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Effects of Low-Dose X-Ray on Cell Growth, Membrane Permeability, DNA Damage and Gene Transfer Efficiency

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Abstract

Background: We aimed to reveal if low dose X-rays would induce harmful or beneficial effect or dual response on biological cells and whether there are conditions the radiation can enhance gene transfer efficiency and promote cell growth but without damage to the cells.

Method: A systematic study was performed on the effects of Kilo-V and Mega-V X-rays on the cell morphology, viability, membrane permeability, DNA damage, and gene transfection of 293 T and CHO cells.

Results: The Kilo-V X-rays of very low doses from 0.01 to 0.04 Gray in principle didn't induce any significant change in cell morphology, growth, membrane permeability, and cause DNA damage. The Mega-V X-ray had a damage threshold between 1.0 and 1.5 Gray. The 0.25 Gray Mega-V-X-ray could promote cell growth and gene transfer, while the 1.5 Gray Mega-V X-ray damaged cells.

Conclusion: The very low dose of KV X-rays is safe to cells, while the effects of Mega-V-X-rays are dose-dependent. Mega-V-X-rays with a dose higher than the damage threshold would be harmful, that between 1.0 -1.5 Gray can evoke dual effects, whereas 0.25 Gray MV X-ray is beneficial for both cell growth and gene transfer, thus would be suitable for radiation-enhanced gene transfection.

Keywords

low dose ionizing radiation, biological effects, KV and MV X-rays, 293 T cell, CHO cell

Introduction

It is well known that high doses of ionizing radiation can lead to various harmful biological and health effects, such as cell mutation, cell killing (necrosis and apoptosis), skin burns, hair loss, birth defects, illness, cancer, and death.[1] But for low doses of ionizing radiation, their biological and health effects are still in dispute. From the conventional radiobiological point of view, the low-level dose effects can be extrapolated from high-level dose information, so even exposures in the level of 0.01 Gy are believed harmful.[2] However, in the last 2 decades, there was increasing evidence reporting that low dose ionizing radiations also have beneficial effects on living organisms, including immune enhancement,[3] anti-inflammatory,[4-6] radiation hormesis,[7] cell growth stimulation,[8] lower mortality rate and cancer frequency.[9,10] Therefore, whether low dose radiations are harmful or beneficial, or they may induce dual responses, questions still need to be answered. The underlying molecular mechanisms of the effects remained largely unclear as well.

For most people living in the earth, they seldom have the chance to be exposed under a high dose of ionizing irradiation, whereas each of us is under low dose radiation every moment from background exposure, or by X-ray diagnostics and imaging when taking a medical examination. So we should pay more attention to the biological effect of low dose ionizing radiation. Especially for the people working in or living near ionizing radiation facilities, or high natural background radiation environment, it is important to know the truth about the biological and health effects of low dose ionizing radiation.

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Accordingly, we performed a systematic study on the immediate effects of low dose ionizing radiation on 2 kinds of living cells at both cellular and molecular levels. Since we mainly concern about the dose-response of very low and low dose ionizing radiations, we chose two kinds of X-rays for our investigation. One was that from a 120 KV X-ray generator with a very low dose (from 0.01 to 0.04 Gy). Such X-rays are generally used for different kinds of X-ray diagnostics from dental to chest examinations and medical imaging. Similar doses of KV X-rays with various KV values are also widely used for industry non-destructive testing, security inspection, XRF((X-Ray Fluorescence), and NDT (Non-Destructive Testing) imaging. The other was generated by a 6 MV Medical Linear Accelerator with doses from 0.25 to 1.50 Gy and taken as the low dose group for the present study. The X-rays from this kind of generator are mainly used for medical treatments. The two groups of radiation are the typical ionizing radiations that members of the public would meet in medical diagnosis, radiotherapy, and some occupational exposures, while their effects on cells are not well understood. So we selected the radiations for our study, we hope that it will not only let us have a better understanding on the dose-response of biological cells to the ionizing radiation that people may have the most opportunity to be irradiated in their lifetime but also help to evaluate that of background exposures by extrapolating their effects to other ionizing radiations using the concept of dose equivalent. As we know that, in some areas of high background exposure and occupational exposure in the world, the annual exposures of the residents are about a similar dose (0.002-0.05 Gy) as that of the KV X-rays in this study but with much lower dose rates(about 3.5×10^{-7} Gy/h).[11,12]

The cells chosen for the study were CHO and 293 T cells. The reason to use the cells was that CHO (Chinese hamster ovary) cells are often employed in biological and medical researches and well-characterized as to DNA repair and DDR(DNA damage response), the cells are also one of the most suitable mammalian hosts for production of recombinant protein therapeutics. Similarly, 293 cells, a specific cell line originally derived from human embryonic kidney cells grown in tissue culture, are also widely used in cell biology researches because of their reliable growth and propensity for gene transfection.

In this study, we will investigate the effects of very low and low dose X-rays on the cells' morphology, viability, membrane permeability, apoptosis, and DNA mutation. We will also explore the effect of X-rays on the plasmid-based GFP transfer efficiency of the cells for it can provide further information about the radiation-related effects on membrane permeability and DNA stranded breaks/repair/recombination machinery. On the other hand, gene transfection into cells is a rate-limited step of gene therapy, it needs a simple, effective, and safe method for gene transfection. Although some previous researches had found the irradiation of ionizing radiation could enhance gene transfer efficiency in living cells, the enhancement was generally achieved using doses that would induce a decline of cell viability.[13-17] Therefore, if we can find out some irradiation conditions that the X-rays can enhance gene transfer efficiency but at the same time promoting cell growth, we will make gene transfection and therapy more practical. We hope that such a study can help to answer the questions of what kind of effect would be induced by the very low and low dose X-ray radiations, and which dose of the radiation would induce a harmful or beneficial effect or dual response on biological cells. At the same time, it would reveal the capability of the radiation as a tool to stimulate cell growth and gene transfer efficiency.

