[UCI]I804:24011-200000264160

2014 년 2 월

석사학위 논문

miR-22 regulates DNA double-strand break repair through silencing of MDC1

조선대학교 대학원

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이 논문을 석사학위신청 논문으로 제출함

2013 년 10 월

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2013년 11월

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국문초록

miR-22에 의한 DNA손상 복구 반응 조절 기전

정 서 연

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세포에 DNA 손상 자극 시 자동적으로 손상을 체크할 수 있는 시스템이 작동해 세포주기 휴지, 노화, 세포사멸과 같은 현상을 유도한다. MDC1은 이런 DNA 손상 반응의 중요 요소로써, DNA 손상 시 손상 복구와 세포주기 확인점을 활성화 할 수 있게 하는 유전자로 알려져 있다. MicroRNA 는 small non-coding RNA로 전사후의 표적 유전자의 표현을 negative하게 조절하는 역할을 한다. 우리는 miR-22의

타겟으로 MDC1을 동정하고, miR-22가 MDC1의 발현을 조절하여 DNA손상 복구를 조절함을 증명하였다. 또한, 세포 노화 시 miR-22가 증가된다는 기존의 보고를 토대로 우리는 노화세포에서 miR-22 발현이 증가되고, 이를 통해 MDC1 발현을 억제하고 DNA 손상 복구에도 결핍을 초래하는 것을 확인 할 수 있었다. ASO-miR22 또는 MDC1을 과발현 시켰을 때 MDC1 발현, MDC1 foci, DNA손상 복구가 회복됨을 확인하였다. 따라서 본 연구는 노화 시 DNA 손상 복구의 결핍이 miR-22에 의해 MDC1 기능억제에 의함을 증명한 중요한 연구라 사료된다.

INTRODUCTION

The maintenance of an intact genome is crucial for cellular homeostasis. DNA double-strand breaks (DSBs), generated by ionizing radiation (IR) and radio-mimetic drugs, are the most cytotoxic Lesions. Failure to repair DSBs causes genomic instability and can lead to tumorigenesis and other age-related diseases upon DSB induction.[1] Thus, cells have mechanisms responsible for recognition of DNA damage and activation of cell cycle checkpoints leading to DNA repair. The generation of DSBs triggers the relocalization of many DNA damage response (DDR) proteins such as MRE11/NBS1/RAD50, MDC1, 53BP1, and BRCA1 to nuclear foci where these proteins colocalize and interact with γ-H2AX.[2] Mediator of DNA damage checkpoint protein 1(MDC1) contains protein-protein interaction domains, such as the FHA and the BRCT domain, it is believed that MDC1 functions as an adaptor protein. Recent studies suggest that MDC1 regulates many aspects of the DNA damage-response pathway,

such as intra-S phase checkpoint, G2/M checkpoint and radiation-induced apoptosis.[3]

In recent year, microRNAs(miRNAs) have receive wide attention as important regulators of gene expression. miRNAs are a class of naturally occurring small noncoding RNAs that negatively regulate the stability and translation of target protein coding mRNAs at the 3' untranslated region (UTR).[4] A single miRNA can regulate multiple target genes simultaneously partly due to imperfect base pairing, thereby making a global impact on gene expression. Many studies have shown that miRNAs play an important role in a wide range of biological processes such as proliferation, development, differentiation, and apoptosis.[5]

Cellular senescence as the finite replicative life span of human fibroblasts in culture.[6] Because the cells underwent many divisions before arresting growth in a stable postmitotic state, this process is termed replicative senescence. In

human fibroblasts, replicative senescence correlates with gradual shortening of telomeres and accumulation of DNA damage. Telomere shortening because of the end replication problem is believed to be the prime intrinsic cause that limits the cell proliferation capacity because critically shortened telomeres serve to activate the senescence process. However, various stresses such as DNA damaging agents, oxidative stress can also induce cellular senescence. Oxidative stress such as sub-lethal doses of hydrogen peroxide (H_2O_2) has been shown to induce premature senescence in young replicating cells by telomere-independent mechanisms.[7; 8; 9]

