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Radiation increases the cellular uptake of exosomes through CD29/CD81 complex formation

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ABSTRACT

Exosomes mediate intercellular communication, and mesenchymal stem cells (MSC) or their secreted exosomes affect a number of pathophysiologic states. Clinical applications of MSC and exosomes are increasingly anticipated. Radiation therapy is the main therapeutic tool for a number of various conditions. The cellular uptake mechanisms of exosomes and the effects of radiation on exosome–cell interactions are crucial, but they are not well understood. Here we examined the basic mechanisms and effects of radiation on exosome uptake processes in MSC. Radiation increased the cellular uptake of exosomes. Radiation markedly enhanced the initial cellular attachment to exosomes and induced the colocalization of integrin CD29 and tetraspanin CD81 on the cell surface without affecting their expression levels. Exosomes dominantly bound to the CD29/CD81 complex. Knockdown of CD29 completely inhibited the radiation-induced uptake, and additional or single knockdown of CD81 inhibited basal uptake as well as the increase in radiation-induced uptake. We also examined possible exosome uptake processes affected by radiation. Radiation-induced changes did not involve dynamin2, reactive oxygen species, or their evoked p38 mitogen-activated protein kinase-dependent endocytic or pinocytic pathways. Radiation increased the cellular uptake of exosomes through CD29/CD81 complex formation. These findings provide essential basic insights for potential therapeutic applications of exosomes or MSC in combination with radiation.

1. Introduction

Exosomes, bilipid membrane vesicles (30–100 nm in diameter) that originate in multi-vesicular bodies and are released into the extracellular milieu upon fusion with the plasma membrane, are attracting increased attention [1]. Exosome secretion is a cellular mechanism for delivering cargo to mediate intercellular communication and to affect biologic function by the exchange of proteins and lipids, or the delivery of genetic materials to recipient cells [2]. Exosomes are also involved in various other cellular functions and pathophysiologic states, and thus could potentially provide a new approach for detecting noninvasive disease and predicting disease progression [3]. Moreover, exosomes have properties that can be exploited for therapeutic interventions as a new drug delivery system and a novel therapeutic tool in various conditions, including cancer, inflammation, ischemia, and regeneration [4].

Tumor cells and the cancer-associated microenvironment, comprising fibroblast-like cells, extracellular matrix, and inflammatory cells, secrete exosomes between them, allowing for crosstalk that leads to the promotion or inhibition of tumor progression, but the precise mechanism of communication is poorly understood [5,6]. Mesenchymal stem cells (MSC), clusters of multipotential fibroblast-like cells present in every organ as well as in the tumor stromal microenvironment, have regenerative and protective effects for injured tissues, and inhibit or promote tumor metastasis with their secreted exosomes, but the underlying mechanism is not clearly understood [6]. Potential applications of MSC and their secreted exosomes are currently attracting attention in a number of medical fields, such as oncology, immunology, and radiation therapy [7,8].

Radiation and drug therapy are currently the main therapeutic tools for a number of diseases. Radiation therapy not only acts on target cells, but also affects the stromal microenvironment. Thus, understanding how radiation affects cellular uptake and the secretion of exosomes between target cells and stromal cells is crucial.

Recent studies of exosome biogenesis revealed that exosomes originate from endosomal proteins involved in membrane transport and fusion in processes requiring heat shock proteins,

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integrins, and tetraspanins, and that the source of exosomes defines their function [7]. For therapeutic application of exosomes, especially those derived from MSC, the target cells must effectively internalize the exosomes. Several mechanisms of exosome uptake involving their surface molecules have been described and two distinct modes of internalization have been suggested [1]. In monocytes and macrophages, exosome internalization depends on the actin cytoskeleton and phosphatidylinositol 3-kinase regulated by dynamin2, and non-phagocytic cells require an energy-dependent pathway, including caveolae, macropinocytosis, and clathrin-coated vesicles [9,10]. The effects of radiation on exosome uptake processes, however, remain unknown. More detailed knowledge of the mechanisms of cellular uptake and the effects of radiation on these processes is needed to promote the effective use of exosomes and MSC as potential therapeutic tools. A better understanding of the processes involved will be instructive for modifying exosomes to be preferentially targeted in pathologic conditions by bioengineering. Here, we address several essential questions relating to the basic cellular uptake of exosomes and how radiation regulates that process, with a focus on target cell ligands. Our findings revealed that radiation leads to the colocalization of integrin (CD29) and tetraspanin (CD81) and increases the cellular uptake of exosomes.

