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Caveolin-1 was involved in the reduced adiosensitivity to X-ray in human mammary epithelial MCF10A cells

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► ***Original article***

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ABSTRACT

Background*:** X-ray chest fluoroscopy is a compulsory component of the health examination procedure in China. The radiation dose from chest fluoroscopy is the largest in X-ray examination. More than half of the women in their twenties with breast cancer have been given X-ray fluoroscopy. Studies have shown that Caveolin-1 is involved in the repair of damage DNA in tumor cells induced by irradiation. However the mechanism and role of Caveolin-1 in normal human mammary epithelial cells are not clear. ***Materials and Methods*:** Here, normal human mammary epithelial cells (MCF10A) and the cells with Caveolin-1 knockdown ($MCF10A^{CE}$) were exposed to X-ray radiation to investigate the role of Caveolin-1 in the enhancement of radiosensitivity in these cells and the associated mechanism. ***Results: Decreased survival rate and a significantly higher level of cell arrest at the G1 and G2 phases, as well as reduced activation of the DNA damage repair proteins ATM and p53, and the stress protein p38MAPK were manifested by $MCF10A^{CE}$ cells compared to MCF10A cells, following exposure to X-ray radiation. Furthermore, binding between Caveolin-1 and Mdm2 in $MCF10A^{CE}$ cells was also lower than in MCF10A cells. ***Conclusion:*** Overall, the finding indicated that Caveolin-1 played an important role in decreasing the radiosensitivity of human mammary epithelial cells.

Keywords: *Caveolin-1, mammary epithelial cell, radiosensitivity enhancement, DNA damage repair.*

INTRODUCTION

Chest fluoroscopy is a commonly used in X-ray examination, and the dose of radiation applied for chest fluoroscopy is the largest in X-ray examinations. It is about 10 times the dose normally received from conventional X-ray examination. The chromosome aberration and micronucleus rates caused by DNA error repair induced by X-ray would increase in the cells half an hour after a chest X-ray is taken. According to the latest estimation of International Radiation Protection Committee, a city with a population of 10 million is predicted to have as many as 350 people with induced cancer, leukemia or other genetic diseases each year as a result of receiving an X-ray examination. X-ray is harmful to the human body, but the diagnosis of many diseases requires X-ray examination, making it a double edged sword. Among all the methods of X-ray examination, the harm inflicted by chest fluoroscopy is very clear. The United States, Japan and other developed countries have banned the use of chest fluoroscopy. Few countries are still using chest fluoroscopy, but the countries that do, are also trying to reduce its usage. Unfortunately, the chance of a patient receiving a chest fluoroscopy is very high in China, because chest fluoroscopy is a routine health examination component for new employees gaining entrance into a company or institution, either private or government owned.

Breast cancer has become a great social concern as it is considered to be a serious

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disease that threatens women's health at both physical and psychological levels. According to a report by the British "Sunday Telegraph", breast cancer is thought to be a result of patients receiving an X-ray in their early twenties. These patients have been exposed to large doses of X-ray irradiation, which generally does not have a significant immediate impact, but is enough to cause cancer decades later [1].

DNA is a key target of irradiation, so there should be some correlation between the irradiation sensitivity of the cells and level of DNA damage sustained by the cells. DNA damage caused by ionizing radiation includes double strand breaks (DSBs), single strand breaks (SSBs), base changes, protein cross-linking and the formation of a dimer. Among them, DSBs is very damaging because it can lead to the loss of the gene coding sequence, destroying the communication between the gene coding region and its regulatory region, adversely affecting chromosome structure and blocking DNA replication [2]. Therefore, DSBs are closely related to irradiation sensitivity. Most irradiation sensitive cells are accompanied by defects in DSB repair. The radiosensitivity of tumor cells is closely related to DSB repair, and the repair rate of DSBs is negatively correlated with the radiosensitivity of the cells. Thus, it has been considered that susceptibility to DNA damage repair could well be the main cause of cell radiosensitivity [3, 4].

Ataxia-telangiectasia mutated (ATM) is the key protein of the HR repair pathway triggered in response DSBs. It plays important roles in the induction and transfer of DNA damage signals as well as in initiating DNA damage repair [5]. When DNA damage occurs, ATM is autophosphorylated, which–would activate and regulate a series of proteins related to DNA damage repair, such as histone family 2A variant (H_2AX), RAD51, p53, and murine double minute 2 (Mdm2), enabling these proteins to aggregate at the site of DNA damage to further activate the downstream proteins and begin DNA repair [6].

