulated by miR-34a in order to demonstrate the role it can play in tumor radio-response or normal tissue radiotoxicity as a radiosensitizer or radioprotector. MiR-34a is poised to assert itself as an important player in radiobiology and should become more and more important in radiation therapy management.

### Keywords

miR-34a; ionizing radiation; radiotherapy; biodosimetry; radiotoxicity; biomarkers; oncology

## 1. Introduction

MicroRNAs (miRNAs) are a family of 21–25-nucleotide small RNAs that initially were discovered as regulators for the timing of *Caenorhabditis elegans* development (Lee et al., 1993; Wightman et al., 1993; Reinhart et al., 2000; Pasquinelli et al., 2000). Since, discoveries showed that the functions of miRNAs are not limited to the regulation of developmentally timed events, but regulate many others aspects of biological processes in

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### Conflict of interest statement

None

animals and plants (Bartel, 2009; He and Hannon, 2004) such as cell death, cell proliferation or hematopoiesis and patterning of the nervous system (Ambros, 2004). Consequently, these last few years, many studies have investigated the role of miRNAs in cancer and have shown that miRNAs can repress the expression of cancer-related genes, making them functioning as tumor suppressors or oncogenes (Esquela-Kerscher and Slack, 2006; Lin and Gregory, 2015). MiRNA-expression profiling of human tumors has identified signatures associated with diagnosis, staging, progression, prognosis and response to treatment (Calin and Croce, 2006). As such, miRNAs are promising therapeutic targets and might prove useful in the “war” against cancer.

With almost two-thirds of all cancer patients treated by any forms of radiation therapy during the course of treatment (Begg et al., 2011), radiation biology is a keystone for understanding radiation-related cellular events, and so improving radiotherapy efficiency while enabling more individualized treatment plans. Several methods can be used to improve the therapeutic index of radiotherapy such as complementary therapies by using radiosensitizing agents for creating a better tumor response, or selecting patients based on their intrinsic radiosensitivity or tumor radioresistance to tailor a personalized treatment (Lacombe et al., 2013). However, all these options need to study deeply the cellular response to ionizing radiation (IR). Hence, many studies have investigated the tumor radioresistance (Baumann et al., 2008) and normal tissue radiosensitivity (Barnett et al., 2009; Bentzen, 2006), usually focusing on genetic instability, microenvironment influence or protein alterations. With their large spectra of functions, miRNAs can regulate and interfere with all these processes. Some of them were already shown to be involved in many fields of radiation oncology (Czochor and Glazer, 2014; Korpela et al., 2015; Gandellini et al., 2014) such as potential predictive biomarkers of tumor radio-response, or as a target for treatments increasing radiosensitivity, thus offering interesting clinical perspectives in tumor radiotherapy (Zhao et al., 2012).

The 12 different let-7 family members (let-7a-1, 7a-2, 7a-3, 7b, 7c, 7d, 7e, 7f-1, 7f-2, 7g, 7i, and mir-98) have become the most studied miRNAs and they have attracted attention of researchers in various fields, such as developmental biology, stem cells biology, aging, and metabolism (Roush and Slack, 2008; Su et al., 2012). Consequently, this family has been also widely studied in radiation biology. Several studies showed the let-7 family of miRNAs is overrepresented in a class of miRNAs exhibiting altered expression in response to IR (Chaudhry, 2009; Simone et al., 2009; Weidhaas et al., 2007). Let-7g is particularly an important regulator since several studies have shown its expression could enhance the radiosensitivity (Wagner-Ecker et al., 2010) by suppression of KRAS (Jeong et al., 2009) and NFκB1 (Arora et al., 2011). Let-7a seems to play the same role as its overexpression also decreases expression of KRAS and it can radiosensitize lung cancer cells (Oh et al., 2010). As the let-7 family of miRNA is usually repressed following exposure to radiation in multiple cell lines, a recent study has investigated if this radiation-induced reduction in expression could be a part of the cellular response to oxidative stress (Saleh et al., 2011). Results showed a radiation-induced decrease in let-7a and let-7b expression in ATM and p53-positive cell lines, as well as in radiation-sensitive tissues *in vivo*. In contrast, this decreased expression is not observed in p53 knock-out mice suggesting that p53 directly

repress let-7 expression. As the let-7 genes can also regulate the cellular response to stress, they could be good candidates for participating in radiation-induced bystander effect.

MiR-21 is another prevalent miRNAs and has emerged as the miRNA most frequently associated with cellular dysfunction, especially in many oncogenic pathways (Sicard et al., 2013). As it is also involved in apoptosis, cell cycle, DNA damage repair, autophagy or hypoxia, not unexpectedly, miR-21 is one of the most studied miRNA in radiation biology demonstrating its important role in the regulation of radiosensitivity and radioresistance (Liu et al., 2014). Indeed, miR-21 is presently known to promote the radioresistance of various cancers such as breast (Anastasov et al., 2012), glioma (Gwak et al., 2012), nasopharyngeal (Zhu et al., 2015) or lung cancers (Liu et al., 2013; J. Zhang et al., 2015), and its silencing is a promising alternative therapy for radiosensitizing tumors. Moreover, recent studies have shown that miR-21 is also involved in radiation-induced bystander effects (Xu et al., 2014), especially through the regulation of oxidative stress (Jiang et al., 2014; Tian et al., 2015). As bystanders effects play an important role in radiation-induced toxicity (Mothersill et al., 2004), miR-21 is also a promising target to decrease normal tissue radiosensitivity.

A few years ago, miR-34a has emerged in the field of radiobiology with many studies revealing its role in cellular response to IR, and its potential interest as a therapeutic target. In this paper, after a general introduction, we will emphasize miR-34a different roles in radiation response. First, we will review the effect of IR on its expression. Then, we will discuss about its influence on tumor radioresistance and its potential role as radiosensitizer. We will also highlight the different biological processes regulated by miR-34a and which of them may be involved in normal tissue radiation-induced toxicity in order to discuss the role of miR-34a as a therapeutic target or a radioprotector of radiation injury. Finally, we will conclude by discussing the future implication miR-34a could play in radiation therapy.

