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# EGCG enhances cancer cells sensitivity under $^{60}$Coγ radiation based on miR-34a/Sirt1/p53

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ABSTRACT

Ionizing radiation (IR) resistance and toxicity to normal cells are the main problems in radiotherapy for cancer. In this study, we demonstrated that epigallocatechin gallate (EGCG) could inhibit effectively IR-induced damage to mouse normal hepatic cells AML-12, and improve dramatically the radiosensitivity of mouse hepatoma cells H22 to $^{60}$Coγ. In addition, the different effects of EGCG and underlying molecular mechanisms based on microRNA-34a (miR-34a) and apoptosis-related proteins were investigated by cells viability analysis, quantitative realtime PCR (qRT-PCR), Western blot and cells transfection. The results indicated EGCG played the key role of radiosensitization on H22 cells by activating the miR-34a/Sirt1/p53 signaling pathway. Besides, EGCG could down-regulate the expression of anti-apoptotic protein Bcl-2, and up-regulate the expression of pro-apoptotic proteins Bax and Caspase-3 in H22 cells. Interestingly, EGCG showed contrary results on AML-12 cells. Therefore, radiation protection and radiosensitization of EGCG were associated with apoptosis regulated by miR-34a/Sirt1/p53 signaling pathway.

## 1. Introduction

Radiotherapy is a widely used therapeutic modality for patients (Lai et al., 2015a; Choi et al., 2018). However, its efficacy can be limited by a number of factors, including resistance to ionizing radiation (IR), normal tissue injury and increased side effects. Radiosensitizers have attracted wide attention for their ability to increase the radiosensitivity of cancer cells and reduce the side effects on normal cells. Many drugs used in classical chemotherapy tend to be expensive, non-specific and may lead to severe systemic side effects (Zhang et al., 2017a). Thus, ingredients from traditional plants can provide an attractive alternative due to their safety, easy availability and better efficacy (Hu et al., 2013).

Natural products are being investigated in terms of radiosensitization and protection against IR-induced damage for their high efficiency and safety (Xu et al., 2018; Ji et al., 2018; Zhang et al., 2018). Epigallocatechin gallate (EGCG) is the major active component of catechins in green tea (Xu et al., 2018). Previous investigations suggested that EGCG displayed multiple anti-tumor effects against liver, ovarian, breast and bladder cancer (Luo et al., 2017; Gan et al., 2018). Furthermore, evidences support that EGCG has antioxidant and pro-oxidant effects, which plays a role in radiation protection and radiosensitization (Choi et al., 2016; Tiwari et al., 2017; Lambert and Forester, 2010). Therefore, these studies suggested that EGCG potentially served as a natural radiosensitizer for cancer treatment.

MicroRNAs (miRNAs) are a class of small endogenous non-coding RNA with a length of roughly 22-nucleotides (Gottwein et al., 2007). Studies have confirmed that miRNAs played an important biological role in cells proliferation, differentiation and apoptosis (Cheng et al., 2016). Besides, more and more studies have verified that IR was involved in the regulation of miRNAs in cancer and normal cells. For example, miR-449b sensitized cancer cells to IR treatment and miR-21 was up-regulated in human or mouse hepatocytes after exposure to IR (Zhu et al., 2010; Ji et al., 2018).

In recent years, the role of microRNA-34a (miR-34a) in IR has received great attention. MiR-34a was up-regulated by IR and induced cells apoptosis by regulating the downstream molecular mechanism, both in normal and cancer cells (Stankevicins et al., 2013; Lacombe and Zenhausern, 2017). Studies even suggested that miR-34a could be used as an indicator of IR (Liu et al., 2011; Halimi et al., 2016). In our previous study, the differential expression of miRNAs in the liver tissues of mouse under $^{60}$Coγ radiation have been screened by high-throughput sequencing (Lu et al., 2016). It was also found that miR-34a was

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significantly up-regulated by $^{60}$Coγ radiation. Therefore, miR-34a is an important target for exploring the mechanism of radiation protection and radiosensitization.

Notably, miR-34a is the direct target gene of p53, and one of miR-34a targets is Sirtuin 1 (Sirt1), which can inhibit p53-dependent apoptosis by deacetylating all major p53 acetylation sites (Tian et al., 2016). Thus, the miR-34a/Sirt1/p53 signaling pathway forms a positive feedback loop, such as Bcl-2, Bax and Caspase-3, playing an important role in cells proliferation and apoptosis. Studies have shown that the activation of miR-34a/Sirt1/p53 signaling pathway leads to a severe decrease in colon cancer cells migration and invasion (Lai et al., 2015b). However, the role of miR-34a/Sirt1/p53 signaling pathway in radiation protection and radiosensitization remains unclear. Liver is an important metabolic organ for drugs and nutritions, and it is often damaged in the course of radiotherapy for abdominal tumors. Therefore, hepatocyte cells and hepatoma cells were selected to explore the mechanism of radiation protection and radiosensitization of EGCG based on miR-34a/Sirt1/p53 signaling pathway. The results were expected to explain the different responses of normal cells and cancer cells to EGCG and $^{60}$Coγ radiation, which could cause a novel strategy for functional food and natural radiosensitizers development.