Caveolin-1, the major component of caveolae, is a 21-24 kDa integral membrane protein that is involved in material exchanges between intracellular and extracellular environments, assembly of lipid or lipid signaling molecules, and also in several other signaling pathways [7, 8]. Recent studies had shown that Caveolin-1 might be involved in DNA damage repair induced by radiation, affecting the radiation sensitivity of the cells. Our previous study had shown that the expression of Caveolin-1 in human mammary epithelial cells is significantly changed after exposure to a low dose of radiation [9]. Furthermore, we found that the expression level of Ku80, p53 and phosphorylated Mitogen-activated protein kinase (p-MAPK) were changed by knocking down Caveolin-1 [10]. These results suggest that Caveolin-1, which functions as a membrane signal transduction protein, might participate in cellular radiation response and DNA damage repair process following exposure to radiation, and such activities of Caveolin-1 may affect the radiosensitivity of the cells. However, the role and mechanism of Caveolin-1 with regard to the radiosensitivity of normal human mammary epithelial cells have been largely ignored.

In this study, the effect of Caveolin-1 on cell survival and cell cycle arrest were investigated in two different cell lines, normal human mammary cell line MCF10A and its Caveolin-1 knockout derivative, MCF10A^{CE}, following exposure to X-ray radiation. The status of DNA damage and the expression levels of proteins associated with DNA damage repair and radiation stress in these cells were also examined to obtain insights into the role of Caveolin-1 with respect to X-ray irradiation injury and to look for potential targets that may be important for the protection against radiation.

MATERIALS AND METHODS

Materials and reagents

Human mammary epithelial cell line MCF10A was purchased from ATCC (CRL-10317™), MCF10A^{CE} cell line had previously been established in our laboratory for knocking down Caveolin-1 in MCF10A by RNAi technology [11], in which Caveolin-1 was knocked down at about 70%. Dulbecco's modified eagle's medium-F12

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(DMEM/F12). Horse serum was obtained from Hyclone Biotechnology. Insulin, cholera toxin, epidermal growth factor and hydrocortisone were purchased from Sigma, USA. ECL™ reagent was purchased from Amersham Pharmacia Biotechnology. All antibodies used in this study are listed in table 1. Other reagents were obtained locally.

Cell culture

The cell lines were cultured in DMEM/F12 medium containing horse serum (5%), Insulin (10 μg/mL), Choleratoxin (100 ng/mL), epidermal growth factor (EGF, 20 ng/mL), Hydrocortisone (1.4 μmol/L), and supplemented with Penicillin (100 U/mL) and Streptomycin (100 mg/L) before use. The cells were cultured in culture bottle and 24-well plates in incubator with 5% CO_2 at 37°C, and the medium was replaced every 48 h. The cells of logarithmic growth phase were selected for experiments, and the cell survival rates were more than 95% (trypan blue dyeing count method) [11].

Cell irradiation treatment

The X-ray energy used to irradiate the cells was 8 MeV. The dose rate was 3 Gy/min, the source skin distance was 100 cm (SSD=100 cm), and the irradiation field was adjusted with the size of the cell culture plate.

Cell viability assay

Cell number and viability was evaluated by trypan blue exclusion assay. The cells were cultured in 24-well plates at a density of 5000 cells/well. After being irradiated with different doses of radiation (from 0 to 10 Gy, with increment of 1 Gy), the cells were treated with 10% trypan blue reagent, and the viability of the cells after 24 h of incubation was then quantified by counting the number of viable and dead cells using a hemocytometer.

Colony formation assay

Cells were seeded in 60-cm culture dishes followed with X-ray irradiation at different doses (from 0 to 10 Gy, with incensement of 1 Gy) and subsequent culturing for 10-14 d. After that, the cells were fixed with methyl alcohol for 5 min, and then treated with 0.4% crystal violet for 10-15 min. Colonies containing more than 50 cells were identified as surviving cells under a stereomicroscope.

Cell cycle analysis

Cells that had been exposed to 6 Gy X-rays were harvested 24 h or 48 h after the exposure and washed twice with PBS. They were fixed in 75% pre-chilled ethanol for over 24 h at 4 °C and then stained with 0.5 mL propidium iodide (PI) (including 0.3 mg/mL RNAase, 20 ug/mL PI) for 30 min in the dark. Cell cycles were analyzed using a FACS Calibur type flow cytometry.