## 2. MiR-34a, a member of miR-34 family

MiR-34a is a member of the miR-34 family with two others members: miR-34b and miR-34c (Misso et al., 2014). MiR-34a is encoded by its own transcript on chromosome 1 (1p36.22) (Li et al., 2012) and is ubiquitously expressed at higher levels than miR-34b/c, in almost each tissue, with the exception of the lung tissue (Chen and Hu, 2012).

An important role of miR-34a consists on mediating p53 tumor suppressor function as p53 can induce expression of miR-34a in cultured cells (Chang et al., 2007) as well as in irradiated mice (Raver-Shapira et al., 2007). Therefore, miR-34a mainly induces p53-mediated apoptosis, cell-cycle arrest in the G1 phase and senescence (He et al., 2007a; Tarasov et al., 2007). However, it exists a positive feedback between p53 and miR-34a because miR-34a can also increases p53 protein levels and stability (Okada et al., 2014) while miR-34a knockdown diminishes p53 acetylation (Xiong et al., 2015). The complex positive and negative effects of miR-34 on the p53 network suggest that rather than simply promoting the p53 response, miR-34a might act at a systems level to stabilize the robustness of the p53 response to genotoxic stress (Navarro and Lieberman, 2015).

As described later in this paper, miR-34a also regulates a variety of target mRNAs involved in the cell cycle, cell proliferation, senescence, migration, and invasion, such as cyclin-dependent kinase 4/6 (CDK4/6), E2F transcription factor 3 (E2F3), Cyclin E2, hepatocyte growth factor receptor (MET), B-cell lymphoma 2 (Bcl-2), NAD-dependent deacetylase sirtuin-1 (SIRT1), Myc, Notch, and CD44 (Hermeking, 2009; Misso et al., 2014). As ectopic miR-34a induces tumor suppressive mechanisms linked to the inhibition of cancer regeneration, migration, and metastasis (i.e. G1-cell cycle arrest, senescence and apoptosis), not surprisingly, several studies have reported that miR-34a expression is silenced in several types of cancer (X. J. Li et al., 2014), especially due to aberrant CpG methylation of its promoter (Chim et al., 2010; Lodygin et al., 2008). Furthermore, miR-34a has also been associated with regulation of the cancer stem cells function in various cancer types such as prostate cancer, pancreatic cancer, medulloblastoma or glioblastoma (Misso et al., 2014).

### 3. Ionizing radiation increases miR-34a expression

In the recent era of big data several studies performed high-throughput screening analysis for investigating the effect of IR on miRNAs expression in several tissues and species. Some of these studies reveal a radiation-induced alteration of miRNA-34a expression (Table 1).

Results from various experimental models comprising *in vitro*, in normal or cancer cell lines, or *in vivo*, independently of cells or tissue types (i.e. circulating cells or epithelial tissue), all show miR-34a expression level usually increased after IR. In particular, radiation-induced miR-34a expression seems to be delayed because its highest level is at 24 hours after IR while a decrease of its expression level may be observed only a few hours after IR. Only Nikifovora *et al.* showed the opposite trend of miR34a expression level increasing at 4 hours after IR and decreasing at 24 hours (Nikiforova et al., 2011). Moreover, the radiation dose has not any significant effect since irradiation doses ranging from 0.1Gy to 40Gy lead to an increase of miR-34a expression level. Finally, it is also important to note that other high-throughput screening studies assessing the changes in miRNAs expression after IR did not identify any perturbations in miR-34a level. As a majority of these studies used microarray technology, these observations could be explained by the common inherent problems encountered with miRNA microarray techniques, i.e a restricted linear range of quantification, imperfect specificity in some cases for miRNAs that are closely related in sequence, or lack of ability to perform absolute quantification of miRNA abundance easily (Pritchard et al., 2012). Studies which specifically focused on miR-34a also confirmed these observations. According to our bibliographic review, all data showed an increase of miR-34a expression at any doses, time points, and for any types of species (human or not) or tissues samples (Table 2). However, one study reported that miR-34 profiles were modified after similar conditions of IR exposure in HeLa cell culture but not in MCF-7 cell lines, suggesting that genotoxic stress may be cell-type specific (Mert et al., 2012). Moreover, the same study failed to find a modification of miR-34 expression under exposure to Bleomycin (a chemical agent often used to mimic IR effects due to its capacity to inducing DNA damages) suggesting the presence of a cell specific behavior in response to radiation injury (Cellini et al., 2014).

Interestingly, increase of miR-34a expression could also be detected in biological fluids, e.g. in serum (Liu et al., 2011). Molecular 'liquid biopsies' are quickly moving into the clinic, creating a new palette of molecular diagnostics and becoming a central piece in the future of platform technology for precision medicine (Cai et al., 2015). Circulating biosimetry markers collected from liquid biopsies are especially helpful to assess the dose of absorbed radiation in the case of an accidental exposure to IR requiring fast and efficient triage for guiding medical countermeasures. However, even if some recent studies have demonstrated that circulating miRNAs are potential biosimetry markers in mouse model (Jacob et al., 2013) or human (Dinh et al., 2016; Summerer et al., 2013), any correlation between the delivered IR dose and miR-34a expression level could not be established so far.