Materials and Methods

Cells and Reagents

CHO cells and 293 T cells were purchased from Procell Biotechnology Co.(Wu Han, China). The DMEM and Ham's F-12 K (Kaighn's)culture-media, FBS fetal bovine serum, and pancreatin were purchased from Gibco (USA). Double antipenicillin streptomycin and PBS were purchased from Hycolone (USA). The culture medium of CHO cell was prepared with F-12 K, FBS fetal bovine serum, and pancreatin in the ratio of 89:10:1; while the culture medium of 293 T cell was prepared with DMEM, FBS fetal bovine serum and pancreatin in the ratio of 89:10:1. The cells were incubated into 96 ~ well Plates with a cell density of 2000 /200 μL per well for 24 hours until they were ready for irradiation.

Radiation Instruments and Irradiations

Two kinds of X-ray irradiators were used for the irradiation. One was a 120 KV X-ray generator (KXO-50XM, TOSHIBA, Japan) with a dose rate of 0.01 Gy/s, another was a 6 MV Medical Linear Accelerator (Primus M, SIEMENS, Germany) with a dose rate of 2 Gy/min·m. The cells were divided into 8 dose groups, 4 for 6 MV Medical Linear Accelerator: A1- 0.25 Gy, A2- 0.50 Gy, A3- 1.0 Gy, A4- 1.5 Gy; 4 for 120 KV X-ray generator: B1- 0.01 Gy, B2- 0.02 Gy, B3- 0.03 Gy, B4- 0.04 Gy. All of the samples were irradiated with SSD radiation field mode (field size was 15 cm×15 cm).

Morphological Observation

The morphology of the cells was observed and recorded by a Nikon TE 300 microscope (Nikon, Japan) with a PCO camera (PCO. Germany). A homemade artificial intelligent image recognition and analysis system was used for cell automorphological analysis. The system was developed using the custom-designed algorithms in MATLAB (MathWorks, Inc., Natick, MA) with computer vision libraries (OpenCV). It integrated all the functions of pattern recognition and feature extraction and a machine learning algorithm of Least Absolute Shrinkage and Selection Operator for the artificial-intelligent automatically pattern recognition and analysis to identify the cells with nuclear translocation, pycnosis, and aberration. The pattern recognition and analysis were firstly performed on 3 thousand cells on 50 slides to pre-train the system for

machine learning, and then on about 1000 cells from 6 slides of each experimental group.

Cellular Viability Determination

The viability of the cells was examined by Cell Counting Kit-8 (CCK-8) assay. In the assay, both the CHO and 293 T cells were respectively seeded in 96-well plates with a density of 2000 /200 ml per well and incubated for 24 hours. Thereafter, 10 μL of CCK-8 reagent was added into each well to incubate the sample cells for 2 h, and then the absorbing density (OD) of each well was measured using an ELISA Microplate Reader at 450 nm. The cellular viability in each group was determined by using the formula: $[(ODR - ODB)/ (ODC - ODB)] \times 100\%$. In here, ODR is the OD of the irradiated cells, ODB is the OD of the blank, and ODC is the OD of the control. The final data were obtained from the averages of 192 wells for each group of cells.

Membrane Permeability

The activities of the Na^+-K^+-ATPase and Ca^{2+}-ATPase on the membrane of the cells were measured with a hamster and human Na^+-K^+-ATPase ELISA kit and Ca^{2+}-ATPase ELISA kit (both were purchased from JianYang Biotechnology Co., Guang Zhou, China). The intracellular free Ca^{2+} was determined by fluorescence microscopic observation (Nikon, Japan) and flow cytometer (Beckman Coulter, USA) with Calcium fluorescence probe Fluo-3 AM (Beyotime Biotechnology, Jiang Su, China) as the probe.

DNA Damage Assay

The DNA damage of the cells was determined by Comet assay[18] with a DNA damage detecting kit (Cell Biolabs, USA). The DNA DSBs (double-stranded breaks) and their repairing were measured using immunofluorescence γ-H2AX assay. γ-H2AX is a sensitive and early indicator of double-stranded breaks (DSBs) in vitro and in vivo, it allows fluorescent visualization and physical localization of the DSBs.[19] It is also a key factor in the repair process of damaged DNA, when there is a DNA double- stranded break, γ-H2AX is recruited to damage sites, which in turn recruits other DNA repair machinery to repair the damaged DNA.[19] In our experiment, γ-H2AX was measured in a way similar to that described previously.[20] The irradiated cells were washed with PBS three times and then fixed by paraformaldehyde for 20 minutes. Thereafter, the cells were washed with PBS three times again and stained overnight at 4°C by using a specific γ-H2AX antibody (1:1000, Abcam, USA). The next day, samples were incubated for 2 hours in FITC-conjugated goat anti-mouse secondary antibody (1:100, Earthox, USA) at room temperature, washed in PBS three times, and then mounted with mounting medium (Leagene, Beijing) ready for microscope observation.

GFP Transfection

The GFP plasmids were transfected into the cells by incubating the cells with the transfection mixture solution of GFP plasmid solution (5 μL GFP plasmid + 500 μL DMEM solution) and PEI solution (25 μL PEI + 500 μL DMEM solution) for 30 min. The cells were then irradiated by X-ray. After the irradiation, the cells were incubated in a CO_2 incubator for 4 h with the transfection mixture solution. Thereafter the mixture solution was replaced by the culture medium and continually incubated for 48 h. The GFP transfer efficiency was determined by fluorescent microscopy (Nikon TE 300, Japan) and flow cytometry (Beckman Coulter, USA).

Data Processing

F test was used for statistical analysis of the data. $P \leq 0.05$ was considered statistically significant and significant difference was noted with * in figures.

Results

Morphology and Cell Growth of Cho and 293 T Cells

Figure 1 shows the morphology of CHO and 293 T cells vs. time after they were exposed to different doses of X-rays. We can see that there was no morphological difference between the irradiated groups and the control. The AI image analysis also indicated that no nuclear translocation, pycnosis, and aberration was observed in the irradiated cells. Both CHO and 293 T cells could normally grow with cell density and morphology similar to the control, indicating that the irradiation didn't impact the cell morphology and growth to induce mutation.

Cellular Viability

Figure 2 illustrates the cellular viability of both cells under different doses of irradiation. Interestingly, the cellular viability of most of the irradiation groups (except the 1.5 Gy group) was higher than the cells without irradiation (the control group denoted as dose 0 in the figures), especially in Group A1 (0.25 Gy), the cellular viability was 11.7% higher for CHO cells and 12.1% higher for 293 T cells.