2. Materials and methods

2.1. Reagents

Antibodies were obtained from Cell Signaling Technology (glyceraldehyde 3-phosphate dehydrogenase, phospho-p38 mitogen activated protein kinase [MAPK] at Thr180/Tyr182, phos-heat shock protein [Hsp] 27 [Ser82], and integrin (β1/CD29), EPITOMICS (integrin αV/CD51), abcam (CD9), Santa Cruz Biotechnology (dynamin2, CD63, CD81, CD151), BD Bioscience (CD29), or Invitrogen (Alexa Fluor 488, 568, 633). Small interference RNAs (siRNAs) were obtained from Santa Cruz Biotechnology (dynamin2, CD81, CD151, CD29) and Invitrogen (integrin αV/CD51). The p38 MAPK inhibitor (SB203580) was purchased from CST. N-Acetyl-L-cysteine (NAC) was obtained from Sigma–Aldrich.

2.2. Cells

Human bone marrow-derived MSC (immortalized cells) were purchased from the Health Science Research Resource Bank and cultured in modified Eagle's medium-α supplemented with 15% heat-inactivated fetal bovine serum (FBS). Rat small intestinal epithelial cells (IEC6) were maintained in Dulbecco's modified Eagle's medium supplemented with 5% FBS, and insulin. Human umbilical cord vein-derived normal endothelial cells (HUVEC) were cultured as previously described [17]. Exosomes were depleted of FBS by centrifugation at 100,000×*g* for 2 h to eliminate contaminating bovine exosomes.

2.3. γ-Ray irradiation

Cells were irradiated by a cesium-137 (Cs137) gammator (model M Gammator, Irradiation Machinery, Parsippany), at a dose rate of 8.0 Gy/min on a rotating platform.

2.4. Exosome isolation and labeling

Exosomes were isolated from the cell culture medium by ultracentrifugation. Briefly, collected culture medium was centrifuged at 1000×*g* for 20 min at 4 °C, and further filtered using a 0.22-μm filter (NALGENE) to remove cells and debris. Exosomes were then pelleted at 100,000×*g* for 2 h and washed with phosphate-buffered saline (PBS) at 100,000×*g* for 2 h using a swinging-bucket rotor SW28 (Beckman), all at 4 °C. Exosomes were resuspended in PBS and aliquoted, and kept at −80 °C. Purified exosomes were labeled with a PKH67 fluorescent labeling kit (Sigma–Aldrich) or a CellVue® claret far red fluorescent cell linker kit (Sigma–Aldrich). After labeling exosomes according to the manufacturer's recommendations, exosomes were washed twice with PBS at 100,000×*g* for 2 h using a swinging-bucket rotor SW28 (Beckman), all at 4 °C. Exosomes were suspended in PBS and filtered using a 0.22-μm filter (NALGENE) before adding to the medium.

2.5. Exosome uptake assay

Cells (2×10^5) were exposed to 8 Gy irradiation and fluorescence-labeled exosomes were added to culture medium. At 16 h or determined incubation time, cells were intensively washed twice with PBS. Harvested cells were washed and re-suspended in PBS. The fluorescence intensity of cells was detected using a fluorescence cell analyzer (FACSCalibur, Becton Dickinson).

2.6. Effect of exosomes on irradiated-cell viability

Cells (2×10^5) were exposed to 8 Gy of irradiation and then the purified exosomes (20 μL) were added to the culture media (2 mL) 10 min later. The culture was continued for 24 h. The cultured cells were harvested, and their viability was examined using the trypan blue exclusion method. All experiments were repeated three times.

2.7. siRNA transfection

Cells were transfected with each specific siRNA described above using TransMessenger Transfection Reagent (QIAGEN) according to manufacturer's recommendation. The cells were used for exosome uptake assay at 24 or 48 h after transfection. The siRNA efficiency was tested by Western blot or FACSCalibur.

2.8. Immunoblotting

Harvested cells were lysed directly in Laemmli sample buffer and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. Membranes were blocked and probed with primary antibody and peroxidase-conjugated secondary antibodies followed by detection using an ECL Western blotting substrate (Millipore). Images were detected using Image Reader LAS-400 Fugifilm.