MiR-34 induction post-radiation has been reported to depend on p53 induction. However, the totality of miR-34a studies, through different cell lines, show that miR-34a overexpression is induced by irradiation as in wild-type p53 cell lines (MCF-7, MCF-10A, HeLa, A549, HCT116, TK6) as in p53-mutated cell lines (WTK1, NH32, CNE1–2) (Table 2). These results are also supported by *in vivo* data in *C. Elegans* where miR-34 expression is radio-induced as in wild-type animals as in cep-1/p53 mutants (Kato et al., 2009). Hence, the miR-34a radio-induction appears more complex than expected and does not seem only p53-dependent. Nevertheless, it is interesting to note miR-34a is not radio-induced in totally p53-deficient mice highlighting the important role of p53 presence yet (He et al., 2007b). A recent report proposed a novel mechanism to explain how rapid miR-34 activation and a pool of mature miR-34 in cells that lacks a 5'-phosphate can be inactivated (Salzman et al., 2016). Following exposure to IR (4Gy), the inactive pool of miR-34 is rapidly activated through 5'-end phosphorylation in an ATM- and Clp1-dependent manner, enabling loading into Ago2. Importantly, this mechanism of miR-34 activation occurs faster than, and independently of, *de novo* p53-mediated transcription and processing. Note, although ultraviolet (UV) are non-ionizing radiations, and so out of the scope of this review, UVs seem to have the same effect as IR. Indeed, several publications which investigated miRNAs expression after UV exposure also highlighted an increase of miR-34a expression level in several cell lines (Table 3).

#### 4. MiR-34a and tumor radiosensitivity

Reducing tumor radioresistance and improving tumor radiosensitivity are key challenges in clinical tumor radiotherapy. Many studies already showed that miRNAs levels are associated with cancer radioresistance (Methetraitur and Slack, 2013).

In the previous section we established that the level of expression of miR-34a is affected by irradiation, but this alone doesn't demonstrate a causal role of miR-34a in the tumor radioresponse. However, other studies showed miR-34a could enhance radiosensitivity via several pathways (Figure 1).

First, Liu *et al.* found that miR-34a played a critical role in radiosensitivity variations in different tissues by enhancing cell apoptosis and decreasing cell viability. These authors demonstrated that one possible downstream target of miR-34 leading to different radiosensitivity was Bcl-2 (Liu et al., 2011). When Bcl-2 expression is decreased by

knockdown in brain glioblastoma and colon cancer cell lines, both the apoptosis is enhanced after IR and the radio-protective role of miR-34a inhibitor is attenuated.

A second study identified LyGDI as a target of miR-34a which could be involved in radiosensitivity regulation (Duan et al., 2013). In non-small cell lung cancer (NSCLC) cell lines with low miR-34a expression, it was shown that restoration of miR-34a expression inhibited cell growth, enhanced irradiation sensitivity, and downregulated LyGDI gene expression. Furthermore, this downregulation of LyGDI expression by miR-34a suppressed COX-2 expression, promoted Rac1 activation and membrane distribution resulting into IR-induced apoptosis enhancement and cellular radiosensitization.

There have also been many investigations related to the use of flavonoids as radiosensitizing agents. For example, Kang et al assessed the effect of two flavonoids drugs, rhamnetin or cirsiolol, in combination with radiation on NSCLC cells (Kang et al., 2013). It was shown that the drugs could reduce the cellular proliferation through the suppression of radiation-induced Notch-1 expression. Interestingly, rhamnetin or cirsiolol also increases the expression of miR-34a in a p53-dependent manner in NSCLC cells. Moreover, the expression of miR-34a mimics decreased Notch-1 protein levels and miR-34a, which could directly bind to the 3'-UTR of Notch-1, showing that miR-34a negatively regulate Notch-1 expression in NSCLC cells. As inhibition of Notch-1 expression increases apoptosis and radiosensitivity of NSCLC cells, taken together these results indicate miR-34a sensitizes NSCLC cells to radiation via Notch-1 pathway. Furthermore, the authors established miR-34a-mediated suppression of Notch-1 expression by rhamnetin and cirsiolol could also reduce epithelial-mesenchymal transition (EMT) in NSCLC cells. Finally, they confirmed the effect of the two drugs *in vivo* by showing correlated increase of radiosensitization and decrease of EMT in a xenograft mouse model.

Recently, a study identified RAD51 as a new direct target of miR-34a able to increase radiosensitivity in NSCLC cells (Cortez et al., 2015). The authors found that miR-34a overexpression promoted both the downregulation of RAD51 and the reduction in radiation-induced RAD51 foci formation. As overexpression of miR-34a also sensitized the cells to radiation by suppressing DNA repair, the authors confirmed that reintroduction of the RAD51 could rescue this phenotype. By examining the effect of miR-34a on radiosensitivity in two mouse models of human lung cancer, it was determined the delivery of miR-34a could be exploited therapeutically. Results indicated that the administration of miR-34a led to repression of RAD51 in lung tumors and consequently enhanced the effects of radiation on tumor growth inhibition.

All these results highlight the potential role of miR-34a to be a promising radiosensitizer. However, further studies are needed to investigate the role of miR-34a mimics or inhibitors by comparing the effects, *in vitro*, on a parental and radioresistant cell line after fractionated irradiation or, *in vivo*, with mouse xenograft model that humanizes radioresistant/radiosensitive tumor cell lines.



## 5. MiR-34a and normal tissue radiation-induced toxicity

A key challenge in radiotherapy is to maximize radiation doses to cancer cells while minimizing damage to surrounding healthy tissue. As severe toxicity in a minority of patients limits the doses that can be safely given to the majority of the patients population, there is interest in better understanding the underlying mechanisms involved in the radiation-induced toxicity development in order to identify new molecules as therapeutic targets or to develop a test to measure an individual's radiosensitivity before treatment (Barnett et al., 2009). These underlying mechanisms involved in the pathogenesis of radiation-induced normal tissue injury have not been fully elucidated, but some evidences show that inflammation and chronic oxidative stress, depletion of tissue stem cells and progenitor cells or damage to microenvironment (i.e. vascular endothelial microvessels) could play an important role (Barker et al., 2015; Kim et al., 2014; Zhao and Robbins, 2009). Interestingly, several studies showed that miR-34a regulates some of these biological processes and so, could be involved in the establishment of radiotoxicity and be a potential therapeutic target (Figure 2).