We also found that both kinds of cells could normally grow and proliferate for weeks without any mutation and apoptosis after the irradiation. They could be passaged by repeatedly frozen and recovery as the normal cells and survive to more than 4 subcultures.

Membrane Permeability

The activities of the Na^+-K^+-ATPase and Ca^{2+}-ATPase as functions of irradiation dose are shown in Figure 3. We can see that in the second hour after irradiation, the activities of both the enzymes decreased in all the groups, indicating that they were inhibited by the radiation. However, at the

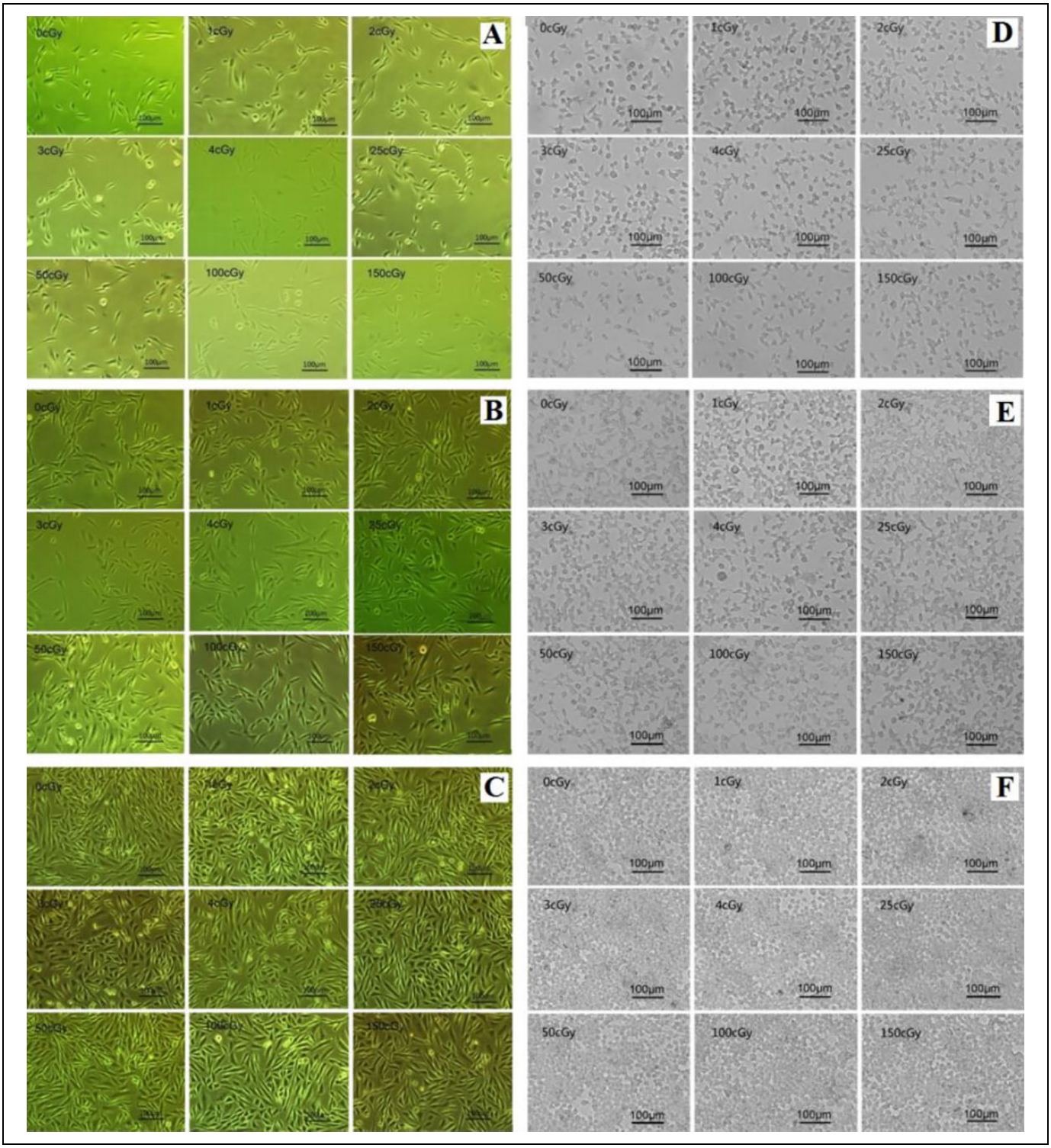

Figure 1. The morphology of CHO and 293 T cells vs. time after irradiation with different doses of X-ray. (A), (B), (C) are CHO cells, (D), (E), and (F) are 293 T cells at the 0, 12th, and 48th h after irradiation respectively.

24th hour after irradiation, their activities restored and even higher than the control for most of the irradiated groups except the 1.0 Gy group for Na^+-K^+-ATPase. The results suggested an improvement of membrane permeability after the irradiation. It was further demonstrated by the increase of free Ca^{2+} in the cells. As we can see from Figure 4, both the fluorescence efficiency and intensity of the intracellular free Ca^{2+} in the irradiated CHO cells and 293 T cells were higher than the control (except the 0.02 Gy group for 293 T cells).

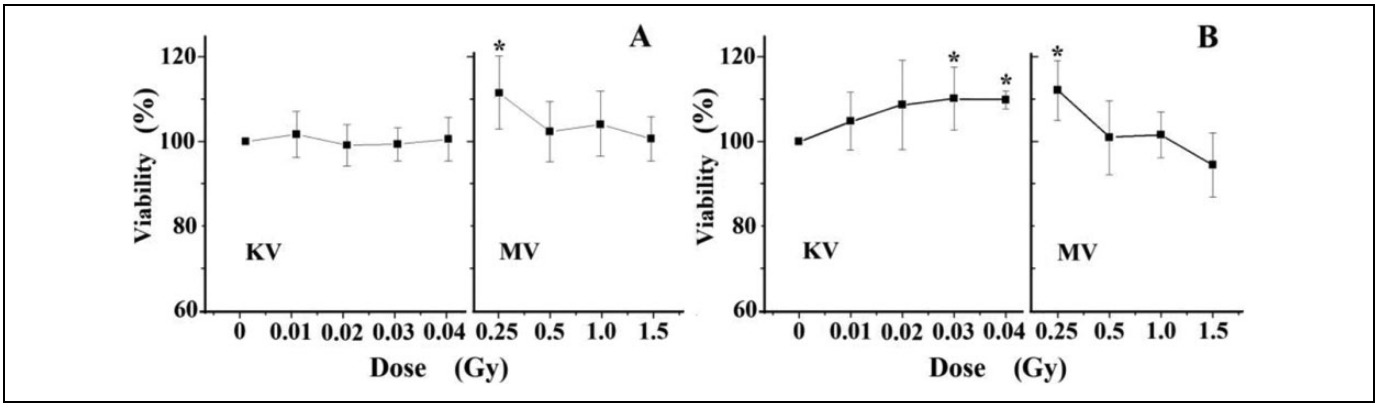

Figure 2. The viability of (A) CHO cells (B) and 293 T cells under different doses of irradiation.