Western blot

The harvested cells were washed twice with cooling phosphate-buffered saline (PBS) and then lysed in radio immunoprecipitation assay (RIPA) buffer. Proteins were resolved by 10% sodium dodecyl sulphate polyacrylamide gelelectrophoresis (SDS-PAGE), transferred to a polyvinylidene fluoride (PVDF) membrane. The membranes were probed with primary antibodies at 4˚C overnight and secondary antibody at 37˚C for 1 h. Signals were detected using enhanced chemiluminescence (ECL) detection reagents and scanned with the Storm Phosphor Imager. The density of each band was quantitated using Image Quant analysis. Equal loading was assessed by immunoblotting with a marker, i.e., β-actin [11].

Immunofluorescence

Cells were fixed in 4% paraformaldehyde in PBS for 10 minutes. After being permeabilized with 0.4% Triton X-100 for 10 minutes at room temperature, cells were blocked in 4% bovine serum albumin (BSA) supplemented PBS for 1 hour and incubated overnight at 4°C with the primary antibody. After 3 washes in PBS, the cells were labeled with TRITC-conjugated secondary antibody [11].

Statistical analysis

Data analysis was performed using the *t-test* and analysis of variance. Data were expressed as

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means ± SDs, and significant differences were considered at the *($^{\Delta}$)P<0.05 or **($^{\Delta\Delta}$)P<0.01 level.

Table 1. List of antibodies used in this study and their sources.

Antibodies	Sources
Anti-b-Actin monoclonal antibody.	Wuhan boster Biological Engineering Co., Ltd.
Goat anti-Rabbit IgG-HRP antibody and anti-Mouse IgG-HRP antibody.	Beijing Zhongshan Jinqiao Biological Engineering Co.
Anti-Caveolin-1 polyclonal antibody, anti-ATM monoclonal antibody, anti-p53 monoclonal antibody, anti-γH_2AX monoclonal antibody, and anti- Mdm2 monoclonal antibody.	Santa Cruz Biotechnology Inc.

RESULTS

Effects of Caveolin-1 knockdown on the radiosensitivity of human mammary epithelial cells

The ability of MCF10A and MCF10A^{CE} cells to withstand X-ray irradiation was investigated by assessing the survival of the cells following X-ray exposure using colony formation assay. The survival of the cells decreased with increasing doses of radiation, with almost complete zero survival for MCF10A^{CE} cells after receiving 10 Gy of X-ray, while MCF10A cells showed better resistance to X-ray irradiation (figure 1A). The number of colonies formed by MCF10A^{CE} and MCF10A following X-ray exposure clearly decreased with increasing doses of X-ray, with MCF10A^{CE} cell showing fewer colonies than MCF10A cells (figure 1B). As for individual colonies, MCF10A exhibited more compacted cells, with the cell masses consisting of more tightly packed cells. Although the size of the colony was smaller with 6 Gy X-ray, the cell density of the colony appeared to remain unchanged (figure 1B). On the other hand, the cells in the colony formed by MCF10A^{CE} cells appeared to be smaller in size, and the cells were not as tightly packed. After treatment with 6 Gy X-ray radiation, the cells became somewhat scattered, with most cells retained in the central part of the colony. MCF10A cells were therefore more resistant to X-ray irradiation than MCF10A^{CE} cells, and since MCF10A^{CE} cells have no functional Caveolin-1 gene, the result suggested that knocking out the Caveolin-1 gene would increase the sensitivity of the MCF10A cells to X-ray radiation.

Effect of Caveolin-1 knockdown on the formation of DSBs induced by irradiation

One of the early events that occurs after DSBs are formed is the phosphorylation of the -COOH terminus of histone H_2AX, and thus phosphorylated H_2AX (gH_2AX) is considered to be a marker for DSBs. The formation of γH_2AX could be clearly observed under a fluorescence microscope after staining with a fluorescence-tagged antibody. To see whether Caveolin-1 knockdown would affect DNA damage following X-ray irradiation, the expression level of γH_2AX was analyzed. The expression level of γH_2AX was higher in MCF10A^{CE} cells than in MCF10A cells, as shown by the higher level of fluorescence signal (figure 2A), indicating the presence of aggravated DSBs in MCF10A^{CE} cells (figure 2B). This again showed that knocking down Caveolin-1 aggravated DNA damage induced by radiation. At the same time, due to the continuous existence of γH_2AX, which represented the presence of unrepaired DNA damage, the result therefore suggested that knocking down Caveolin-1 would impair DNA damage repair.