Late radiation damage in most tissues is characterized by the loss of parenchymal cells and the excessive formation of fibrous tissue. As such, radiation-induced fibrosis (RIF) is probably the most studied form of radiotoxicity. The normal phases of wound healing progress from the injurious stimulus to inflammation, proliferation and finally resolution. By contrast, radiation generates reactive oxygen species (ROS), DNA damage and inflammation as the early stimuli that mediate the activation of a dysregulated proliferative phase. Unlike wound healing, radiation exerts a field effect on the vascular compartment. Endothelial dysfunction leads to a progressive vasculopathy that is characterized by impaired gaseous exchange and the development of tissue hypoxia, which drive an uncontrolled proliferative stage that replaces the normal proliferative phase of wound healing. This may represent a poorly coordinated haemostatic response that aims to preserve tissue oxygenation. The progressive and perpetuating proliferative phase prevents tissue resolution and results in the development of RIF (Barker et al., 2015).

To our knowledge, only one report in the prior art investigated the direct role of miR-34a in RIF. By studying miRNA alterations driving acute and late stages of radiation-induced fibrosis in mice, Simone et al. identified miR-34a as a key player for skin fibrosis development (Simone et al., 2014). First, a large miRNA screening by microarray technique revealed an increase of miR-34a level in mice fibrotic tissue from 7 days to 120 days after 35Gy radiation dose. Then, the study demonstrated the radiation-induced upregulation of miR-34a in cell lines causes inhibition of c-Met production, a known effector of fibrosis. These results suggest miR-34a as a potential target to prevent or treat this devastating side effect of irradiation. However, numerous others studies demonstrated the role of miR-34a in fibrosis. Thus, miR-34a plays a critical role in the progression of cardiac tissue fibrosis especially by downregulating PNUMS (Boon et al., 2013) and Smad4 pathway (Huang et al., 2014). These studies show miR-34a inhibition reduces the severity of experimental cardiac fibrosis in mice whereas miR-34a overexpression results in greater tissue fibrosis (Yang et al., 2015). Moreover, overexpressing miR-34a levels increased the profibrogenic activity of TGF- $\beta$ 1 in cardiac fibroblast, whereas inhibition miR-34a levels weakened the activity.

Another study showed TGF- $\beta$ 1-treated fibroblasts produce microvesicles with high miR-34a level which are able to induce tubular cell apoptosis in fibrotic kidney (Zhou et al., 2014). Furthermore, microRNA profiling also indicates that miR-34a is upregulated in bleomycin-induced pulmonary fibrosis suggesting it could be also play an important role in lung fibrosis (Honeyman et al., 2013; Xie et al., 2011). Although a causal link between chronic oxidative stress and radiation-induced late normal tissue injury remains to be established, a growing body of evidence appears to support the hypothesis that chronic oxidative stress might serve driving the progression of radiation-induced late effects (Azzam et al., 2012; Robbins and Zhao, 2004). Interestingly, several studies showed that miR-34a mimics increased, and antisense miR-34a inhibited, *in vitro* ROS production (Bai et al., 2011; Ferreira et al., 2014; S.-Z. Li et al., 2014; F. Zhang et al., 2015) in several cell lines, suggesting a direct role of miR-34a in oxidative stress. Moreover, a study showed miR-34a was able to inhibit intracellular pathways, such as those involving antioxidative enzymes. Thus, Bai *et al.* demonstrated that aging mesangial cells exhibited upregulation of miR-34a (in association with miR-335) while they marked downregulation of two mitochondrial antioxidative proteins: SOD2 and Txnrd2 (Bai et al., 2011). MiR-34a mimic inhibits Txnrd2 expression whereas antisense miR-34a increased its expression. Li *et al.* also showed that the upregulation of miR-34a (and miR-93) constitutes an inescapable repression of Sirt1 and Mgst1, as well as the transcription factors (Sp1 and Nrf2) controlling their activation and constituting a double dampening regulation at the post-transcriptional level (Li et al., 2011). MiR-34a also enhances ROS-generating enzymes production as NADPH oxidase 2 (NOX2) which produces superoxide ion (S.-Z. Li et al., 2014).

MiR-34a also changes the cytokines expression profile, molecules which play a vital role in the acute and chronic inflammatory responses that drive fibrosis in injured tissues (Borthwick et al., 2013). For example, overexpression of miR-34a mimics in blood monocytes induces tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) production by downregulating AXL expression (Kurowska-Stolarska et al., 2010). Another study also showed that the expression levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) are modulated by miR-34a in human adipose tissue-derived stem cells, as they are strongly increased by miR-34a (Park et al., 2015). However, these recent results are still unclear and need further investigations because they showed IL-6 treatment repressing miR-34a expression while miR-34a can also inhibit IL-6 receptor expression (H. Li et al., 2015; T. Li et al., 2015; Rokavec et al., 2014). It has also been experimentally confirmed that miR-34a directly regulates Interferon- $\beta$  (IFN- $\beta$ ) production by targeting IFN- $\beta$  3' UTR in macaque and human (Witwer et al., 2010; Zhou et al., 2011).

As hypoxia is involved in fibrosis development and can increase mitochondrial ROS generation, it is also interesting to note that hypoxia may downregulate the expression of miR-34a, promoting the epithelial–mesenchymal transition in renal tubular epithelial cells by targeting the Notch signaling pathway (Du et al., 2012). However, the role of miR-34a in hypoxia is still unclear as some studies showed contradictory results, for example hypoxia upregulating miR-34a expression (Doridot et al., 2014).

All these observations indicate that miR-34a has the potential to be a novel mediator and target of radiation injury, radiosensitivity and radioprotection.