DNA Damage and Repair

Figure 5 shows the results of the γ-H2AX assay. We can see that there were almost no γ-H2AX foci in the cells of KV groups, but in the MV groups, the γ-H2AX foci per cell were quite apparent and increased with dose in both cell lines (see Figure 5C and D). Figure 5 also shows the γ-H2AX foci per cell at the 24th h after irradiation in which the number of γ-H2AX foci greatly decreased compared with the cells at the 0.5 h post-irradiation, indicating that the DNA repair process was effective and the cells were almost recovered in most of the irradiated groups except the 1.50 Gy group.

This was further demonstrated by the results of the Comet assay (see supplementary S-1 and S-2). Similar to that of immunofluorescence γ-H2AX assay, in the second hour after irradiation, no significant change was found in the KV groups of CHO cells compared with the control, but there was slight damage in the cells of MV groups as indicated by the short tails appearing in the cells. However, at the 24th hour after the irradiation, except the 1.5 Gy group, all the irradiated groups generally showed no comet tail indicating almost no DNA damage on the cells anymore. The 293 T cells showed a similar situation. This suggested that the very low dose irradiations of X-ray would not induce cell damage. Although the irradiations of 0.25 -1.0 Gy had induced some cell damages in 2 hours after irradiation, the cells would be self-repaired within 24 hours. But the cells with significant damage in the 1.50 Gy group couldn't recover in 24 hours after irradiation.

GFP Transfer Efficiency

The results of the experiment are shown in Figure 6. We can see that for the CHO cells in the 0.03 Gy, 0.04 Gy, 0.25 Gy, and 0.50 Gy groups, the number of cells with GFP fluorescence was greater than that of the control. Similarly, in the 0.04 Gy, 0.25 Gy, and 0.50 Gy groups of 293 T cells, the numbers of the cells with GFP fluorescence were also greater. It suggested that the low doses of irradiation could stimulate GFP transfer efficiency in the two cells. To have a better quantitative knowledge about the GFP transfer efficiency, we also performed flow cytometry on the cells (results shown in supplementary S-3). The detailed information about the GFP transfer efficiencies in the two cells that combined with the results of fluorescent microscopic observation and flow cytometry are given in Figure 6C and D. We can see that the 0.25 Gy groups had the highest GFP transfer efficiency and compared with the control, there was 22% and 23% increment for CHO cells and 293 T cells respectively. The higher gene transfer efficiency of the irradiated cells further demonstrated that the membrane permeability of the cells was improved by the irradiation.

Discussion

We have investigated the effects of two X-rays on the cells' morphology, viability, membrane permeability, apoptosis, and DNA damage of two cell lines in the dose ranges of each of the radiation usually applied in medical diagnostics, imaging, treatments, and some industry and security tests. Compared with the previous researches,[7,21-24] our study was a systematic one on the dose-response of cells to the two X-rays from different biological aspects at both cellular and molecular levels. It provided new information related not only to the effects of low dose KV and MV X-ray irradiations on living cells but also radiation-enhanced gene transfection. We have shown that the very low doses KV X-ray irradiation (doses from 0.01-0.04 Gy) didn't induce any significant change in CHO cells on cell morphology, cell viability, membrane permeability, DNA structure, and GFP transfection. It just led to a slight increase in cellular permeability and viability in 293 T cell but didn't have any significant effect on the cell's morphology, DNA structure, and GFP transfection. Therefore, the very low doses KV X-ray used in medical diagnostics/imaging can be considered not harmful for cells.

The biological effect induced by the irradiation of MV X-ray was dependent on dose. Among the 4 groups of MV irradiation, the effects of 0.25 Gy MV irradiation should be emphasized, for it could induce a significant increase in the cell viability of both CHO and 293 T cells. The irradiated cells could maintain their normal morphology without nuclear translocation, pycnosis and aberration, they had cellular viability 11-12% higher than the control, and could be passaged by