2.9. Cell surface expression analysis using flow cytometry

Cells were cultured for 6 h after 8 Gy irradiation. Harvested cells were washed once in PBS, re-suspended in 100 μL of diluted (1:100 in PBS with 1% vol/vol bovine serum albumin) primary antibody or fluorescein isothiocyanate-coupled antibody, and incubated for 30 min at 4 °C in the dark. The cells were washed once and incubated with anti-rabbit or mouse Alexa Fluor 488 secondary antibody (1:100 in PBS with 1% vol/vol bovine serum albumin) for 30 min at 4 °C in the dark. The cells were washed again and re-suspended in PBS. The fluorescence intensity of 10,000 cells for each sample was then analyzed using FACSCalibur.

2.10. CD29 and tetraspanin family colocalization assay

MSC were exposed to irradiation and incubated for 6 h. The cells were fixed in 4% formaldehyde PBS solution and stained for CD29 and tetraspanins (CD9, CD63, CD81, or CD151) and Alexa Fluor

488- or 568-coupled secondary antibody without permeabilization. A colocalization analysis was performed on confocal sections showing maximum CD29 signals using Metamorph software. Percentage of colocalization of CD29 and CD81 signals was calculated from 14 images obtained from two independent experiments. Where needed, brightness was increased uniformly using Photoshop (Adobe)

2.11. Exosome binding and colocalization assay

For the exosome binding assay, cells (2×10^5) on collagen-I coated glass-bottom dishes (IWAKI) were incubated for 6 h at 37 °C post 8 Gy irradiation, and incubated at 4 °C for 30 min to inhibit endocytosis. Fluorescence (PKH67)-labeled exosomes were added. After incubation for 2 h at 4 °C, cells were intensively washed three times with PBS and imaged using IX70 fluorescence microscope and CCD-camera with a 40× NA 0.95 objective. For flow cytometric analysis, harvested cells were washed and re-suspended in PBS and the fluorescence intensity of cells was detected using FACSCalibur.

For the exosome colocalization assay, cells on collagen-I coated glass-bottom dishes (IWAKI) were incubated for 6 h at 37 °C after 8 Gy irradiation, and further incubated with fluorescence (CellVue) labeled exosomes for 1 h at 37 °C. Following three intensive washes with PBS, the cells were fixed in 4% formaldehyde PBS solution and stained for CD29 and tetraspanin CD81 and Alexa Fluor 488- or 568-coupled secondary antibody without permeabilization. Colocalization analysis was performed on confocal sections showing maximum CD29 signals using Metamorph software. Percentage of colocalization of exosomes and CD29/CD81 complex signals was calculated from eight images obtained from two independent experiments.

2.12. Statistical analysis

The significance of differences between the control and experimental groups was determined using the Mann–Whitney *U* test depending on the data distribution. Statistical analysis was performed using Excel 2010 software program (Microsoft) with the add-in software Statcel 2.

2.13. Image acquisition details

The fluorophores used in the study were anti-rat Alexa Fluor 488 (Invitrogen), anti-rabbit Alexa Fluor 568 (Invitrogen), and anti-mouse Alexa Fluor 568 (Invitrogen). All samples were mounted in PBS and imaged at room temperature. Microscopy images were obtained using an IX70 fluorescence microscope (Olympus) and CCD-camera (ORCA-R2, Hamamatsu). We used the MAC5000 (LEP) controller system and the Metamorph acquisition software for three-dimensional deconvolution. We used an oil immersion objective: 40×, NA 0.95 or 100×, NA 1.4. For some images, brightness and contrast were increased uniformly over all images of the respective assay using Photoshop.

3. Results and discussion

3.1. Radiation increases the cellular uptake of exosomes and enhances the effects of exosomes on irradiated-cell viability

To clarify whether radiation affects the cellular uptake of exosomes, recipient cells (IEC6, MSC, and HUVEC) with or without irradiation were incubated with PKH67-fluorescent labeled-exosomes derived from MSC. Radiation significantly increased the cellular uptake of exosomes based on flow cytometry analysis (Fig. 1A). We investigated the time-course of the cellular uptake of exosomes. Increased exosome uptake was discernible at 6 h after radiation exposure (data not shown). The cellular uptake of exosomes increased in a radiation dose-dependent manner (Fig. 1B). This effect of radiation was observed among various combinations of recipient cells and exosomes (Fig. 1C), suggesting that the radiation-induced cellular uptake of exosomes was not dependent on the recipient cell type.

Next, we investigated whether exosomes from cultured MSC increase the viability of irradiated cells (Fig. 1D). The exosomes from MSC increased the viability of irradiated cells through anti-apoptosis (Fig. S1). Radiation generally increases the cellular uptake of exosomes in MSC *in vitro*, and the uptake of exosomes might reflect the biologic functions of the cellular source. Therefore, in the present study, we addressed the basic cellular uptake of exosomes and how radiation regulates the cellular uptake of exosomes.