## 6. Conclusion

It is now clear IR influences miR-34a expression in cancer and normal cells with miR-34a expression level being increased by IR. Unfortunately, these radiation-induced expression changes do not correlate with the absorbed dose. Indeed, all studies which investigated the miR-34a expression changes after several doses of radiation did not manage to establish a direct correlation between the increase of miR-34a level and the increase of radiation dose, especially for extreme conditions such as very low dose or dose > 10Gy. Moreover, although increase of miR-34a expression after IR is observed in numerous and different cell lines and tissue, these changes seem to be very cell-specific. Indeed, no expression modification are occurred in some cell lines and expression level can be very different among cell lines for the same dose of radiation. However, we cannot conclude if miR-34a could be a good biodosimetry marker or not so further studies are needed. To assess its real power as biodosimetry marker, we suggest to focus on miR-34a expression measurement on just one type of biospecimen, especially those easily collected with minimally invasive procedure (i.e. blood), and evaluate the expression changes for several doses at a determined time point. MiR-34a will not probably be the ideal dosimetry biomarker but it might help to determine the absorbed dose for a specific time after IR exposure. Moreover, it has also been known for a long time that individuals are not equal to their response to IR leading to the concept of individual radiosensitivity. This concept may be an important factor for the study of miR-34a response to IR, especially as a dosimetry biomarker. Studies which analyzed *in vivo* alteration of miR-34a expression after IR in humans have incidentally been confronted to this concept. For example, Kabacik et al. measured miR-34a dose response in T-lymphocytes from two healthy donors. If miR-34a showed an up-regulation for both donors compared to the non-irradiated control samples, it is interesting to note the two curves differ as the second donor displays a different response from radiation dose > 4Gy where miR-34a expression seems decrease. Halimi et al. measured serum miR-34a level before and after radiation treatment (RT) in 40 patients as well. They demonstrated a significant miR-34a overexpression after RT but results also showed a large standard deviation for miR-34a level value revealing an important difference in miR-34a response to IR between the 40 donors. This inter-individual variability does not seem to be sex-specific as demonstrated by Ilnytsky et al. which investigated variability of miRNAs response to IR between male and female mice (Ilnytsky et al., 2008). This study shows that whole body IR exposure results in a significant and sex-specific deregulation of microRNA expression in mouse spleen and thymus tissue. However, interestingly, miR-34a is one of the two miRNAs similarly regulated in male and female, revealing radiation-induced miR-34a expression would not be sex-specific.

By binding the three prime untranslated region of many genes, miR-34a downregulates numerous important biological processes and is an important keystone for major cellular pathways, e.g. p53, SIRT-1, Notch, etc. (Figure 3).

As such, it may regulate several pathways involved in the cellular response to IR. First, miR-34a can act as a radiosensitizer in cancer cells; miR-34a increases apoptosis, decreases double-strand break repairs and so cell viability. As such, several studies already showed the use of miR-34a mimics can enhanced the tumor radiosensitivity. However, this effect has

been only demonstrated in lung cancer, but miR-34a regulates numerous biological processes in numerous different tissue types, and so it might also be a good radiosensitizer candidate for many other tumors. Second, miR-34a can act as a radioprotector in normal cells; while delivery of miR-34a mimics enhances radiosensitivity, inhibition of miR-34a could also protect cells from radiation injury. However, with the exception of Liu *et al.* who performed a miR-34a knockdown on human embryonic kidney cells, demonstrating that radiation-induced apoptosis can be increased, only a few studies have directly investigated the effect of miR-34a inhibition in response to IR in normal cells. Therefore, there is a large field to be investigated in order to evaluate the miR-34a potential as a therapeutic target to prevent radiation injury in normal tissue. As miR-34a overexpression can enhance radiosensitivity and delivery of miR-34a inhibitor increases radioresistance, it might also be possible identifying if miR-34a could serve as predictive biomarker for radiotherapy response. Thus, measurement of miR-34a expression level prior to radiotherapy might predict normal tissue toxicity, or tumor radioresistance, suitable for tailoring a personalized treatment and improving its efficiency. Although it would be interesting to study this potential correlation between miR-34a expression and the clinical outcome, no studies have investigated this point so far.

Therefore, miR-34a could play an important role in radiation oncology and it could be exploited to improve cancer radiation therapy, either in cancer cells as radiosensitizer, or in normal cells as radioprotector or even serve as biomarkers of human radiation exposure. In this context, we predict an increase in the volume of future experimental work related to investigating the role of miR-34a in cellular response to IR whose outputs may lead to a significant impact in more precise radiation therapy treatments.