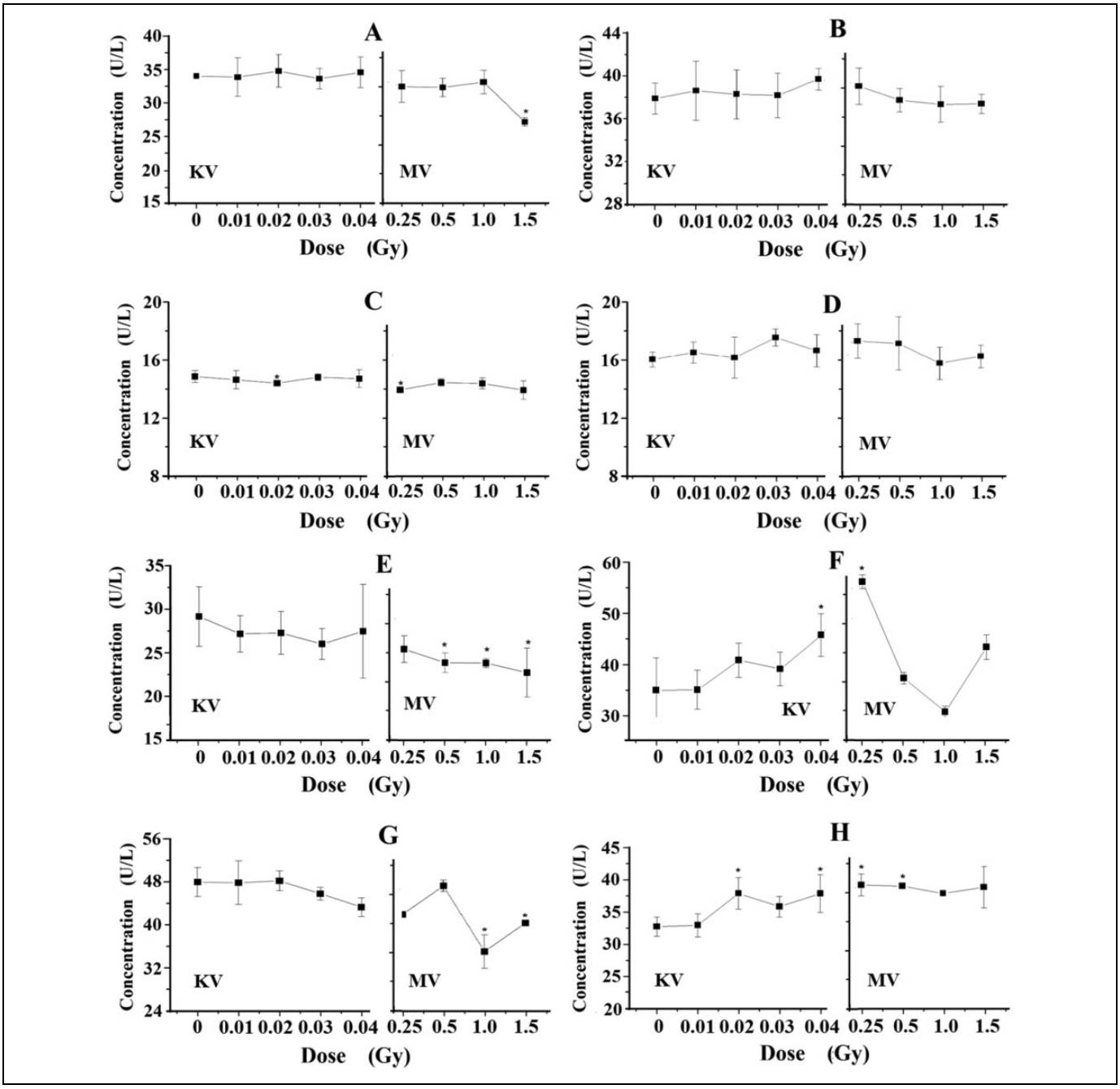

Figure 3. The activities of the Na^+-K^+-ATPase and Ca^{2+}-ATPase as functions of irradiation dose. (A) and (B): Na^+-K^+-ATPase of CHO at the 2nd and 24th h respectively after irradiation; (C) and (D): Ca^{2+}-ATPase of CHO at the 2nd and 24th h respectively after irradiation; (E) and (F) Na^+-K^+-ATPase of 293 T cell at the 2nd h and 24th h respectively after irradiation; (G) and (H) Ca^{2+}-ATPase of 293 T cell at the 2nd and 24th h respectively after irradiation.

repeatedly frozen and recovery as the normal cells and survive to more than 4 subcultures. The irradiation could also stimulate the GFP transfer efficiency in the cells at the same time. So the X-ray of 0.25 Gy can be considered has beneficial effects for cells to not only stimulated cell growth but also enhanced gene transfection. In comparison with the previous studies on gene transfection enhancement by ionizing radiations, this was the first time to propose an optimal irradiation condition that the radiation can stimulate both gene transfer efficiency and cell growth. The X-rays from 0.50 -1.0 Gy seem to have induced dual responses of the cells. They caused DNA double-stranded breaks in the nucleus on a few of the cells, though the damage was slight and could be self-repaired within 24 hours. At the same time, they promoted cell growth, improved membrane permeability, and increased intracellular free Ca^{2+} and GFP transfer efficiency. The 1.5 Gy MV X-ray, however, basically