3.2. Radiation induces the colocalization of integrin β1 (CD29) and tetraspanin (CD81) on the cell surface

Exosome uptake involves at least three steps, including binding to the cell surface, direct fusion with the plasma membrane, and internalization by recipient cells [1]. We investigated whether radiation enhanced the binding capacity of cells to exosomes. To identify characteristic changes in the binding capacity of cells following radiation, an exosome uptake assay was performed at 4 °C to inhibit energy-dependent endocytosis [5]. More exosomes attached to irradiated cells than to non-irradiated cells (Fig. 2A), suggesting that radiation modifies cell membrane surface molecules to more effectively bind with exosomes.

Some studies have reported that exosome targeting of cells is mediated via tetraspanin families, integrin families, or their related-molecules based on their expression on exosomes [11–13]. Tetraspanins with four transmembrane domains are expressed in various cells and are rich in exosomes [14]. They share the ability to associate with different transmembrane receptors and among themselves, forming tetraspanin-enriched microdomains that serve as platforms to regulate the avidity of adhesion receptors for their ligands [12]. Thus, we first examined whether radiation affects the expression levels of tetraspanins, including CD9, CD63, CD81, and CD151, and also integrin β1 (CD29) on target-cell surfaces. Based on flow cytometry, radiation had no effect on the expression levels of the tetraspanin family and integrin β1 (Fig. 2B). Next, we examined the radiation-induced changes in their distribution. Radiation dramatically increased the colocalization of CD29 and CD81 (Fig. 2C and D). In addition, we performed immunoprecipitation analysis to confirm the formation of CD29/CD81 complexes (Fig. S2A). The distribution of CD9, CD63, and CD151 was not altered by radiation exposure (Fig. S2B). We also confirmed the colocalization of CD29/CD81 in HUVEC (data not shown). The findings suggested that radiation exposure increased CD81 tetraspanin-enriched microdomain association with CD29 on MSC and HUVEC.

3.3. Exosomes bind to the CD29/CD81 complex

Next, we examined whether exosomes were dominantly colocalized to a particular tetraspanin (CD9, CD63, CD81, and CD153) or CD29 in irradiated MSC. Microscopic analysis revealed that exosomes did not selectively merge with every tetraspanin or CD29 in irradiated or non-irradiated cells (data not shown). Thus, we focused on the association between exosomes and the CD29/CD81 complex. Microscopic analysis of every slice (0.4 μm in depth) in three-dimensional deconvoluted stacks was performed. Most exosomes were dominantly merged with the CD29/CD81 complex in irradiated cells, whereas a few exosomes were not specifically