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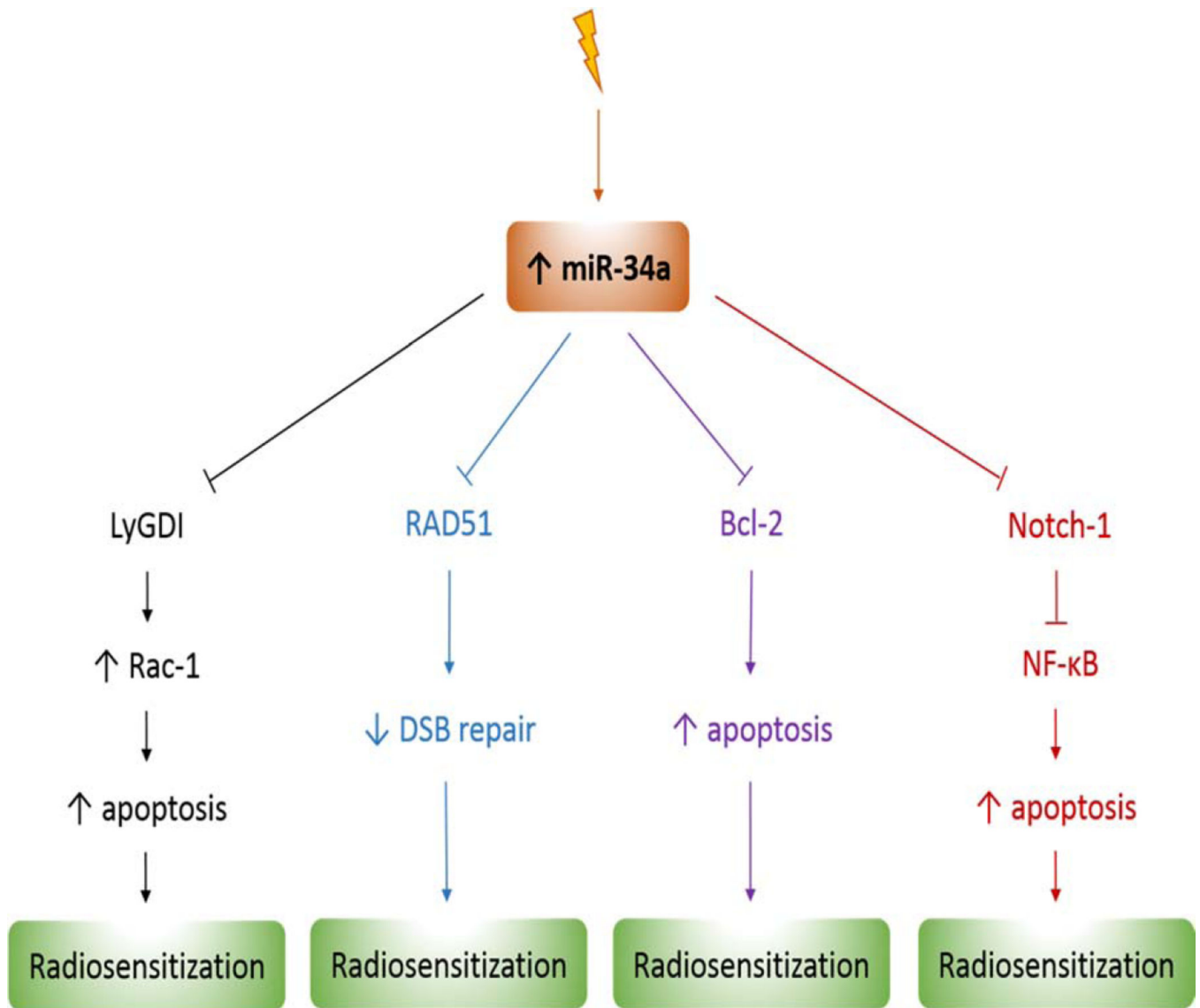


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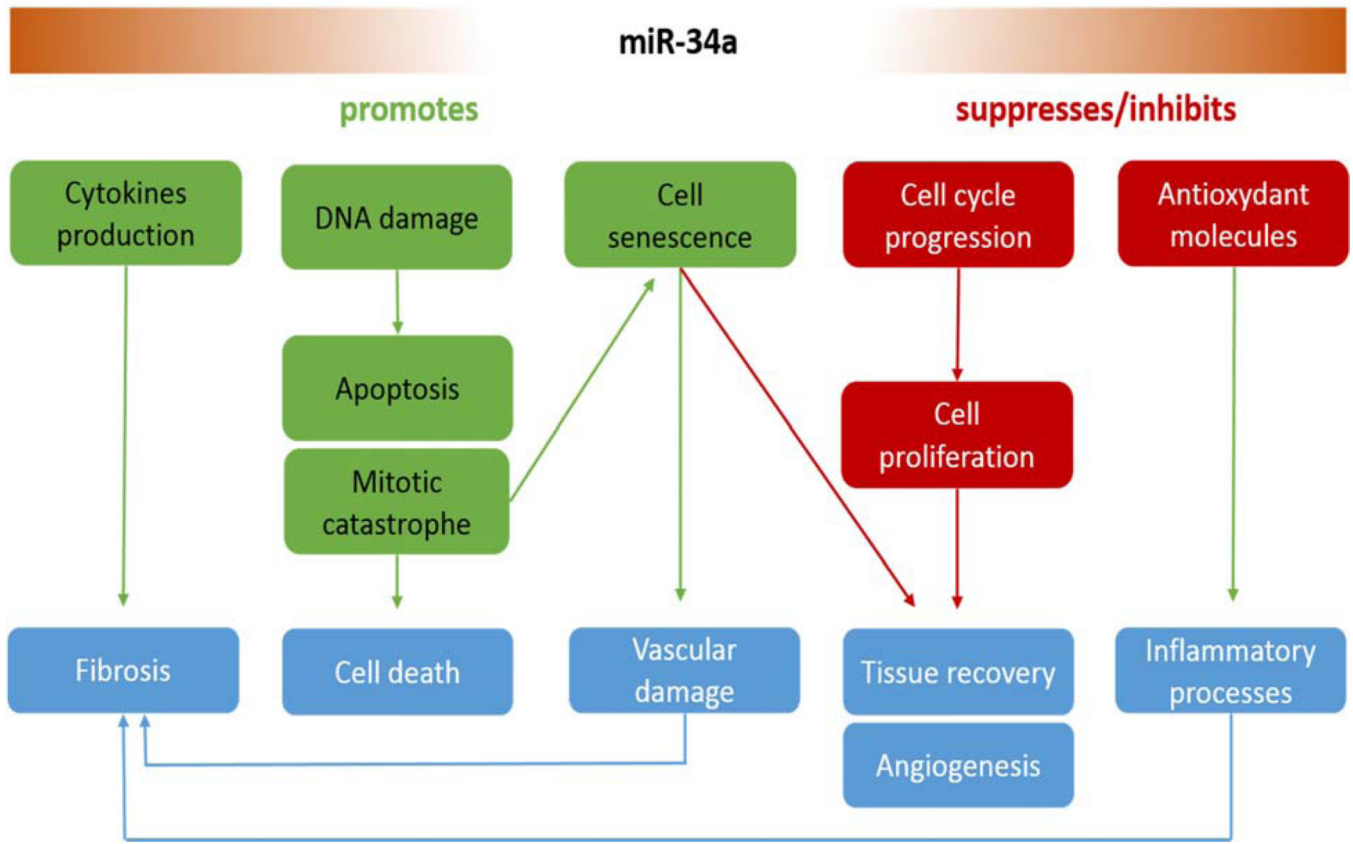
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**Highlights**