## 2. Materials and methods

### 2.1. Materials

The mouse hepatocyte cells line (AML-12) was purchased from Nanjing Sansheng Biotechnology Co., Ltd (Jiangsu, China) and the mouse hepatoma cells line (H22) was purchased from Wuhan Procell Life Science&Technology Co., Ltd. (Hubei, China). ITS Liquid Media Supplement (100×) (containing 10 μg/mL bovine insulin, 5.5 μg/mL human transferrin, 5 ng/mL sodium selenite), dexamethasone and radio immunoprecipitation assay protein lysate containing proteinase inhibitor cocktails were obtained from Sigma (MO, USA). EGCG (95%) was purchased from Shaoxing Dongling Health Food Co., Ltd (Zhejiang, China). MiR-34a mimics, miR-34a mimics control, miR-34a inhibitor and miR-34a inhibitor control were purchased from Guangzhou RiboBio Co., Ltd, (Guangdong, China) and Lipofectamine 2000 were obtained from Invitrogen (CA, USA). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories (Kumamoto, Japan). The PrimeScript RT reagent kit was purchased from Takara (Dalian, China) and UltraSYBR Mixture was obtained from CWBIO (Beijing, China). Primers for miR-34a, Sirt1, p53, Bcl-2, Bax and Caspase-3 were synthesized by Sangon Biotech (Shanghai, China). In addition, all antibodies were purchased from Cell Signaling Technology (MA, USA) except for β-actin (Wuhan, China).

### 2.2. Cells culture

The AML-12 cells were cultured in a Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) culture medium with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, 1% ITS liquid media supplement and 40 ng/mL dexamethasone (Lu et al., 2018). The H22 cells were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) medium containing 10% FBS, 1% penicillin-streptomycin. Two kinds of cells were incubated in a 5% $CO_2$ incubator (Thermo Fisher Scientific, American) at 37 °C.

### 2.3. Cells treatment

Both AML-12 and H22 cells were placed into 96-well plates and cells culture flask. Then, cells were incubated with different concentrations of EGCG (0, 10, 20, 40 μM) continued for 12 h according to the preminary experiment. For radiation, cells received a dose of 4.0 Gy radiation at 2.0 Gy/min using a $^{60}$Coγ Irradiator at Institute of Isotope Research, Henan Academy of Sciences (Zhengzhou, China) (Ding et al., 2019; Xie et al., 2016). After 24 h of $^{60}$Coγ radiation, cells were collected to analyse cell viability and evaluate the related genes and protein expression.

### 2.4. Cells transfection

As previously described, four different groups of treated cells, including miR-34a mimics, miR-34a mimics control, miR-34a inhibitor and miR-34a inhibitor control group, were used for transfection with Lipofectamine 2000 for analyzing the effects of miR-34a/Sirt1/p53 signaling pathway (Xie et al., 2016). The final concentration of each group of cells was 50 nM. After transfection of 6 h, the medium was then replaced with the fresh medium containing EGCG (20 μM) for another 12 h. After 24 h of $^{60}$Coγ radiation, cells were harvested for further analysis.

### 2.5. Cells viability analysis

The effects of EGCG and $^{60}$Coγ radiation on cell viability were determined by CCK-8 reported in previous study (Li et al., 2018). At the end of culture, 10 μL CCK-8 regent was added to each well containing 100 μL fresh medium and then the plates were incubated for 2 h at 37 °C. Finally, the absorbance was determined at 450 nm on a microplate reader (Molecular Devices, USA).

### 2.6. Reverse transcription PCR (RT-PCR) and quantitative realtime PCR (qRT-PCR)

Total RNA was extracted from AML-12 and H22 cells using RNA isolation kit according to the previous reports (Tang et al., 2018). The PrimeScript RT reagent kit was used for cDNA synthesis. The qRT-PCR was performed with UltraSYBR Mixture using RG-3000 (Corbrtt, USA). The primers sequences for miR-34a, Sirt1, p53, Bcl-2, Bax and Caspase-3 were listed in Table 1. Amplification conditions of miR-34a consisted of pre-denaturation at 95 °C for 10 min followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 65 °C for 30 s. The amplification conditions of mRNAs were as followed pre-denaturation at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s. The mRNA level of U6 and GAPDH was used as endogenous control. The relative expression was analyzed by the $2^{-\Delta\Delta CT}$ formula.

### 2.7. Western blot

According to the treatment described above, total proteins from AML-12 and H22 cells were extracted by ice-cold radio immunoprecipitation assay protein lysate containing proteinase inhibitor cocktails. The protein concentration was measured by bicinchoninic acid (BCA) Protein Assay kit and then denatured with loading buffer by boiling for 5 min. Protein samples were separated on 10% SDS-PAGE, transferred onto 0.22 μm polyvinylidene fluoride (PVDF) membranes, and blocked in 5% nonfat milk. The membranes were incubated at 4 °C overnight with the primary antibodies of Sirt1, p53, Bax, Bcl-2, Caspase-3 at a dilution of 1:1000 and β-actin of 1:3000. Then the membranes were incubated with the secondary antibodies for 2 h at 37 °C. The immunoreactive bands were detected by electrochemiluminescence kit using chemiluminescence imaging systems (Syngene, UK) and β-actin was used as control (Zhang et al., 2018).

### 2.8. Statistical analysis

The differences between groups were evaluated through ANOVA analysis and T-test using GraphPad Prism 5.0. The data were presented as mean ± SD (n = 3). Significant difference from the control group and the $^{60}$Coγ radiation control group was designated as $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

**Table 1**
Primers used in this study.

| Gene | Sequence (5'→3') |
|---|---|
| mmu-miR-34a-5p RT primer | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACAACC |
| mmu-miR-34a-5p Forward primer | GCGGCGGTGGCAGTGTCTTAGC |
| mmu-miR-34a-5p Reverse primer | ATCCAGTGCAGGGTCCGAGG |
| U6 Forward primer | GCTTCGGCAGCACATATACTAAAAT |
| U6 Reverse primer | CGCTTCACGAATTTGCGTGTCAT |
| mmu-Sirt1 Forward primer | AGAACCACCAAAGCGGAAA |
| mmu-Sirt1 Reverse primer | TCCCACAGGAGACAGAAACC |
| mmu-p53 Forward primer | TGGAAGGAAATTTGTATCCCGA |
| mmu-p53 Reverse primer | GTGGATGGTGGTATACTCAGAG |
| mmu-Bax Forward primer | CAGGATGCGTCCACCAAGAA |
| mmu-Bax Reverse primer | CGTGTCCACGTCAGCAATCA |
| mmu-Bcl-2 Forward primer | TGTTCCATGCACCAAGTCCAGTA |
| mmu-BCl-2 Reverse primer | CACATGGCCGGCACACTTA |
| mmu-Caspase-3 Forward primer | TGGACTGTGGCATTGAGACAG |
| mmu-Caspase-3 Reverse primer | CGACCCGTCCTTTGAATTTC |
| GAPDH Forward primer | AGGTCGGTGTGAACGGATTTG |
| GAPDH Reverse primer | TGTAGACCATGTAGTTGAGGTCA |