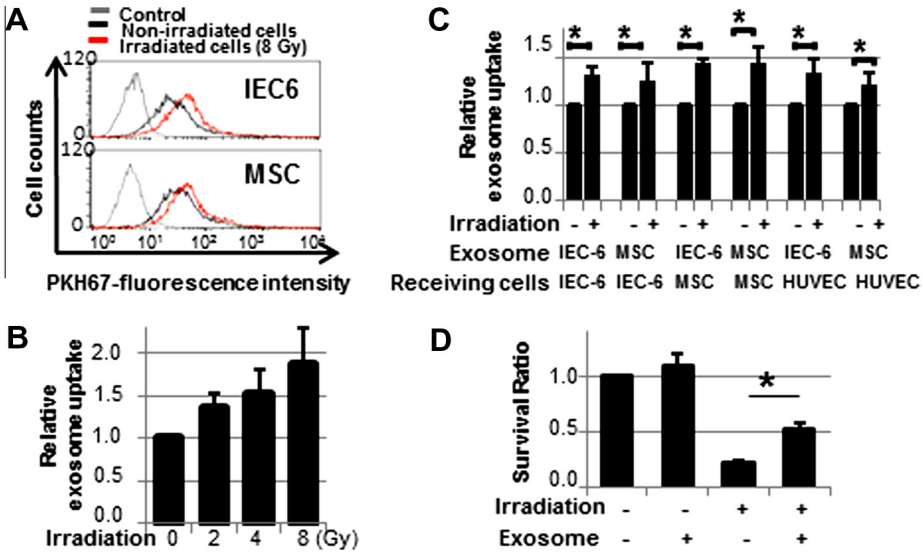

Fig. 1. Radiation increases the cellular uptake of exosomes and effect of exosomes on irradatied cell viability. (A) Irradiated-recipient cells (8 Gy) and labeled exosomes from MSC were incubated for 16 h. Representative cytograms are shown. (B) 2–8 Gy Irradiated-cells were incubated with the labeled exosomes for 16 h, and anlyzed by flow cytometry. (C) Radiation-induced cellular uptake of exosomes was not dependent on the recipient cell type. Experiments were performed three times. Values represent mean ± SD. The non-irradiated control group was set to 100%, and mean fluorescent intensity was calculated. $^{*}p < 0.05$ versus the non-irradiated control group: Mann–Whitney *U*-test. (D) Exosomes from MSC were added and incubated with previously irradiated cells (8 Gy) for 24 h. Viable cells were detected by the trypan blue exclusion method. Cell viability experiments were repeated three times. Data were normalized to non-irradiated control group cells. $^{*}p < 0.05$.

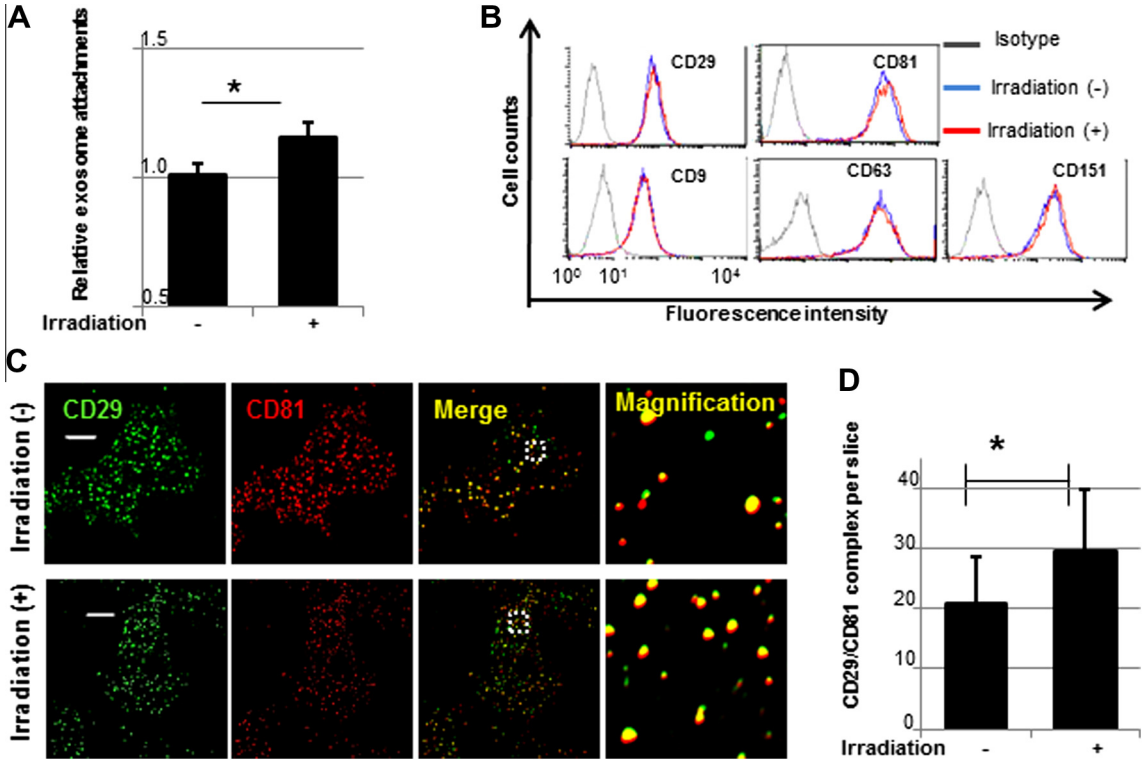

Fig. 2. Radiation induces colocalization of integrin β1 (CD29) and tetraspanin (CD81) on the cell surface. (A) The binding capacity of cells to exosomes following radiation was evaluated at 4 °C. The fluorescence intensity of 10,000 cells for each sample was analyzed by flow cytometry. Results are shown as mean of three independent experiments. $^{*}p < 0.05$ by Student's *t*-test. (B) Expression levels of the tetraspanin family and integrin β1. MSC were exposed to 8 Gy of radiation and incubated for 6 h. Cells were stained for CD29 (green) and tetraspanins (red: CD9 or CD63 or CD81 or CD151) without permeabilization. Representative cytograms are shown. (C) Colocalization analysis of CD29 and CD81 in control and irradiated cells at 6 h after irradiation. (D) Numbers of CD29/CD81complexes per slice are based on 14 images from 2 independent experiments. Error bars represent standard deviation. White bar: 10 μm. $^{*}p < 0.05$ by Student's *t*-test.