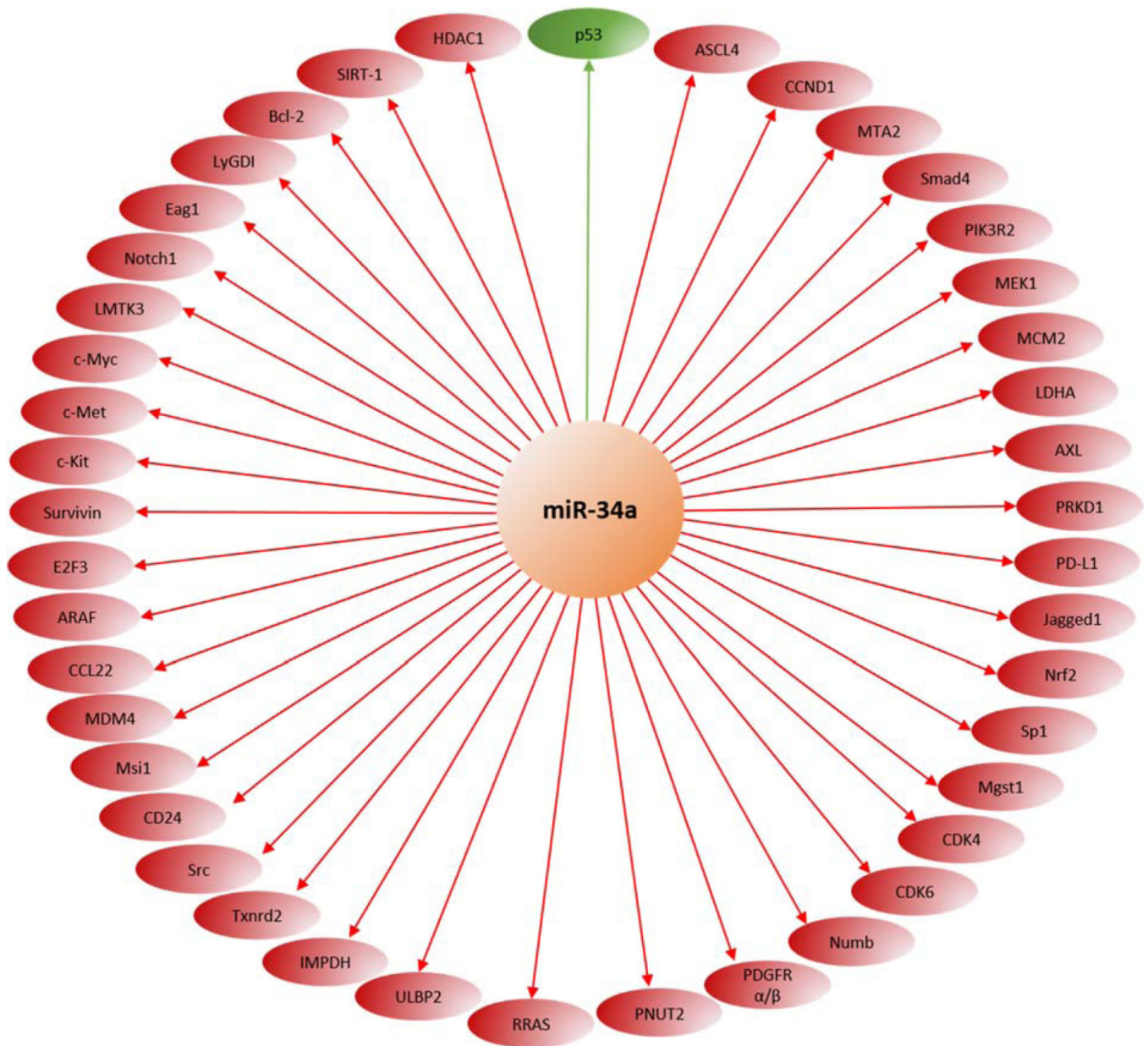
- Ionizing radiation increases miR-34a expression
- MiR-34a enhances tumor radiosensitivity by repressing several important cellular pathways
- MiR-34a regulates numerous biochemical processes which could be involved in development of normal tissue radiation-induced toxicity
- MiR-34a is a promising target for radiation therapy management



**Figure 1.** miR-34a targets which enhance tumor radiosensitivity. DSB: double-strand break



**Figure 2.** Biological processes regulated by miR-34a which might be involved in normal tissue radiation-induced toxicity



**Figure 3.** main miR-34a targets. MiR-34a downregulates many genes by binding their three prime untranslated region (red). MiR-34a is also known as an activator of the major p53 pathway (green).