Effect of Caveolin-1 knockdown on the cell cycle induced by irradiation

The cell cycle checkpoint is one of the key features that are affected by a DNA-damaging agent. To determine the effect of Caveolin-1 on the cell cycle following irradiation of the cells with X-ray, changes in the cell cycles of MCF10A and MCF10A^{CE} cells treated with 6 Gy X-ray were examined over time. In both cells, the cell number in the G1/G2 phase increased, but decreased in the S phase 24 h after irradiation, indicating that the cell cycle was arrested at the G1 and G2 phases (figure 3, table 2). However, the cell number in the G2 phase suddenly

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decreased 48 h after irradiation. Overall, the arrest of the cell cycle at the G1 and G2 phases was more significant for MCF10A^{CE} cells. This suggested that Caveolin-1 might affect the repair of damaged DNA through directly or indirectly regulating the cell cycle.

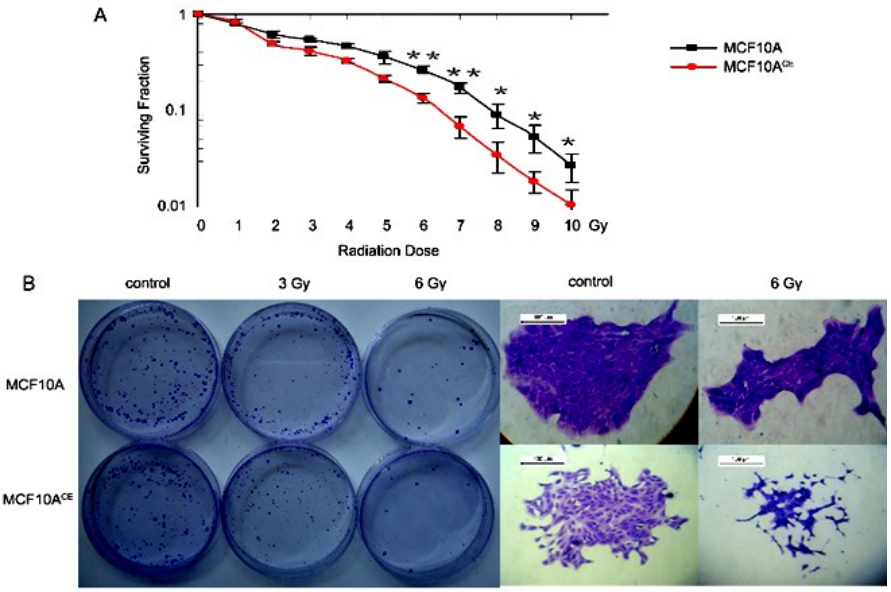


Figure 1. Effect of X-ray irradiation on the survival and colony formation of MCF10A and MCF10ACE cells. **(A)** Survival curve. The cells were cultured for 24 h following X-ray treatment and the number of death and live cells were then determined by Trypan blue assay. Data are the means ± SDs from triplicate determinations. "**" and "*" indicates significantly different from control (non-irradiated) cells at the P<0.01 and P<0.05 levels, respectively. **(B)** Colony formation. The cells were cultured for 10 days following X-ray treatment. The left panel shows the distribution of cell colonies and the right panel shows the appearance of the colonies. Only representative images are shown. n=3.