distributed in either irradiated or non-irradiated control cells (Fig. 3A and B). These findings suggested that radiation not only induced CD29/CD81 complex formation, but also led to preferential attachment of the exosomes to the complex by modifying their molecules or another unknown factor. Findings from transient gene knockdown against CD29 and/or CD81 supported these findings (Fig. 3C and D). In this experiment, an intact-cell population (gate G1) and an impaired-cell population (gate G2) were divided,

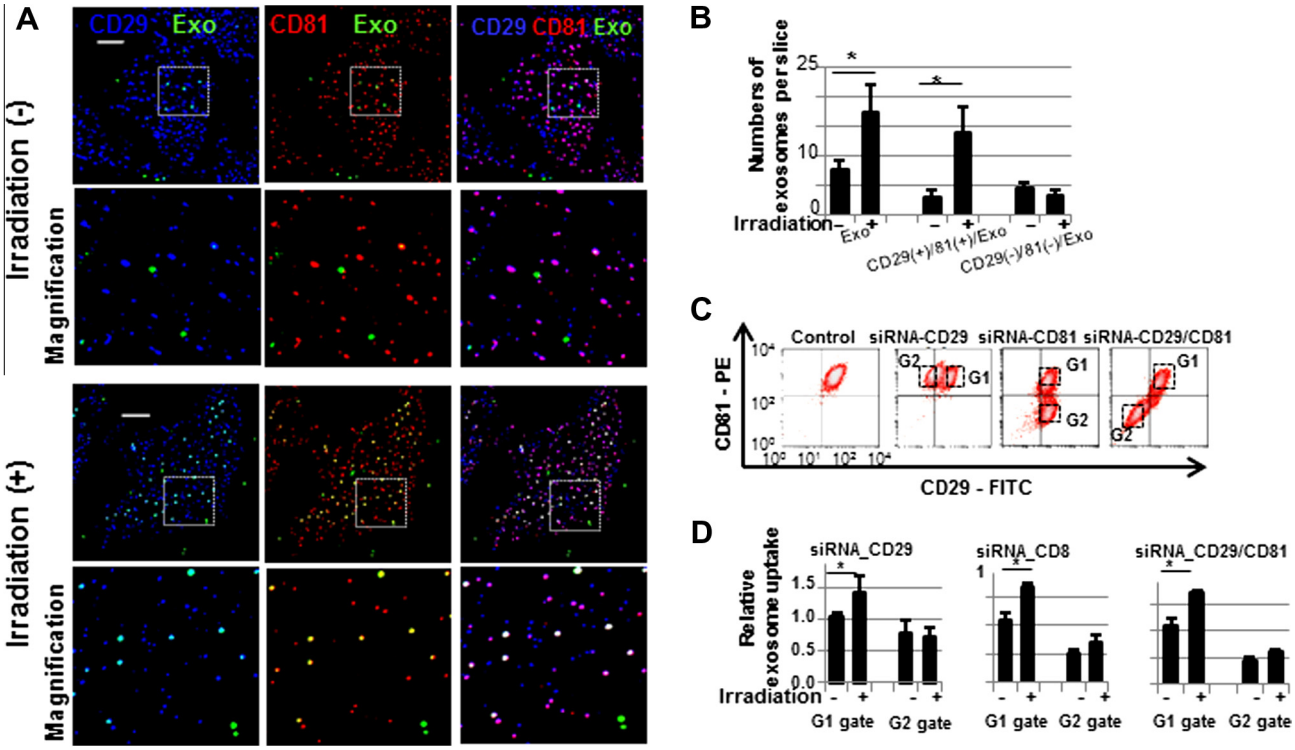

Fig. 3. Exosomes bind to the CD29/CD81 complex. (A) Colocalization analysis of CD29 (blue), CD81 (red), and CellVue-labeled exosomes (green) was performed in control and irradiated cells for 1 h at 6 h after irradiation. Exosomes bound to the CD29/CD81 complex, leading to merged signals (white). (B) Histograms show the numbers of attached-exosomes per slice (1 slice = 1cell). Exosome column: number of exosomes (green) signals per slice, exosomes/CD29+/CD81+ column: number of three merged signals (white) per slice, other column: number of exosomes/CD29(−)/CD81(−) signals per slice. Numbers in each column are based on 8 images from three independent experiment. $^{*}p < 0.05$ by Student's *t*-test. White bar: 10 μm. (C) and (D) MSC were transfected with siRNA against CD29 and/or CD81 for 48 h, and an exosome uptake assay was performed. In this assay, an intact-cell population (G1-square gate: control) and an impaired-cell population (G2-square gate: knockdown) were analyzed ($n = 4$, ±SD). Control siRNA knockdown was set to 100%. $^{*}p < 0.05$ by Student's *t*-test.