## 3. Results

### 3.1. Contrary effects of EGCG on AML-12 and H22 cells viabilities under $^{60}$Coγ radiation

The organs and tissues in the body might be damaged by IR. Liver is an important metabolic organ for drugs and functional factors (Lauschke et al., 2016). The differential expression of miRNAs in the liver tissues of mouse under $^{60}$Coγ radiation have been performed by high-throughput sequencing in our previous study (Lu et al., 2016). Therefore, hepatocyte (AML-12) and hepatoma cells (H22) of mouse were chosen here for detail research. Firstly, the effects of EGCG and $^{60}$Coγ radiation on cells viabilities were studied. As shown in Fig. 1, the AML-12 cells viability was increased with EGCG alone. After $^{60}$Coγ radiation, the AML-12 cells viability was decreased. Interestingly, its viability was recovered after EGCG treatment. Contrarily, the proliferation of hepatoma cells was inhibited by EGCG in a concentration-dependent manner and the inhibition was more effective when EGCG combined with $^{60}$Coγ radiation. Therefore, EGCG played contrary effects on AML-12 and H22 cells.

### 3.2. Effects of EGCG and $^{60}$Coγ radiation on miR-34a/Sirt1/p53 signaling pathway in AML-12 cells

In our previous study, we found that miR-34a was significantly up-regulated in liver tissue of mouse exposed to $^{60}$Coγ of 4 Gy by high-throughput sequencing (Lu et al., 2016). Moreover, miR-34a and its downstream genes Sirt1 and p53 could form a positive feedback loop, which was always related to cells proliferation and apoptosis (Tian et al., 2016). Thus, we examined the expression of miR-34a, Sirt1, p53 and apoptosis-related proteins to investigate the underlying mechanism of EGCG and $^{60}$Coγ radiation involved in AML-12 cells. The results revealed that the expression of miR-34a was increased after $^{60}$Coγ radiation treatment. On the contrary, EGCG treatment could decrease the expressions level of miR-34a in AML-12 cells (Fig. 2A). Afterwards, Sirt1 mRNA expression in EGCG-treated group was increased. However, its level was decreased after $^{60}$Coγ radiation treatment and recovered by EGCG (Fig. 2B). The changes of p53 were contrary to that of Sirt1 (Fig. 2C). It was found that the mRNA expression of anti-apoptotic protein Bcl-2 was significantly increased with EGCG treatment, but the $^{60}$Coγ radiation treatment had an opposite effect on Bcl-2 expression. However, Bcl-2 was reversed by EGCG in $^{60}$Coγ radiation group (Fig. 2D). Additionally, EGCG decreased the mRNA expression of pro-apoptotic proteins Bax and Caspase-3. $^{60}$Coγ radiation increased the expression levels of Bax and Caspase-3, but EGCG combined with $^{60}$Coγ radiation decreased their expression (Fig. 2E and Fig. 2F). The above proteins determination results showed similar changes with mRNA detections (Fig. 2G). These data indicated that apoptosis was activated through miR-34a/Sirt1/p53 signaling pathway by $^{60}$Coγ radiation, but inhibited by EGCG in AML-12 cells.

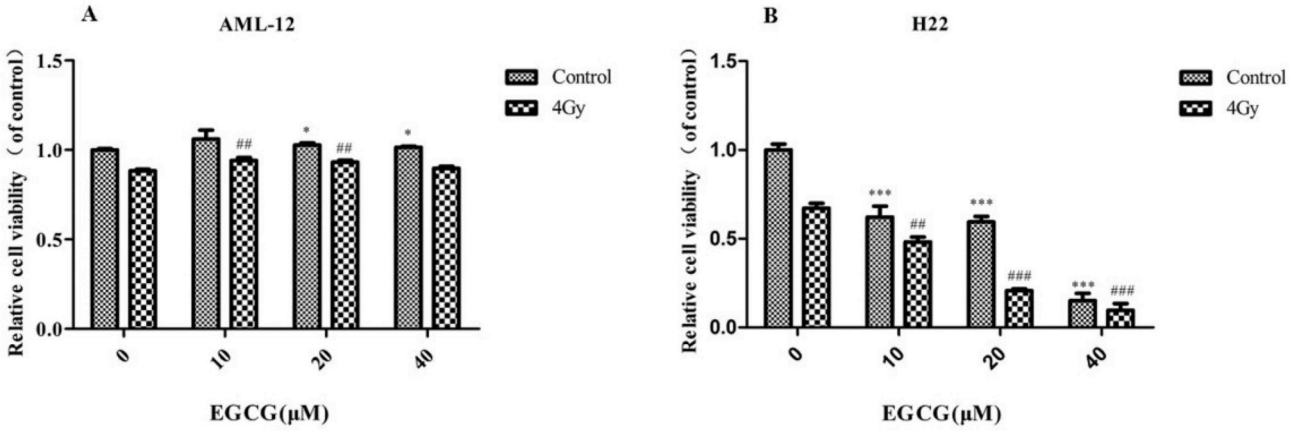


**Fig. 1.** Contrary effects of EGCG on AML-12 and H22 cells viabilities. CCK-8 kit was performed to evaluate the effects of EGCG and $^{60}$Coγ radiation on the cells viabilities for AML-12 **(A)** and H22 **(B)**. All the data were presented as mean ± SD (n = 3). Significant difference from the control group was designated as $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, and significant difference from the $^{60}$Coγ radiation control group was designated as $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$, respectively.