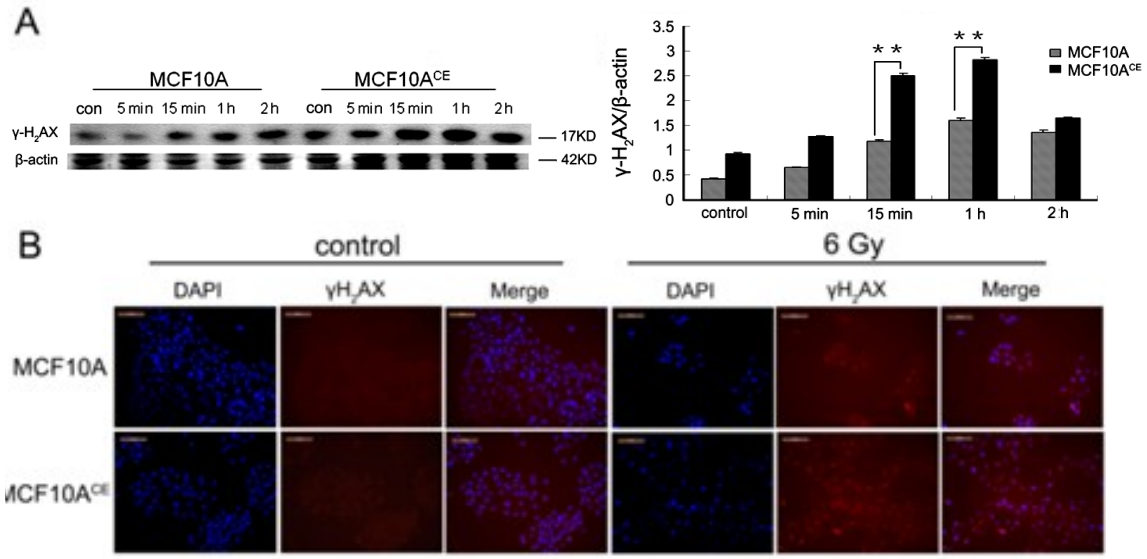


Figure 2. Effect of X-ray irradiation on γH2AX level in MCF10A and MCF10ACE cells. **(A)** γH2AX level as detected by Western blot. Data are the means ± SDs from triplicate determinations. "**" indicates significantly different at the P<0.01 level. **(B)** γH2AX level as detected by immunofluorescence. The cells were cultured for 24 h following X-ray treatment. Representative images are shown. n=3.

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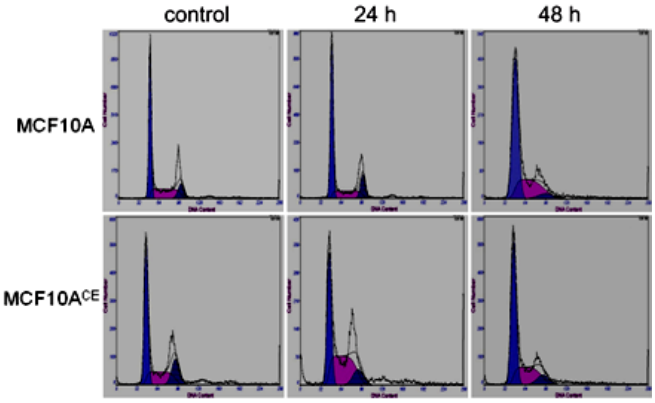


Figure 3. Effect of X-ray treatment on cell cycle of MCF10A and MCF10ACE cells. Non-irradiated cells (control) and those irradiated with 6 Gy X-ray were subjected to flow cytometry analysis. Flow cytometry charts showing the distribution of cells within the different cycles. Non-irradiated cells (control) and irradiated cells collected at 24 h or 48 h after irradiation. n=3.

Table 2. Comparison of the percentages of MCF10A and MCF10A^{CE} cells at different stages of the cell cycle after X-ray treatment.

		Percentage of cells		
Cell	**Time (h)**	**G1**	**S**	**G2**
MCF10A	con	53.1±0.95	33.3±0.84	13.6±0.79
	24	59.8±0.67**	24.1±0.43**	16.1±0.52*
	48	61.7±0.86$^{\Delta}$	32.4±0.88$^{\Delta\Delta}$	5.91±0.65$^{\Delta\Delta}$
MCF10A^{CE}	con	41.6±0.42	46.8±0.49	11.5±0.64
	24	51.7±0.56**	28.6±0.64**	19.5±0.58**
	48	59.0±0.57$^{\Delta\Delta}$	31.7±0.87$^{\Delta}$	9.19±0.43$^{\Delta\Delta}$

Effects of Caveolin-1 knockdown on the activation of DNA damage repair pathway induced by irradiation

ATM is an important member of the HR DNA repair pathway, and its expression level directly reflects the repair capacity of the cell. To obtain further insight into the effect of Caveolin-1 on the activation of DNA repair pathway induced by irradiation, changes in the expression levels of phosphorylated ATM (p-ATM) and ATM in MCF10A and MCF10A^{CE} cells following irradiation with 6 Gy X-ray were measured over time. The level of p-ATM in both cell lines increased significantly 15 min after irradiation, and then decreased sharply at 1h after irradiation, although on the whole, MCF10A^{CE} cells exhibited a significantly lower level of p-ATM than MCF10A cells (figure 4A). In addition to ATM, X-ray treatment also caused similar changes to the expression of p53, which is a downstream target gene of ATM (figure 4B). The results showed that Caveolin-1 did have an effect on the activation of DNA damage repair pathway in a cell following irradiation.

Mdm2 is a negative regulator of p53. When DNA damage occurs, p53 would dissociate from Mdm2 and the free Mdm2 then proceeds to negatively regulate the process of DNA damage repair. The expression of ATM is known to be regulated by Caveolin-1 when DNA is damaged [12]. Thus the connection between Caveolin-1 and Mdm2 was further investigated by probing whether the two proteins are colocalized. The results showed that there was no Caveolin-1 localized with Mdm2 in the control group, but showed a significantly high level of Caveolin-1 colocalized with Mdm2 in MCF10A cells at 12 h following irradiation with 6 Gy X-ray (figure 4C). This suggested that the colocalization of Caveolin-1 with Mdm2 might be induced by X-ray irradiation, which would effectively relieve the antagonistic action of Mdm2 toward p53.