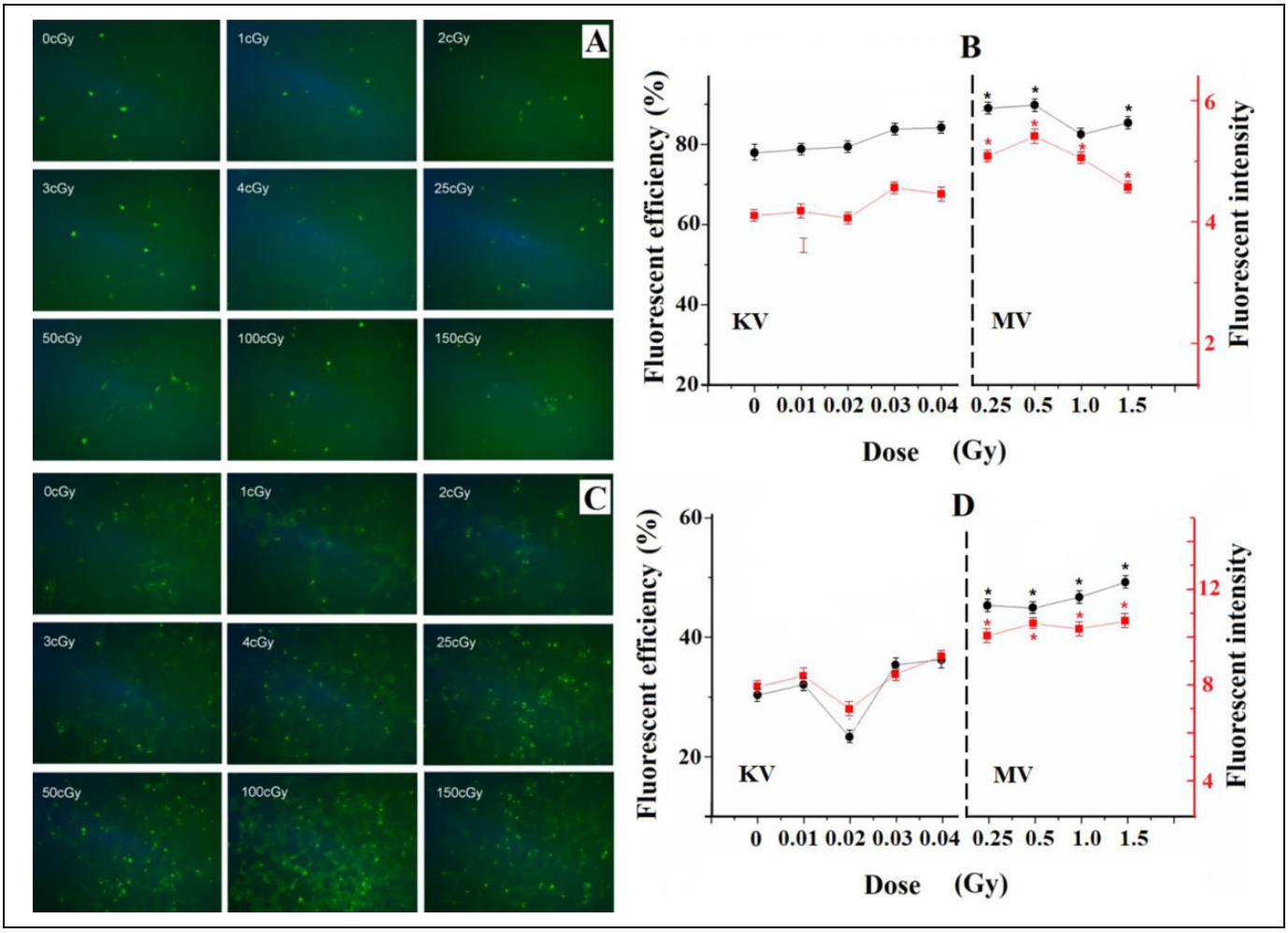

Figure 4. The free Ca^{2+} in the cells.(A) The images of CHO cells; (B) The fluorescence intensity(■) and efficiency(●) as functions of irradiation dose in CHO cells; (C) The images of 293 T cells; (D) The fluorescence intensity(■) and efficiency(●) as functions of irradiation dose in 293 T cells. Data were obtained from the averages of 3000 cells for each group.

presented a negative effect on the cells. It induced significant DSBs and cell damage to result in lower cell viability.

The biological and health effects of low doses of ionizing radiation are currently broadly studied and debated. Compared with that of the harmful effects, the underlying molecular mechanism regarding their effect in stimulating cell growth is not clear yet. Owing to the results of this study, we have a better understanding of the effects of different doses of KV and MV X-rays on cells, so here we can give some explanations for the possible mechanism of the effects of the radiation on cells.

It is well-known that ionizing radiation can induce direct and indirect actions on biological cells. In the effects of direct ionization of cellular macromolecules, ionizing radiation can lead to a large number and different types of molecular damage in DNA by breaking the S–H, O–H, N–H, and C–H bonds of the molecules.[25] The damages include single-strand breaks (SSB), double-strand breaks (DSB), base damage of various types and DNA-protein cross-links, and local combinations of all of these. However, since the DNA molecules make up just a small part of the cell, the probability of the radiation interacting with the DNA molecules is very small unless the irradiation dose is high enough to have quite a lot of X-ray photons for the interaction. Apart from the direct action, the ionizing radiation can induce indirect action on the cell as it irradiates the cellular water at the same time. Since most of the cell's volume is made up of water, there is a much higher probability of radiation interacting with it. During the process of the interaction, the reactive oxygen species (ROS) hydroxyl radical (*OH) and ionized water (H_2O^+), as well as reductants hydrogen radical (H*) and hydrated electrons (eaq^-) are generated, these species also cause some damages in DNA. The DNA damages induced by both the direct and indirect effects are powerful inducers of cell death by apoptosis.[26] However, if the DNA damage is not strong enough to induce direct cell death, the cell cycle progression would stop to repair the damaged DNA. The cells that successfully performed an effective DNA repair thus can reenter the cell cycle and continues their normal growth.[26]

Therefore, the fate of the irradiated cells depends on the level of radiation-induced DNA damage which was proven to