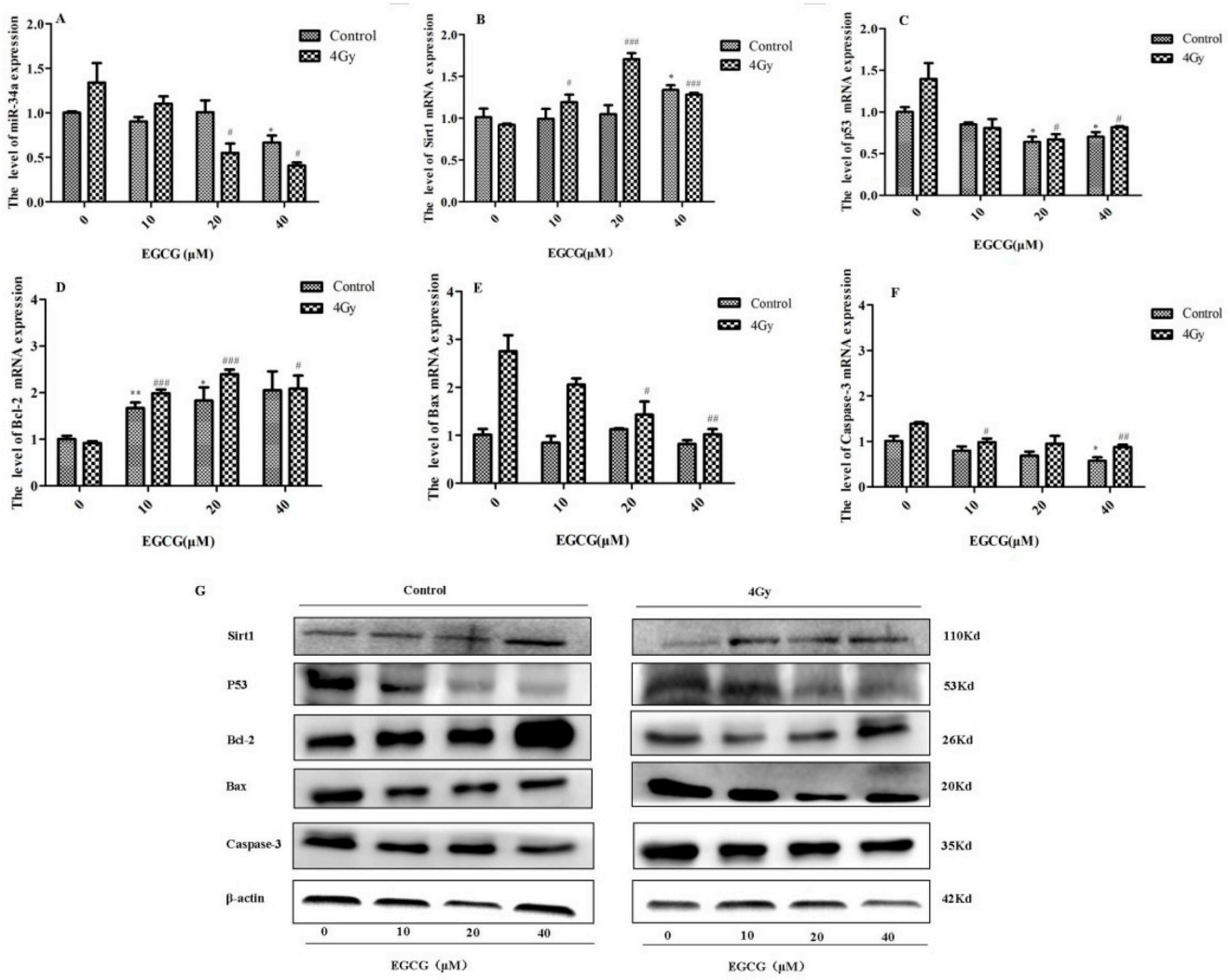


**Fig. 2.** EGCG inhibited the miR-34a/Sirt1/p53 signaling pathway in AML-12 cells. The expression of miR-34a **(A)** and the mRNA expressions of Sirt1 **(B)**, p53 **(C)**, Bcl-2 **(D)**, Bax **(E)** and Caspase-3 **(F)** were measured by qRT-PCR in AML-12 cells treated with EGCG and $^{60}$Coγ radiation. All the data were presented as mean ± SD (n = 3). Significant difference analysis was the same as Fig. 1. The protein expression of Sirt1, p53, Bcl-2, Bax and Caspase-3 in AML-12 cells treated with EGCG and $^{60}$Coγ radiation were assessed by Western blot **(G)**.

### *3.3. Effects of EGCG and $^{60}$Coγ radiation on miR-34a/Sirt1/p53 signaling pathway in H22 cells*

To compare the regulating mechanisms of EGCG on normal cells and cancer cells under $^{60}$Coγ radiation, the expression levels of miR-34a, Sirt1, p53 and apoptosis-related proteins in H22 cells were examined, respectively. The results showed that EGCG or $^{60}$Coγ radiation treatment could up-regulate the expressions of miR-34a in H22 cells, and increased more significantly when EGCG combined with $^{60}$Coγ radiation (Fig. 3A). The qRT-PCR analysis indicated that Sirt1 was decreased by EGCG or $^{60}$Coγ radiation. When the two treatments were combined, the decrease was more severe (Fig. 3B). However, the results of p53 were just opposite to that of Sirt1 (Fig. 3C). Apoptosis related proteins results indicated that the mRNA expression of Bcl-2 was significantly down-regulated by EGCG or $^{60}$Coγ radiation, and the trend was more significant when EGCG combined with $^{60}$Coγ radiation (Fig. 3D). The mRNA expression of pro-apoptotic proteins Bax and Caspase-3 were contrary to Bcl-2 (Fig. 3E and Fig. 3F). Western blot analysis further confirmed these results (Fig. 3G). Therefore, the apoptosis was activated by EGCG or $^{60}$Coγ radiation through miR-34a/Sirt1/p53 signaling pathway, and the effect was more obvious when EGCG combined with $^{60}$Coγ radiation in H22 cells.