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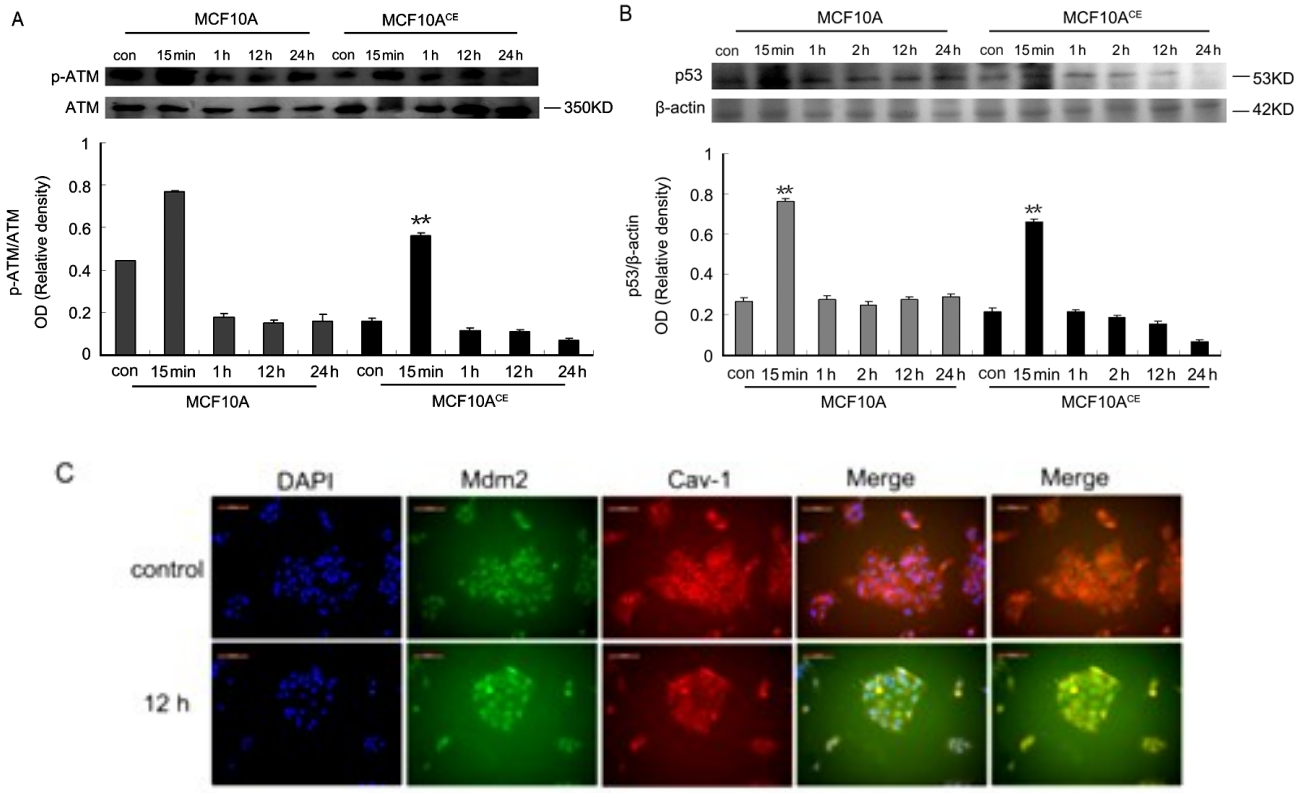


Figure 4. Effect of X-ray on the expressions of p-ATM, p53 and Mdm2 in MCF10A and MCF10A^{CE} cells. Cells were irradiated with 6 Gy X-ray and the levels of p-ATM **(A)**, and p53 **(B)** following different times (15 min, 1 h, 12 h and 24 h) of incubation were determined by western blot. The plot below the blot in A shows the intensity of the phosphorylated band relative to that of the non -phosphorylated band, whereas the plot below the blot in B shows the intensity of the p53 band relative to that of the β-actin band for each time point. Data are the means ± SDs from triplicate determinations. "**" indicates significantly different from control (non-irradiated) cells at the P<0.01 level. **©** Co-localization of Mdm2 and Caveolin-1 in MCF10A cells as detected by immunofluorescence analysis. Representative images are shown. n=3.