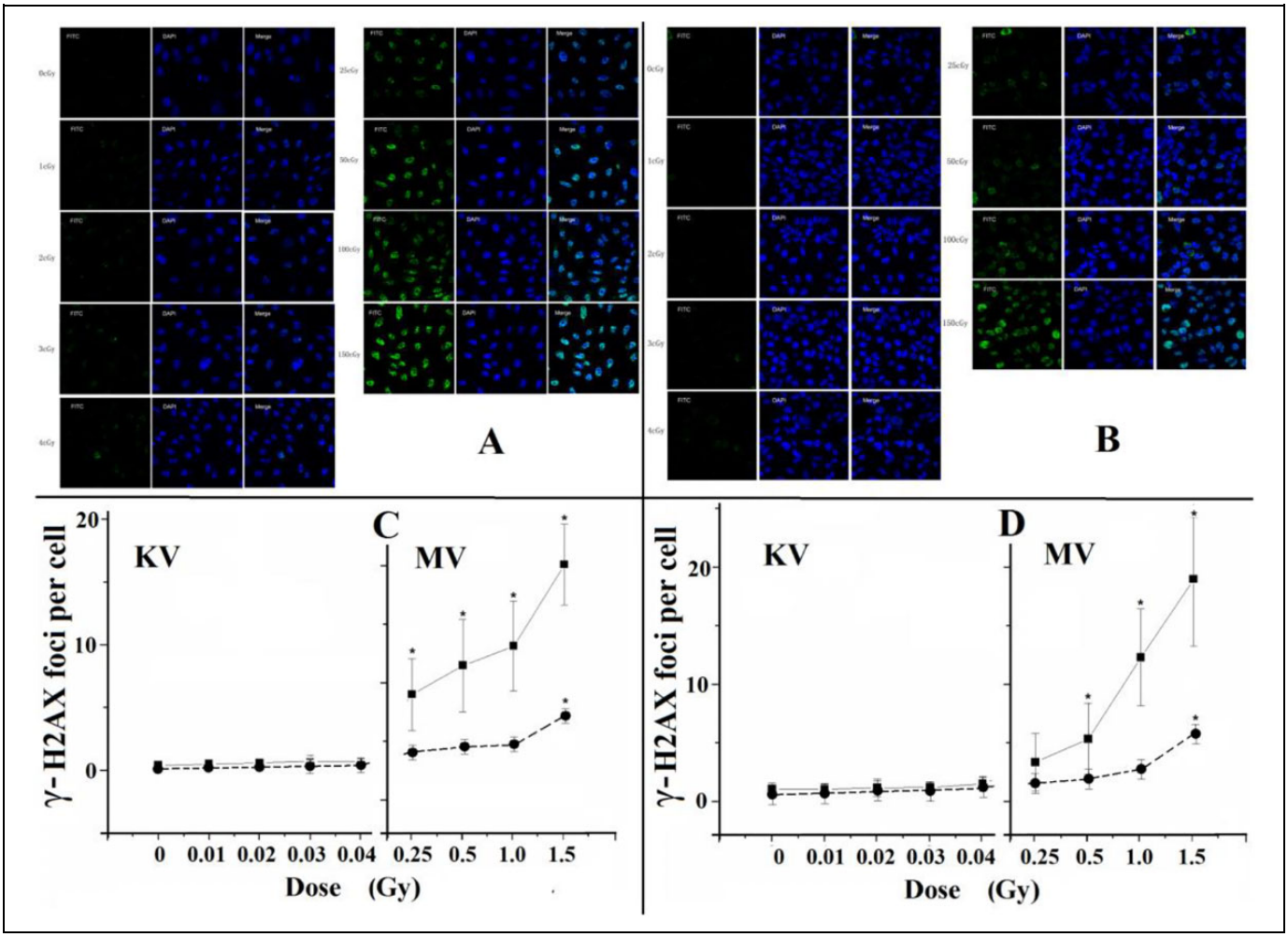

Figure 5. The FITC labeled γ-H2AX foci in the cells. (A) CHO cells; (B) 293 T cells; (C) The H2AX foci per cell vs. irradiation dose in CHO cells, ■: 0.5 h, ●:24 h after the radiation; (D) The H2AX foci per cell vs. irradiation dose in 293 T cells, ■: 0.5 h, ●: 24 h after the radiation. Data were obtained from the averages of 3000 cells for each group.

be dose-dependent by the results of our DNA damage experiment and that reported by the previous researches.[27] As we can see from Figure 5 that, since the doses of the irradiations in KV groups were so low that the absorbed ionizing radiation energy was insufficient to induce DSB or DNA damage, almost no γ-H2AX foci were found in the cells. At the same time, the irradiation didn't induce a significant change in the membrane permeability of the cells (see Figures 3 and 4). So the growth of the cells in the KV groups was not affected by the irradiation and the irradiated cells didn't show any death and mutation or even damage as shown in the results of the observations on cell morphology and viability (Figures 1 and 2) as well as comet assay (S-1 and S-2).

For the MV X-rays, since with higher doses and shorter wavelength, the passage of their ionizing tracks could penetrate more cells to have a higher probability to directly damage the cell DNA as suggested by the apparent γ-H2AX foci that indicated the induced DSB (see Figure 5). But the induced cell effects were dose-dependent. For the doses ranging from 0.25 to 1.0 Gy, the absorbed radiation energy of the cells was insufficient to induce strong DNA damage, so the damaged DNAs could be still repaired by the DNA repair machinery. The decreased number of γ-H2AX foci (Figure 5) and almost no cell DNA damage shown in the comet assay (S-1 and S-2) at the 24th h post-radiation supported this assumption. On the other hand, the ionizing radiation could also cause lipid peroxidation and oxidation of the -SH groups of membrane proteins.[28,29] The induced conformational change of membrane protein then led to an improvement of membrane permeability to transport more electrolytes such as Ca^{2+}, Na^{+}, and K^{+} across the cell membrane(see Figures 3 and 4). The combined action of free Ca^{2+} with the second messenger Diacylglycerol (DAG) thereby induced activation of protein kinase C (PKC) and then promoted cell growth.[30,31] The extracellular signal-regulated kinase (ERK)1/2 was also activated by the irradiation through the mediation of growth factor tyrosine phosphorylation.[22-24,32] The activation of (ERK)1/2 could induce gene expression related to DNA damage repair or cellular viability and facilitate DNA repair by remodeling the chromatin structure[32] and therefore promoted cell growth. Since almost no cell