### *3.4. Radiation protection mechanism of EGCG by inhibiting miR-34a/Sirt1/p53 signaling pathway*

To further confirm the miR-34a/Sirt1/p53 signaling pathway involved in radiation protection of EGCG, AML-12 cells were transfected with miR-34a mimics or miR-34a inhibitor, and then treated with EGCG and $^{60}$Coγ radiation. Results verified that $^{60}$Coγ radiation could increase the expression of miR-34a, while EGCG could reduce its expression (Fig. 4A). Subsequently, we examined the effects of miR-34a, EGCG and $^{60}$Coγ radiation on AML-12 cells viability. MiR-34a overexpression or $^{60}$Coγ radiation could inhibit significantly AML-12 cells viability, while recovered by EGCG. AML-12 cells viability was increased when miR-34a was inhibited, and the increase was more obvious with EGCG treatment (Fig. 4B). Similarly, the qRT-PCR results showed that Sirt1 and anti-apoptotic protein Bcl-2 were significantly down-regulated by miR-34a mimics or $^{60}$Coγ radiation treatment, while EGCG could up-regulate their expression. Besides, their expression were up-regulated by miR-34a inhibitor, and the trend was more significant in combination with EGCG (Fig. 4C and Fig. 4E). However, the results of p53, Bax and Caspase-3 were contrary to those of Sirt1 and Bcl-2 (Fig. 4D, Fig. 4F and Fig. 4G). Western blot results of Sirt1, p53, Bcl-2, Bax and Caspase-3 also showed similar changes with mRNA (Fig. 4H). The data validated

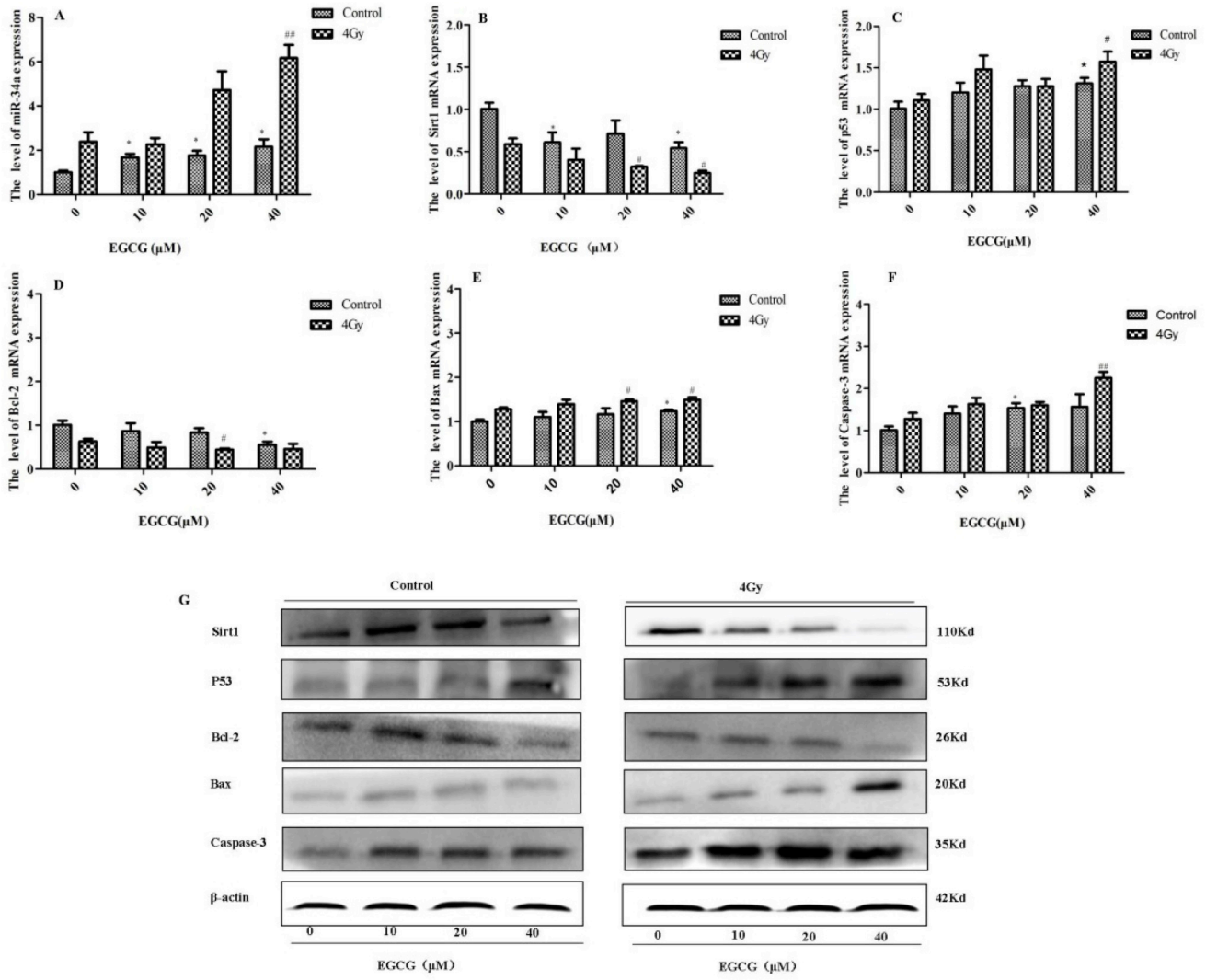


**Fig. 3.** EGCG activated the miR-34a/Sirt1/p53 signaling pathway in H22 cells. The expression levels of miR-34a **(A)** and the mRNA expression levels of Sirt1 **(B)**, p53 **(C)**, Bcl-2 **(D)**, Bax **(E)** and Caspase-3 **(F)** were measured by qRT-PCR in H22 cells treated with EGCG and $^{60}$Coγ radiation. All the data were presented as mean ± SD (n = 3). Significant difference analysis was the same as Fig. 1. The protein expression of Sirt1, p53, Bcl-2, Bax and Caspase-3 in H22 cells treated with EGCG and $^{60}$Coγ radiation were assessed by Western blot **(G)**.