Several studies implicate miRNAs in senescence and aging, which includes the observation that overexpression of miRNA *lin-4* results in a modest increase in the adult lifespan in *C. elegans*.[10] A role for miRNAs in cellular senescence is also supported by the observation that depletion of Dicer induces senescence through the activation of the p53 pathway.[11] Interestingly, recent studies in-

dicated that distinctive miRNA expression profiles were associated with quiescence, oxidative stress-induced or replicatively senescent fibroblast.[12] These results suggest that miRNA expression profiles might be dependent upon the status of cell cycle arrest and the types of senescence.

Here we have investigated a connection between miRNAs and DNA repair. In this study, we have found that miR-22 controls DSB repair and genomic integrity via direct targeting of MDC1. miR-22 mediated downregulation of MDC1 is contributed to impaired DSB repair in senescent cells. Thus, miR-22 plays on important role in the regulation of MDC1 expression and may represent a therapeutic target for senescent disease.

MATERIALS AND METHODS

1.Cell culture

U2OS, Hela cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and streptomycin (0.1 mg/ml), penicillin (100units/ml). MRC-5 cells were cultured in Minimum essential medium supplemented with streptomycin (0.1 mg/ml), penicillin (100 units/ml), and 10% fetal bovine serum. Cell growth was monitored under an inverted microscope. Upon reaching 70-80 % confluency, cells were digested with 0.5 % trypsin-EDTA before being passaged. All cell lines were maintained at 37 ° C and in 5 % CO_2 at saturated humidity. Cells in exponential growth were harvested for subsequent experiments.

2. RNA extraction and qRT-PCR

Total RNA was isolated from cells following the Trizol manufacturer' s protocol (Invitrogen). 1 μg of total RNA was reverse transcribed into cDNA using an first strand synthesis kit with oligo-dT primers according to the manufactur-

er' s instructions (Takara). miRNA first-strand synthesis was using Mir-X™ kit(Clontech). A synthesized cDNA was subsequently used for each qRT-PCR reaction. PCR amplification was performed using specific primer pairs. qRT-PCR was performed using the SYBR Green based fluorescent method and the MX3000P® qRT-PCR system using cycling parameters. The microRNA expression results were normalized against house keeping gene U6, and the data was analyzed using ⊿⊿C(t) and starting quantity methods.

3. Western blot

Cells were lysed in RIPA buffer (50mM Tris-HCl (pH 7.5), 150mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate) with protease inhibitors (Roche Diagnostic Corp.). Equal amounts of protein were separated by 6-15% SDS-PAGE followed by electrotransfer onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). The membranes were blocked for 1 hr with TBS-t (10mM Tris-HCl (pH 7.4), 150mM NaCl and 0.1% Tween-20)

containing 5% skim milk and then incubated at 4° C with primary antibodies(1:1000). The blots were washed four times for 15 min with 0.1% Tween 20 containing TBS-t and then incubated for 2 hr with peroxidase-conjugated secondary antibodies (1:4000). The membranes were washed four more times and developed using an enhanced chemiluminescence detection system (ECL; GE Healthcare, Buckinghamshire, UK).

4. Luciferase Assay

A fragment of the 3'UTR of MDC1(starting after the TGA stop codon and extending for 658bp) containing the miR-22 response element was cloned into pMIR-REPORT luciferase vector (Applied Biosystem). Deletion mutant of the miR-22 response element was made by GENEART Site-Directed Mutagenesis kit (invitrogen). Hela cells were plated in 6-well plates. After oneday, pMIR-REPORT luciferase vector including 3'UTR of MDC1 WT or MT and miR-22 or control oligonucleotides were transfected into cells using Lipofectamine 2000

(Invitrogen). After 48h after transfection, luciferase assay were performed using the dual luciferase reporter assay system (promega). Data were normalized to *Renilla* luciferase by cotransfection of pRL *Renilla* luciferase control reporter vector.