Effects of Caveolin-1 knockdown on the activation of radiation stress and apoptosis pathways induced by irradiation

P38MAPK pathway plays an important role in inflammation and stress response, and also participates in the process of cell survival, differentiation and development. In order to determine how Caveolin-1 might affect the pathway involved in the repair of DNA damage induced by irradiation, the levels of p-p38MAPK and p38MAPK in MCF10A and MCF10A^{CE} cells were measured following irradiation with 6 Gy X -ray. The level of p-p38MAPK in the MCF10A^{CE} cells was significantly lower than that in MCF10A cells (figure 5A), suggesting that knocking down Caveolin-1 might affect the activation of the radiation stress pathway.

To further explore the involvement of Caveolin-1 in apoptosis while participating in the repair of damaged DNA induced by irradiation, the expressions of apoptosis related proteins Bcl-2 and in the two cell lines were also measured after irradiation with X-ray. The expression of Bcl-2 in both cell lines was down regulated after irradiation with 6 Gy X-ray, but again, MCF10A^{CE} cells displayed a lower level compared to MCF10A cells (figure 5B). Furthermore, the apoptotic pathway mediated by Caspase-3 was not activated (figure 5C), suggesting that knocking down Caveolin-1 had no obvious effect on the apoptosis pathway.

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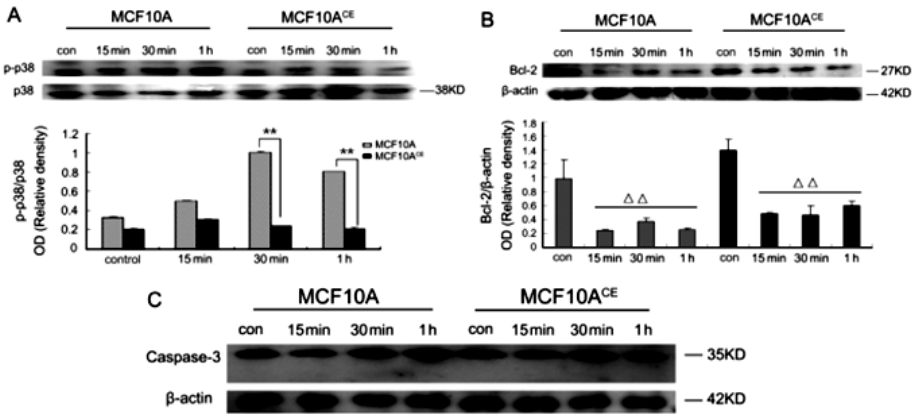


Figure 5. Effect of X-ray irradiation on the expression levels of p-p38, Bcl-2 and Caspase-3 in MCF10A and MCF10ACE cells. Cells were irradiated with 6 Gy X-ray and the levels of p-p38 **(A)**, Bcl-2 **(B)** and Caspase-3 **(C)** following different times (15 min, 30 min and 1 h) of incubation were determined by western blot. The plot below the blot in A shows the intensity of the phosphorylated band relative to that of the non-phosphorylated band, whereas the plot below the blot in B shows the intensity of the Bcl-2 band relative to that of the β-actin band for each time point. Data are the means ± SDs from triplicate determinations. "**" indicates significantly different at the P<0.01 level. "△△" indicates significantly different from control (non-irradiated) cells at the P<0.01 level. n=3.

DISCUSSION

Numerous studies have shown that Caveolin-1 expression is either decreased or absent in most tumor cells. This suggests that it might perform a variety of cellular functions (e.g., acting as a tumor inhibitor) that are closely related to the transformation, proliferation, differentiation, invasion, metastasis and apoptosis of tumor cells [8]. Clinical study has found that pancreatic cancer tends to be more resistant to the killing effect of irradiation during the course of radiotherapy. Cordes *et al.* reduced the expression of Caveolin-1 in pancreatic cancer cells via siRNA and found that the survival rate of these cells decreased significantly after X-ray irradiation [13]. These authors thought that Caveolin-1 might interact with intracellular integrin and focal adhesion protein, and therefore by decreasing the expression of Caveolin-1 in the cell, it would decrease the association between the cell and extracellular matrix, consequently having an impact on survival when the cell is exposed to radiation. Other investigators have found that loss of Caveolin-1 can cause the hyper-proliferation of intestinal crypt stem cells in mice given 6 Gy of radiation, resulting in reduced self renewal ability for the stem cells, but with increased apoptosis [14]. The colony formation assay showed that the survival ability of MCF10A^{CE} cells, which expressed a lower level of Caveolin-1, was indeed lower than that of the MCF10A cells after irradiation (figure 1). This observation was consistent with previous results, indicating that the role of Caveolin-1 cannot be ignored although the mechanism of its participation in the radiation-induced damage response in a cell is not clear.