EGCG played the role of radiation protection by inhibiting the miR-34a/Sirt1/p53 signaling pathway, up-regulating the expression of anti-apoptotic protein Bcl-2 and down-regulating the expression of pro-apoptotic proteins Bax and Caspase-3.

### *3.5. Radiosensitization mechanism of EGCG by activating miR-34a/Sirt1/p53 signaling pathway*

Similarly, H22 cells were transfected with miR-34a mimics or miR-34a inhibitor, and then were treated with EGCG and $^{60}$Coγ radiation, to verify the miR-34a/Sirt1/p53 signaling pathway involved in radiosensitization of EGCG. Results clarified that both $^{60}$Coγ radiation and EGCG could increase the expression of miR-34a in H22 cells (Fig. 5A). The effects of miR-34a, EGCG and $^{60}$Coγ radiation on H22 cells viability were detected. Results showed that miR-34a overexpression, $^{60}$Coγ radiation or EGCG significantly inhibited H22 cells viability, and the effect was more significant when these treatments were combined. However, its viability was increased when miR-34a was inhibited (Fig. 5B). The qRT-PCR results showed that Sirt1 and anti-apoptotic protein Bcl-2 were significantly down-regulated by miR-34a mimics, treatments of $^{60}$Coγ radiation or EGCG, and the trend was more obvious when these treatments were combined. Furthermore, their expression were up-regulated by miR-34a inhibitor (Fig. 5C and Fig. 5E). In addition, the results of p53, Bax and Caspase-3 were contrary to those of Sirt1 and Bcl-2 (Fig. 5D, Fig. 5F and Fig. 5G). Western blot results of Sirt1, p53, Bcl-2, Bax and Caspase-3 showed similar changes with mRNA (Fig. 5H). Date indicated that EGCG exerted radiosensitization on H22 cells by activating miR-34a/Sirt1/p53 signaling pathway, down-regulating the expression of anti-apoptotic protein Bcl-2 and up-regulating the expression of pro-apoptotic proteins Bax and Caspase-3.

## 4. Discussion

Radiotherapy is an effective treatment for many types of cancers. However, IR could generate reactive free radicals, bring DNA damage, chromosomal aberrations and cells death. So the side effects of IR are a main obstacle to the effective application of radiotherapy (Ding et al., 2016). Radiosensitizers, having radiation protection on normal cells and radiosensitization on cancer cells, play an important role in radiotherapy (Xu et al., 2018). EGCG was known to have various beneficial effects, such as radiation protection, chemo-preventive, anti-apoptotic, and anti-cancer effects (Avadhani et al., 2017). Here the radiosensitization of EGCG and its mechanism were studied, which will provide a basis for the development of natural radiosensitizers for