5. comet assay

For the comet assay, MRC5 and U2OS cells were treated with 10 Gy of γ-ray by followed by incubation in culture medium at 37° C. The alkaline version of the comet assay (single cell gel electrophoresis) was performed as described by Singh et al. Briefly, 100 μL of cells were taken, homogenized with low-melting point agarose, spread on a microscope slide pre-coated with normal-melting-point agarose and covered with a coverslip. After 30 min at 4° C, the coverslip was removed from the slides. and they were immersed in cold lysing solution (2.5 M NaCl; 100 mM ethylenediaminetetraacetic acid (EDTA); 10mM Tris, 10% dimethylsulfoxide and 1% Triton-X, pH 10) for 24 h. After lysis, the slides were

placed in an electrophoresis chamber, covered with electrophoresis buffer (300 mM NaOH, 200mM EDTA, pH >13) and left for 20 min for the DNA to unwind. The electrophoresis ran for 30 min (1 V/cm tank length), after which the slides were and fixed in 70% ethanol for 5 min. air-dried and neutralized slides were stained with 30-50 ul ethidium bromide (20 mg/ml). Cells were screened per sample in a fluorescent microscope. Average comet tail moment was scored for 40-50 cells/slide using a computerized image analysis system (Komet 5.5; Andor Technology, South Windsor, CT, USA).

6. Immunofluorescence microscopy

To visualize nuclear foci, cells were grown on glass coverslips and were irradiated with 10 Gy of ionizing radiation. cells were then washed twice with PBS, fixed with 4% paraformaldehyde for 10 min and ice-cold 98% methanol for 5 min, followed by permeabilization with 0.3% Triton X-100 for 15 min at room temperature. Next, the cover slips were washed three times with PBS and then

blocked with 5% BSA in PBS for 1hr. The cells were single or double immunostained with primary antibodies against various proteins overnight at 4° C. Next, the cells were washed with PBS and then stained with Alexa Fluor 488 (green, Molecular Probes) or Alexa Fluor 594 (red, Molecular Probes) conjugated secondary antibodies, as appropriate. After washing, the cells were mounted using Vectashield mounting medium with 4,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA, USA). Fluorescence images were taken using a Zeiss Axioplan 2 imagingepifluorescent microscope equipped with a charge-coupled device camera and ISIS software (MetaSystems, Altlussheim, Germany).

7. Senescence-associated β-galactosidase staining

For cellular senescence analysis, MRC5 cells were processed and stained with the senescence marker b-galactosidase staining kit (sigma-aldrich) according to manufacturer protocols. Wash the cells twice with 1 ml of 1X PBS per

well/plate. Carefully remove the entire wash solution by aspiration, so the cells do not detach. Add 1.5 ml per well of 1X Fixation Buffer and incubate the plate for 6−7 minutes at room temperature. During the fixation process prepare the Staining Mixture as described in the Preparation Instructions. Rinse the cells 3 times with 1 ml of 1X PBS per well/plate. Add 1 ml of the Staining Mixture per well. Incubate at 37 ° C without CO2 until the cells are stained blue (2 hours to overnight). Observe the cells under a microscope.

RESULT

1. miR-22 targets MDC1

To identify miRNAs undergoing DDR regulation and potentially contributing to the declining DSB repair, we analyzed the 3' UTR MDC1 using TargetScan. We found top four miRNAs, including miRNA-124, miR-22, miR125a-5P, and miR-98(Fig1A. upper panel). To examine whether these four regulate MDC1 expression, we examined the MDC1 protein levels by immunoblot analysis after transiently trnasfecting these miRNAs into U2OS cells. The levels of MDC1 protein were significantly reduced in miR-22 transfected cells, while no changes of MDC1 level were observed for other miRNAs(Fig 1A). In order to confirm that MDC1 is a target of miR-22, performed luciferase assay. We transfected Hela with a luciferase reporter construct which contains the wild type of MDC1 3' UTR or mutant of MDC1 3' UTR. The relative luciferase activity of the wild type construct of MDC1 3' UTR was reduced by overex-