There is no doubt that the DNA damage is the main detrimental biological effect of radiation, in particular, ionizing radiation, which can induce DNA break in irradiated cells directly or indirectly, consequently leading to cell death, loss of proliferation, and mutation [15]. Double-strand break (DSBs) is the most serious form of DNA damage, and it can seriously compromise the ability of the cell to survive [16]. Several studies have demonstrated that the γH$_2$AX is correlated with DSBs within a 1:1 ratio, so it can be used as an effective index for detecting the presence of DSBs [17, 18]. MacPhail *et al.* also found that the formation of the focal point of γH$_2$AX induced by ionizing radiation is not dependent on the cell type, and the γH$_2$AX focus is rapidly formed after the occurrence of DSBs, and then gradually disappears after DNA repair [17]. Our results showed that knocking down Caveolin-1 aggravated DNA damage and affected DNA damage repair following exposure

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to radiation (figure 2). ATM is a kinase whose activation is caused by the phosphorylation of Ser1981 by DSBs, and H_2AX is the substrate of the enzyme. The formation of γH_2AX mediated by ATM must be related to the presence of DSBs. Thus, simultaneous activation of ATM and γH_2AX in the same cell is strong evidence of the presence of DSBs [19]. In our experiments, ATM was activated in both cell lines, indicating the occurrence of DSBs, which was consistent with the results of γH_2AX.

Although complex and precise regulatory mechanisms are present in the cell to help maintain genome integrity, the damage inflicted on the DNA by radiation is not always reparable. If the damaged DNA cannot be repaired quickly, the cell cycle checkpoint will be activated, which will cause the cell to become arrested to buy time for the DNA repair process to repair the damaged DNA [20, 21]. Knocking down Caveolin-1 increased the cell cycle arrest after the cells were treated with 6 Gy X-ray (figure 3), suggesting that Caveolin-1 may be involved in DNA damage repair through directly or indirectly regulating the cell cycle.

When DSBs occurs, the DNA damage repair process will be activated, which subsequently leads to the recruitment of DNA repair factors to the sites of DNA damage. These DNA repair factors will activate ATM kinase, which will then become autophosphorylated. The activated ATM kinase will phosphorylate a variety of important functional substrate proteins, which then accumulate at the damaged DNA, and further activate downstream proteins to initiate the DNA repair [22, 23]. p53 is a downstream protein of ATM, and it regulates the cell cycle and induce periodic cell arrest, as well as recruiting other repair factors to the broken ends of the DNA in order to start the repair process [24]. Mdm2 is a negative regulator of p53, and both proteins would interact with each other to form a negative feedback. In normal cells, Mdm2 and p53 are maintained in a balanced stage, but when DNA damage occurs, they would dissociate and negatively regulate the expression level of each other [25]. Caveolin-1 would combine with Mdm2 and activate the p53 -mediated aging signaling pathway when the cells are under the condition of cellular oxidative stress [26]. Increased colocalization of Caveolin-1 with Mdm2 following exposure of the cells to X-ray radiation relieved the inhibitory action of Mdm2 on p53 (figure 5). This would then allow p53 to induce cell cycle arrest in favor of DNA repair, or to induce apoptosis if the damage to the DNA is severe or cannot be repaired.

On the other hand, as Caveolin-1 is located on the cell membrane, it will probably regulate the phosphorylation of ATM. Previous study has suggested that Caveolin-1 may work in conjunction with serine/threonine protein phosphatase (PP2A), the inhibitor of ATM [27]. Volonte *et al.* found that the level of phosphorylated ATM in Caveolin-1 deficient mouse fiber cells was lower than the level in normal mouse fiber cells when these cells were subjected to oxidative stress [12]. These authors thought that Caveolin-1 might interact with PP2A, thereby regulating the activation of ATM. However, knocking down Caveolin-1 resulted in reduced phosphorylation of ATM after the cells were treated with 6 Gy X-ray (figure 4A), consistent with the above results. Thus the findings of this study suggested that Caveolin-1 plays an important role in protecting mammary cells against the damaging effect of X-ray radiation by contributing to the DNA repair process. However, DNA damage repair by Caveolin-1 appears to be a complex process, and the specific mechanism of this pathway requires further work.

CONCLUSION

Caveolin-1 played an important role in decreasing the radiosensitivity of human mammary epithelial cells.

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Conflicts of interest*: *Declared none.

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