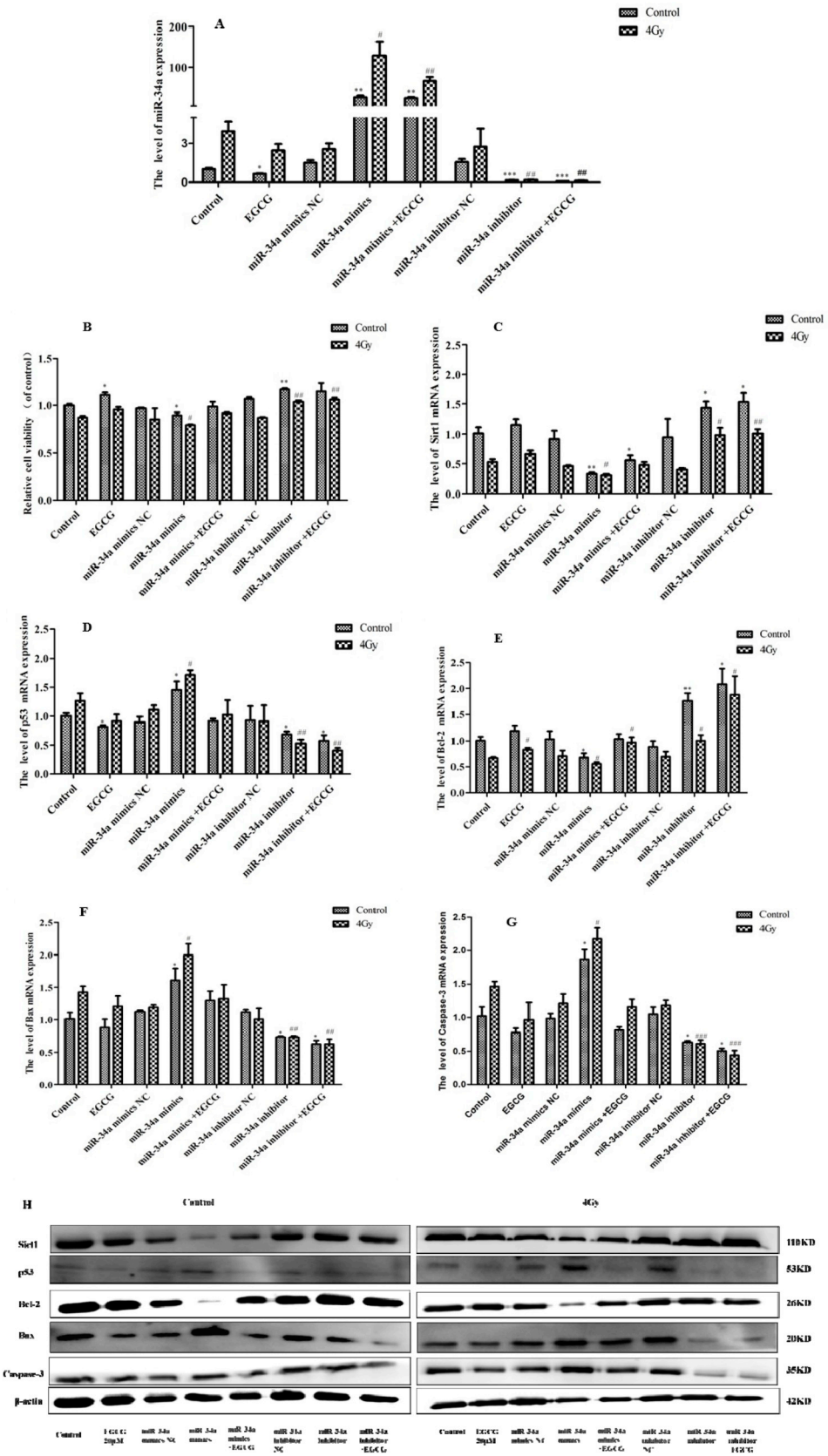


**Fig. 4.** EGCG played the role of radiation protection by inhibiting miR-34a/Sirt1/p53 signaling pathway. AML-12 cells were transfected with miR-34a mimics or miR-34a inhibitor, and then treated with EGCG and $^{60}Co\gamma$ radiation. The expressions of miR-34a **(A)**, Sirt1 **(C)**, p53 **(D)**, Bcl-2 **(E)**, Bax **(F)**, Caspase-3 **(G)** were measured by qRT-PCR. The cell viability of AML-12 **(B)** was assessed by CCK-8. Significant difference analysis was the same as Fig. 1. The protein expression of Sirt1, p53, Bcl-2, Bax and Caspase-3 were assessed by Western blot **(H)**.

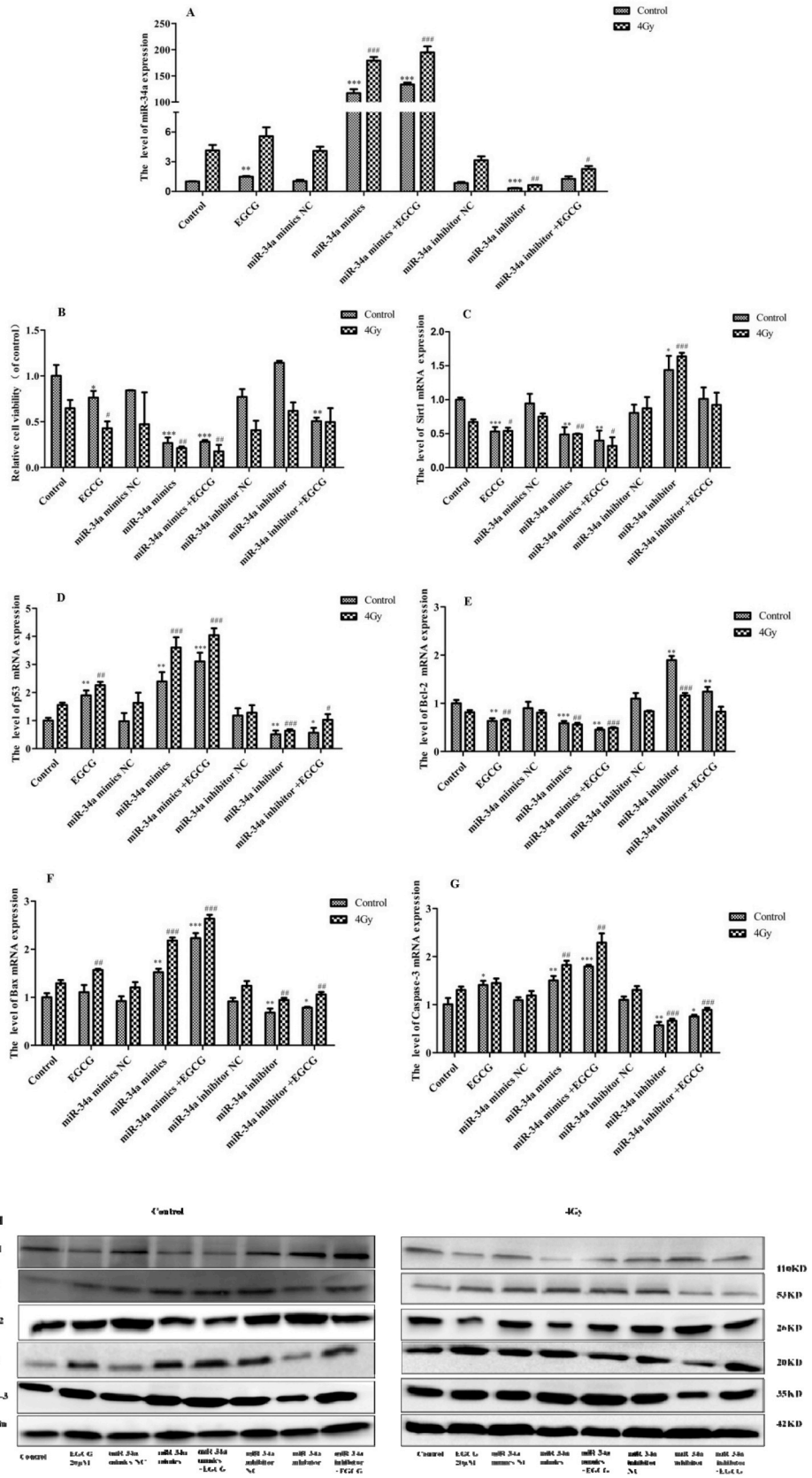


**Fig. 5.** EGCG played the role of radiosensitization by activating miR-34a/Sirt1/p53. H22 cells were transfected with miR-34a mimics or miR-34a inhibitor, and then treated with EGCG and $^{60}Co\gamma$ radiation. The expressions of miR-34a **(A)**, Sirt1 **(C)**, p53 **(D)**, Bcl-2 **(E)**, Bax **(F)**, Caspase-3 **(G)** were measured by qRT-PCR. The cells viability of H22 **(B)** was assessed by CCK-8. Significant difference analysis was the same as Fig. 1. The protein expression of Sirt1, p53, Bcl-2, Bax and Caspase-3 were assessed by Western blot **(H)**.