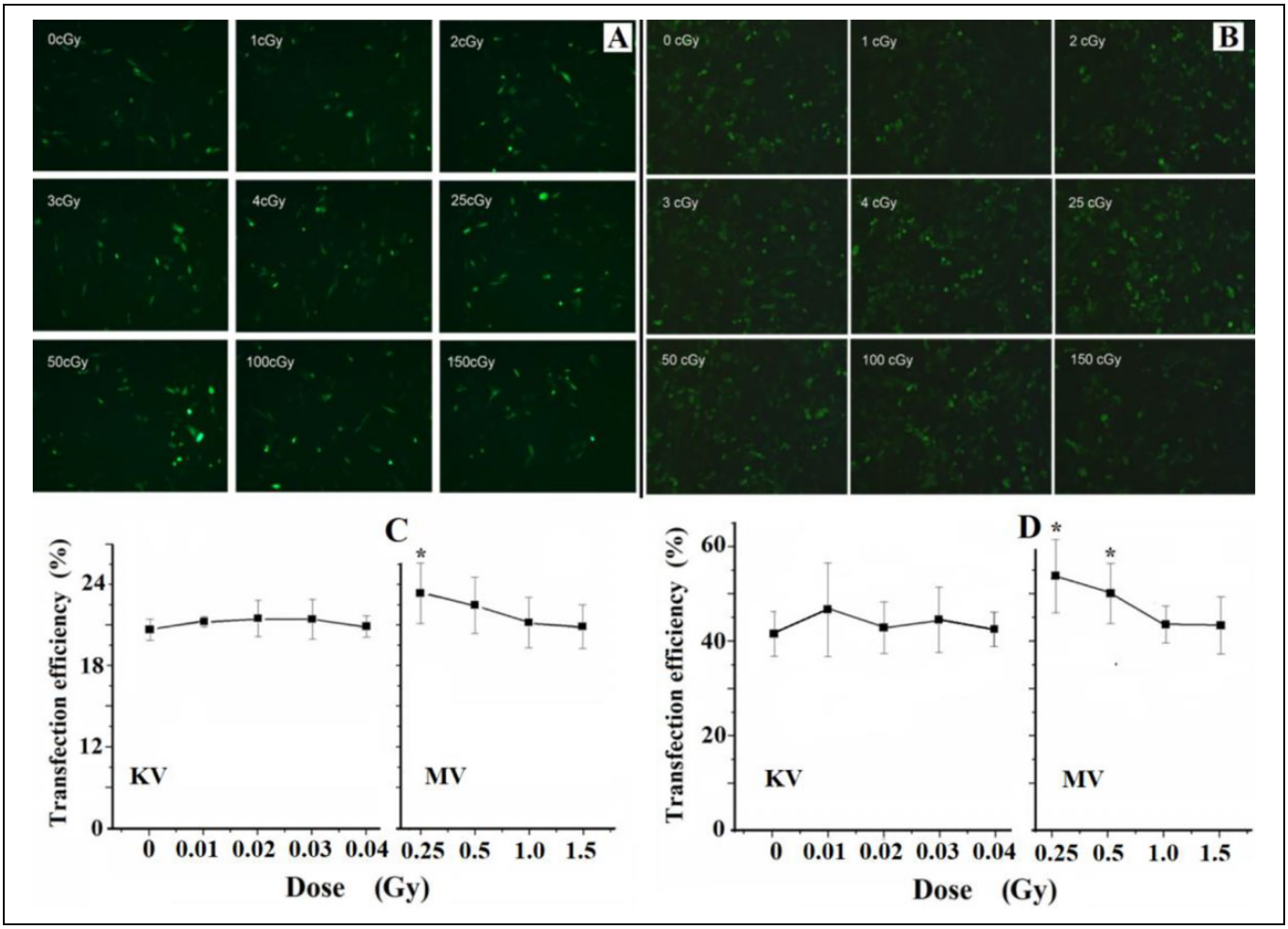

Figure 6. The GFP transfer efficiency of the irradiated cells. (A) and (B) The GFP fluorescence images of CHO cells and 293 T cells respectively; (C) and (D) The transfection efficiency vs. irradiation dose for CHO cells and 293 T cells respectively. Data were obtained from the averages of 3000 cells for each group.

or only a few cells were damaged while the cell growth was promoted at the same time by the improvement of membrane permeability, the cells in 0.25 -1.00 Gy MV groups had higher cellular viability than the control and exhibited a dual response to the irradiation. Among all the groups, the cells in 0.25 Gy group were exposed with a lower dose of radiation, their DNA damages were less severe and had fewer DSBs needed to be repaired, thus they had the highest cellular viability. In contrast, the cells in 1.50 Gy group were exposed by a higher dose of radiation so the induced DNA damage was too strong to be repaired by the DNA repair machinery as indicated by the γ-H2AX foci and the comet assay at the 24th h after irradiation (Figure 5, S-1, and S-2), so they had lower cellular viability than the control.

Unlike the previously reported irradiations that would induce a decline of cellular viability when they were used for gene transfer enhancement,[13-16] the 0.25 Gy MV X-ray could enhance plasmid-based gene transfer efficiency in cells but without impeding the cells' viability. And even better, it promoted cell growth at the same time. This was probably because of the permeability increase of the cell membrane induced by the radiation. The improvement of membrane permeability not only promoted more Ca^{2+} getting into the cells to stimulate cell growth but also allowed an efficient transfer of donor-targeting DNA to the cell nucleu.[28] The enhancement of the gene transfection might be also through the induction of DNA repair/recombination machinery. The irradiation-induced DNA stranded breaks and the subsequent reparation of the breaks would regulate the duration of radiation-induced inhibition of replicon initiation,[29] so the exogenous DNA molecules can be easier to express and increase their expression rate.[30] Since the DBS was slight and the DNA repair process was effective to prevent damage on the cells as indicated by the results of our immunofluorescence assay γ-H2AX and comet assay mentioned above. The irradiation of 0.25 Gy MV X-ray could enhance plasmid-based gene transfer efficiency and promote cell growth. Therefore, using 0.25 Gy MV X-ray to facilitate the delivery of foreign DNA into target cells would be a simple, effective, and safe method for gene transfection. It may provide a powerful means not only for basic research but also for cell

therapeutics, as gene transfection into cells is an important step of gene therapy.

Since our study was based on a short-term model, it just focused on changes and processes in cell morphology, viability, membrane permeability, and DNA damage that take place during and shortly after irradiation. It also has limitations in just studying the effect of single time irradiation of the X-rays, but not repeated irradiations with additional dosages over some time so that it couldn't provide information if there is a cumulative effect by the irradiations. Nevertheless, it presented information about the dose-response relation for the selected endpoints for understanding the effects of dose by the two X-rays with distinct radiation qualities. It also provided us a view about what kind of biological effect would be induced by the radiations and which doses of each of the X-rays are harmful, safe, or has dual effects on biological cells. Though the information was given by the study on two cell lines, a similar response of the cells to the irradiations suggested that the effects of low dose X-ray that found from these cells were most probably also applied to other mammalian cells, or at least to the similar cells.

Conclusion

According to our research on the effects of KV and MV X-rays, the KV X-ray in the studied dose range from 0.01 to 0.04 Gy in principle didn't lead to any significant change in cells. The effects of MV X-rays depended on dose and were quite different in the studied doses. The irradiation of 0.25 Gy could markedly promote cell growth and gene transfection while without inducing cell dead or mutation. The irradiations ranging from 0.5 to 1.0 Gy evoked dual responses of the cells. They would induce slight DNA double-stranded breaks while promoting cell viability, membrane permeability, and GFP transfection. Whereas the 1.5 Gy MV X-ray was harmful, for it would bring about significant cell damage and lead to lower cell viability. With the information presented by this study, people should be confident the ionizing radiation that they have the most opportunity to be exposed in the common medical examinations and imaging by KV X-rays are safe as the radiation wouldn't induce any observable effect on cells. The people working in or living near ionizing radiation facilities, or in a high natural background radiation environment, also should be relieved, since according to the biological effectiveness (RBE) of the radiation type or dose equivalent, their annual exposures are usually just similar to the doses of the KV X-rays used in our study but with much lower dose rates. However, medical-physicists should be more careful in radiotherapy treatment planning to ensure the planned dose of MV X-rays for cancer treatment should be high enough to kill the cancer cells but not that might induce dual effects or even stimulating effect. The capacity of some ionizing radiations such as the 0.25 Gy MV X-ray in enhancing plasmid-based gene transfer efficiency while promoting cell growth recommended that the ionizing radiation is well suited for radiation-enhanced gene transfection.

Authors' Note

YXH conceived and designed the experiments. ZW, MYL performed the experiments. ZW and YXH analyzed the data. YXH wrote the paper.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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