**Table 1**  
Overall screening analyses that showed an alteration of miRNA-34a expression after ionizing radiation.

Species	Cell type	Cell lines	Irradiation dose (Gy)	Collection time after radiation	Screening method	MiR-34a expression	Ref.
Human	Thyroid	Primary cells from biopsies	1 & 10	4 & 24h	Microarray and qRT-PCR	↑ at 4h after 1 & 10Gy ↓ at 24h after 1 & 10Gy	(Nikiforova et al., 2011)
Human	Prostate	LNCaP PC3	1×5 & 1×10 10×0.5 & 10×1	24h after last dose	Microarray and qRT-PCR	↑ after 10×0.5 & 1Gy in LNCaP ↑ after 10×1Gy in PC3	(John-Aryankalayil et al., 2012)
Human	Lung	A549	40	24h	Microarray	↑	(Shin et al., 2009)
Human	Lymphoblast	IM9	10	24h	Microarray	↑	(Cha et al., 2009)
Human	Coronary artery	HCAEC	1×10 & 5×2	6 & 24h after last dose	Microarray	↑ at 24h after 1×10Gy ↑ at 6 & 24h after 5×2Gy	(Palayoor et al., 2014)
Human	Glioblastoma	A172	2×15 & 2×30	-	qRT-PCR	↑ after 2×30Gy ↓ after 1×15Gy	(Sasaki et al., 2012)
Human	Lymphoblast	TK6	2	4, 8, 12 & 24h	Sequencing	↑ at 8 & 24h ↓ at 4 & 12h	(Chaudhry et al., 2013)
Human	Lymphocyte	Whole blood	0.2 & 2	4 & 24h	Microarray and qRT-PCR	↑ at 24h after 0.2 & 2Gy ↓ at 4h after 0.2Gy	(Girardi et al., 2012)
Human	T-lymphocyte	Whole blood	1, 2, 3, 4 & 5	15 & 30min, 1, 1.5, 2, 4, 5, 6 & 24	qRT-PCR	↑ at 24h after 1, 2, 3, 4 & 5Gy ↑ at 3, 4, 5, 6 & 24h after 2Gy	(Kabacik et al., 2015)
Mouse	Liver	Kunming	4 (WBI)	14 days	Sequencing and qRT-PCR	↑	(Lu et al., 2016)
Mouse	Skin Muscle Subcutaneous tissue	CH3	35 (right hind leg)	7, 14, 50, 90 & 120 days	Microarray and qRT-PCR	↑ at 7, 14 & 120 days	(Simone et al., 2014)
Mouse	Spleen	C57BL/6	2.5 (WBI)	6h	Microarray and qRT-PCR	↑	(Illytskyy et al., 2008)
Rat	Retinal ganglion	RGC-5 cells	6	5 days	Microarray and qRT-PCR	↑	(Wang et al., 2015)

Species	Cell type	Cell lines	Irradiation dose (Gy)	Collection time after radiation	Screening method	MiR-34a expression	Ref.
Rat	Lung	Wistar	20 (right lung)	12 weeks	Microarray	↑	(Xie et al., 2014)
Rat	Mammary gland	Long-Evans	0.1 & 2.5 (WBI)	6 & 96h 4 & 24 weeks	Microarray and qRT-PCR	↑ at 96h & 24 weeks after 0.1 & 2.5Gy	(Wang et al., 2013)
Rat	Mammary gland	Long-Evans	0.1 & 2.5 (WBI)	96h	Microarray	↑ after 0.1 & 2.5Gy	(Luzhna and Kovalchuk, 2014)

WBI: Whole-Body Irradiation

**Table 2**  
miRNA-candidate studies that showed an alteration of miRNA-34a expression after ionizing radiation.

Species	Cell type	Cell lines	Irradiation dose (Gy)	Collection time after radiation (h)	Expression	Ref.
Human	Breast	HMEC	0.1 & 2.5	6, 12, 24, 48 & 96	↑ at 96h after 0.1 & 2.5Gy	(Wang et al., 2013)
Human	Lymphoblast	TK6 NH32 WTK1	0.73, 1.5, 3 & 6	6, 12, 24, 48 & 72	↑ at 48h after all doses for TK6 ↑ at 48h after 3 & 6Gy for NH32 & WTK1 ↑ at 24 & 48h after 0.73Gy for TK6	(Chen et al., 2012)
Human	Nasopharynx	CNE1 CNE2	5×2 & 4×2.33 5×2 & 4×2.43	-	↑ for all cell lines & all doses	(Long et al., 2014)
Human	Cervix Breast	HeLa MCF-7	2 & 5 Gy	24	↑ in HeLa after 2 & 5Gy	(Mert et al., 2012)
Human	Breast	MCF-7 MCF-10A TD-47	0.003, 0.012, 0.048 & 5	4 & 24	↑ in MCF-10A at 4h after 4 all doses & at 24h after 0.012Gy ↑ in MCF-7 at 24h after 0.003Gy	(Stankevicius et al., 2013)
Human	Lung	A549	4	6, 12, 18, 24 & 36	↑ at 18, 24 & 48h	(Salzman et al., 2016)
Human	Serum	In vivo	RT: 50Gy to the tumor (25×2Gy)	24 after the end of RT	↑	(Halimi et al., 2016)
Mouse	Spleen	In vivo	6	4 & 8	↑	(He et al., 2007b)
Mouse	Spleen Thymus Liver Serum	BALB/c C57BL/6	2, 7 & 12 (WBI)	4, 8, 24 & 48	↑ in all 3 tissue at 8h after 7Gy ↑ in spleen & serum at 8h after 2, 7 & 12Gy ↑ in serum at 8h after 2, 7 & 12Gy ↑ in serum at 4, 8, 24 & 48h after 7Gy	(Liu et al., 2011)
Human	Colon	HCT116	2, 4 & 8	4, 8, 24 & 48	↑ at 8h after 2, 4 & 8Gy ↑ at 4, 8 & 24h after 4Gy	(Liu et al., 2011)
<i>C. elegans</i>	Wild-type N2 <i>cep-1/p53</i> mutants	In vivo	200 (WBI)	3, 6 & 9	↑ at 3h	(Kato et al., 2009)

RT: Radiation treatment; WBI: Whole-Body Irradiation

**Table 3**  
Studies that showed an alteration of miRNA-34a expression after non-ionizing ultraviolet radiation

Species	Cell type	Cell lines	Irradiation type	Irradiation dose	Collection time after radiation (h)	MIR-34a expression	Ref.
Human	Keratinocytes	Primary	UVB	30 mJ/cm <sup>2</sup>	4 & 24	↑ at 4 & 24h	(Zhou et al., 2012)
Human	Fibroblasts Cervix	Primary HeLa	UVC	8 J/ cm <sup>2</sup>	4 & 24	↑ at 24h in fibroblasts	(Pothof et al., 2009)
Human	Liver	HepG2	-	50, 70 & 100 J/m <sup>2</sup>	2, 4 & 12	↑ after 50, 70 & 100 J/m <sup>2</sup> ↑ at 2, 4 & 12h after 50 J/m <sup>2</sup>	(Liang et al., 2014)
Human	Cervix	HeLa	-	20 J/ cm <sup>2</sup>	2, 4 & 8	↑	(Pawlicki and Steitz, 2008)