allowing us to analyze radiation-induced exosome uptake involving CD29 and/or CD81 in a single experimental unit (Fig. 3C). Predictably, CD29 knockdown completely abolished the radiation induced-effects on exosomes (Fig. 3D). These findings are consistent with those of a previous study in which exosome binding to fibroblasts and extracellular matrix components, such as collagen-I and fibronectin, was inhibited in a CD29 antibody experiment [11]. Similarly, the CD81-knockdown experiment revealed suppression of radiation-induced uptake as well as the basal uptake of exosomes (Fig. 3D). Thus, we concluded that CD81, cooperating with CD29, plays a central role in the radiation-induced increase in the uptake of exosomes in MSC. In addition, the depletion of CD151 not colocalized with CD29 or CD81 based on microscopy did not alter the effect of radiation on the cellular uptake of exosomes or the expression of CD29 (Fig. S3A). To further confirm the role of CD29 in exosome uptake induced by radiation, we also performed knockdown analysis in HUVEC. Both basal and radiation-induced uptake of exosomes was inhibited in HUVEC with knockdown of CD29 or CD81 (Fig. S3B)

3.4. Integrin αV and dynamin2 do not contribute to the radiation-induced increase in exosome uptake

The dynamin family regulates the internalization of clathrin-coated vesicles and the uptake of caveolar microdomains in the process of endocytosis [15]. Recipient cells internalize integrin αV through clathrin-coated vesicles and plasminogen activator inhibitor 1 induces its internalization [16]. Integrin αV is not only a CD81-associated transmembrane molecule but it is also a cell surface receptor that binds to CD29 [12]. Radiation increases integrin αV expression [17]. These findings suggest that integrin αV is a key candidate regulator among exosomes, cellular attachment, internalization, and radiation. We first used integrin αV knockdown to examine the role of this molecule in the radiation-induced increase in exosome uptake (Fig. 4A). Unexpectedly, integrin αV knockdown had no significant effect, and only slightly reduced basal exosome uptake in irradiated cells, whereas radiation increased both integrin αV and PAI-1 expression (Fig. 4A and B), consistent with a previous report [16]. These findings suggested that integrin αV does not have a key role in the radiation-induced cellular uptake of exosomes.

Cellular internalization of exosomes through phagocytosis in macrophages is inhibited by the knockdown of dynamin2 or the overexpression of a dominant-negative form of dynamin2 [9]. Furthermore, radiation increases dynamin2 expression [9]. Therefore, we next addressed whether dynamin2 is involved in the radiation-induced increase in exosome uptake. In contrast to previous findings, the dynamin2 expression levels did not differ significantly between irradiated and non-irradiated cells (Fig. 4B). Analysis of dynamin2 knockdown revealed an increase in both radiation-induced and basal uptake of exosomes (Fig. 4C). This opposite effect is likely related to compensatory mechanisms by other endocytic pathways [18]. These results suggested that regulation of the radiation-induced increase in exosome uptake is independent of the dynamin2-associated endocytic pathway.

3.5. Radiation-increased exosome uptake is independent of oxidative stress and stress-induced p38 MAPK intracellular signaling

Exosome internalization via endocytosis and pinocytosis is regulated by oxidative stress-induced p38 MAPK [3]. Radiation produces intracellular reactive oxygen species (ROS), which