cancer cells. In this study, we found EGCG selectively protected normal cells and radiosensitized cancer cells. Our results showed that EGCG increased the growth of mouse normal hepatic cells AML-12 after $^{60}Co\gamma$ radiation, which was consistent with previous findings (Tiwari et al., 2017). The antioxidant activity of EGCG may account for radiation protection (Katiyar et al., 2001). EGCG provides a mild oxidative stress environment, improves the activity of antioxidant enzymes, and opens up the antioxidant defense mechanism for normal cells (Lambert and Forester, 2010). On the other hand, we also found that EGCG has radiosensitization on hepatoma cells through inhibiting significantly the growth of H22 cells. Researches have revealed that EGCG is involved the generation of reactive oxygen species, which can induce cellular oxidative DNA damages, apoptosis, regulate transcription factors and signaling pathways (Li et al., 2010; Chen et al., 2015). Moreover, the opposite effect of EGCG on H22 cells may be related to its pro-oxidant activity according to previous studies (Ni et al., 2018; Tsai et al., 2018).

Apoptosis, one form of programmed cell death, is considered to be a major process of the radiation-induced cell death (Rajagopalan et al., 2018). Therefore, the regulations of apoptosis-related pathways are the important mechanisms of radiation protection of normal cells and radiosensitization of cancer cells. MiRNAs can regulate cells proliferation, differentiation and death and some of them play important roles in the tumor radiation resistance, normal tissues toxicity or as predictive biomarkers of IR (Xu et al., 2014; Aryankalayil et al., 2018). MiR-34a is an emerging miRNA in recent radiobiology studies (Halimi et al., 2016; Lacombe and Zenhausern, 2017). Sirt1, an NAD-dependent deacetylase, could regulate apoptosis in response to oxidative and genotoxic stress. Moreover, Sirt1 has been confirmed to be a direct miR-34a target. Previous studies showed that Sirt1 modulated apoptosis through deacetylation of molecular targets that include p53 (Xiong et al., 2015). In addition, p53 could target of miR-34a and plays an important role in DNA damage response, DNA repair, cell cycle regulation, and triggering apoptosis after cell injury (Reynolds et al., 2018). Thus, the miR-34a/Sirt1/p53 signaling pathway may play a crucial role during cell death and radiosensitization. In our study, $^{60}Co\gamma$ radiation activated the miR-34a/Sirt1/p53 signaling pathway in normal cells, while played opposite effect in cancer cells.

Moreover, it is known that mitochondrial-mediated pathway mediates the regulation of apoptosis and Bcl-2 family and Caspase-3 are essential players in the pathway (Zhang et al., 2017b). The anti-apoptotic protein Bcl-2 blocks the release of cytochrome which can inhibit Caspase-3 (Zhu et al., 2018). In addition, the Bcl-2 family has been shown to be a p53 target (Beberok et al., 2018). P53 inhibits the function of Bcl-2 by inhibiting its transcription, while p53 can bind to Bax promoter to initiate mitochondrial-mediated apoptotic pathway. Our data showed that $^{60}Co\gamma$ radiation decreased the expression of anti-apoptotic protein Bcl-2 and increased pro-apoptotic proteins Bax and Caspase-3 by activating the miR-34a/Sirt1/p53 signaling pathways in normal and cancer cells. EGCG increased the expression of Bcl-2 and decreased the expression of Bax and Caspase-3 by inhibiting miR-34a/Sirt1/p53 signaling pathway in normal cells. In cancer cells, EGCG decreased the expression of Bcl-2 and increased the expressions of Bax and Caspase-3 by activating miR-34a/Sirt1/p53 signaling pathway.

## 5. Conclusions

In summary, EGCG has different effects on mouse hepatocyte and hepatoma cells. EGCG exerts radiation protection by inhibiting miR-34a/Sirt1/p53 signaling pathway, up-regulating the expression of anti-apoptotic protein Bcl-2, and down-regulating the expression of pro-apoptotic proteins Bax and Caspase-3 in AML-12 cells. However, EGCG has radiosensitization by activating miR-34a/Sirt1/p53 signaling pathway, down-regulating the expression of Bcl-2, and up-regulating the expression of Bax and Caspase-3 in H22 cells. Therefore, EGCG could be used as a potential natural radiosensitizer to make radiotherapy more effective and reduce IR damage to normal cells. Further studies are necessary for investigating mechanisms of EGCG through animal experiment.

## Declaration of competing interest

The authors declare that they have not known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Abbreviations

| | |
|---|---|
| BCA | Bicinchoninic acid |
| CCK-8 | Cell Counting Kit-8 |
| DMEM/F12 | Dulbecco's Modified Eagle Medium/Nutrient Mixture |
| EGCG | Epigallocatechin gallate |
| FBS | Fetal bovine serum |
| IR | Ionizing radiation |
| MiRNAs | MicroRNAs |
| MiR-34a | MicroRNA-34a |
| PVDF | Polyvinylidene fluoride |
| qRT-PCR | Quantitative realtime PCR |
| RPMI-1640 | Roswell Park Memorial Institute 1640 |
| Sirt1 | Sirtuin 1 |

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