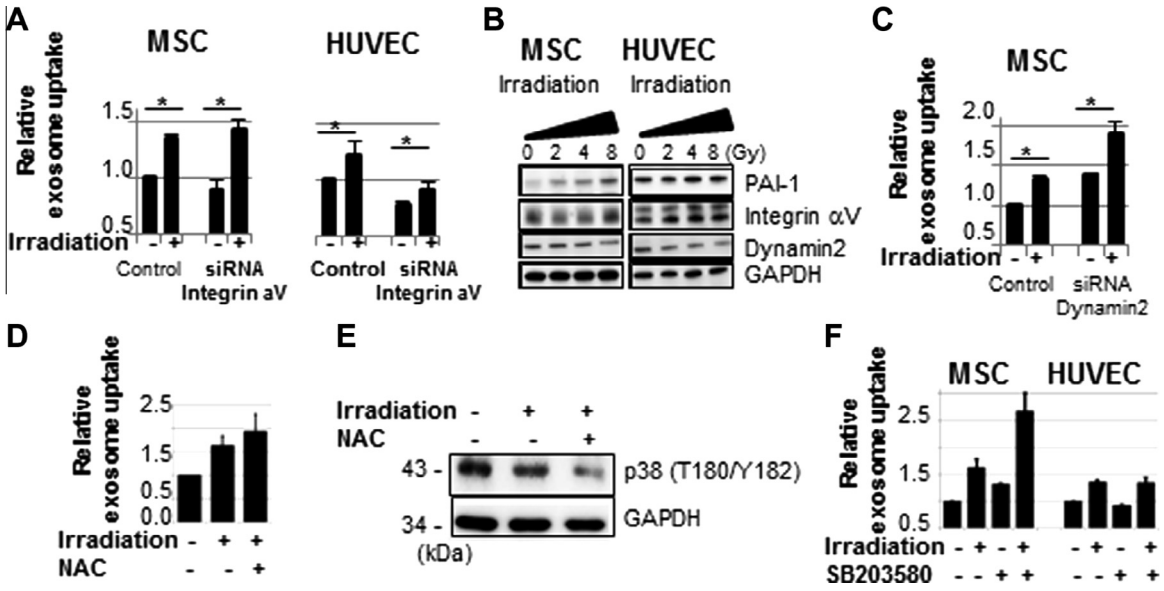

Fig. 4. Radiation-increased exosome uptake is independent of integrin αV, dynamin2, oxidative stress, and stress-induced p38 MAPK intracellular signaling. (A) An exosome uptake assay was performed following knockdown against integrin αV. Exosome uptake of control siRNA was set to 100%. $^{*}p < 0.05$ by Student's *t*-test. (B) Expression levels of PAI-1 and integrin αV in MSC and HUVEC are shown by immunoblotting. Representative data are shown. (C) An exosome uptake assay was performed following knockdown of dynamin2. $^{*}p < 0.05$ by Student's *t*-test. (D) NAC did not inhibit the radiation-induced exosome uptake ($n = 3$, ±SD). Exosome uptake rate of non-irradiated control was set to 100%. (E) Immunoblotting showing the effects of radiation with NAC on p38 MAPK activity (phosphorylated form) at 16 h after irradiation. Representative data are shown. (F) Treatment with SB203580 (p38 MAPK inhibitor) did not inhibit exosome uptake in irradiated-cells ($n = 4$, ±SD). The exosome uptake rate of control was set to 100%.

phosphorylate p38 MAPK (Thr180/Tyr182) to the active form [19]. To clarify the involvement of this type of endocytosis, we investigated whether the ROS generated by radiation contribute to and regulate p38 MAPK-dependent exosome endocytosis. In this study, radiation increased the intracellular levels of ROS, whereas NAC, added as an antioxidant agent, inhibited ROS generation (Fig. S4A). In the exosome uptake assay, NAC did not inhibit the radiation-induced increase in exosome uptake (Fig. 4D). The amount of phosphorylated-p38 MAPK following NAC treatment was decreased in irradiated cells (Fig. 4E). In addition, treatment with SB203580, a p38 MAPK inhibitor, increased rather than decreased exosome uptake in irradiated cells (Fig. 4F). The levels of phosphorylated-p38 MAPK were identical for up to 20 h post-irradiation (data not shown). Phosphorylated Hsp27 (Ser82) was not significantly increased by radiation (Fig. S4B). Taken together, our results demonstrated that radiation induces an increase in exosome uptake independent of ROS generation, and p38 MAPK-dependent endocytosis and pinocytosis.

3.6. Conclusions

We demonstrated that radiation increased the cellular uptake of exosomes. Radiation markedly enhanced the initial cellular attachment to exosomes and induced colocalization of CD29 and CD81 on the cell surface without affecting their expression levels. Exosomes dominantly bound to the CD29/CD81 complex. Other possible exosome endocytosis processes, dynamin2, and ROS and their evoked p38 mitogen-activated protein kinase-dependent endocytic or pinocytic pathways were not involved in the radiation-induced changes. Therefore, radiation increases the cellular uptake of exosomes through CD29/CD81 complex formation. These findings provide essential basic insights for potential therapeutic application of exosomes and MSC in combination with radiation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.03.067.

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