pression of miR-22, whereas such effects of miR-22 on luciferase activity were not observed with the mutant construct of MDC1 3' UTR(Fig 1B). This result indicates that the miR-22 binding site within MDC1 3' UTR mediates miR-22 translational repression. Next we investigated whether the miR-22 controls role of MDC1 in DSB repair. miR-22 overexpressed-U2OS cells displayed significantly decrease of MDC1 protein (Fig 1C). Because recruitment of MDC1 in response to DNA damage is the major signal of DDR, we evaluated the effect of miR-22 in MDC1 foci formation following DNA damage. Immunofluorescence analysis revealed that miR-22 reduced the percentage of cells containing MDC1 foci after exposure to IR (Fig 1D). We measured DNA repair activity in control or miR-22 overexpressed U2OS cells using comet assays. miR-22 overexpressed U2OS cells exhibited longer comet tails, compare with control cells(Fig 1E), suggesting that miR-22 is a negative regulation of MDC1 function.

As a result of the above, To confirm whether this effect on MDC1 is specifically attributable to miR-22, miR-22 anti-sense oligonucleotide(ASO-mir-22) was transfected into U2OS cells in the presence of miR-22. Expression of ASO-miR-22 rescued miR-22 mediated decrease in MDC1 protein(Fig 2A). We measured the residual DSBs after IR, as an indicator of unrepaired damaged DNA, by γ-H2AX staining and single-cell electrophoresis(comet assay). γ-H2AX is a marker of double-strand breaks. After irradiation with ionizing radiation(IR), H2AX is rapidly phosphorylated and there is always a constant number or percentage of γ-H2AX formed per DSB.[13] miR-22 transfection enhanced γ-H2AX level compared with control-transfected cells. The depletion of miR-22 by transfection with ASO-miR-22 reduced level of γ-H2AX, Probably due to the repair of DNA breaks(Fig 2B). After DNA is repaired, γ-H2AX is dephosphorylated.[14] Comet assay also showed that inhibitory effect of DSB repair by miR-22 was fully rescued by transfecting ei-

ther ASO-miR-22(Fig 2C), Indicating that miR-22 regulates DSB repair by controlling MDC1.

To confirm whether this effect of miR-22 is specifically to MDC1, we co-transfected miR-22 and MDC1 in cells and analyzed the ability of cells to repair DSB. Expression of MDC1 recued miR-22 mediated decrease in MDC1 protein(Fig 3A). Immunofluorescence analysis revealed that decrease of MDC1 by miR-22 was rescued by transfecting MDC1 expression plasmid(Fig 3B). In addition, MDC1 overexpressed cells, the number of bright γ-H2AX foci decreased after IR(Fig 3C). We performed comet assay for detected DSBs repair. This result also showed that overexpression of MDC1 reduced defect of DSB repair by miR-22(Fig 3D), indicating that MDC1 overexpression causes rescue of the MDC1-mediated DNA repair.

2. miR-22 mediated downregulation of MDC1 suppress DSB repair in senescence cells.

Replicative senescence was first described in human fibroblasts as state of permanent cell cycle arrest resulting from serial passage in culture due to a limited proliferative lifespan.[15] Sub-lethal oxidative stress such as hydrogen peroxide(H_2O_2) treatment can cause massive acute DNA double-strand breaks. Much of this DNA damage can be repaired and thus the cell can re-enter the cell cycle, however some of the DNA damage persists which will eventually trigger premature senescence. Such persistent DNA damage can be increased substantially by H_2O_2 treatment, thus resulting in a high induction of premature senescence.[16; 17] Senescent cells can be distinguished by the presence of a biomarker-senescence associated beta-galactocidase (SA-β-gal), which is detectable at pH6.[18] We adopted H_2O_2 treatment to induce premature cellular senescence, 150uM H_2O_2 for 1hr with very few lethal ef-

fects under the conditions growing 7days and replication senescence used in this study. Previous studied that miR-22 expression was increased in senescent cells.[19] Given miR-22 was abundant in senescent cells and MDC1 is a target of miR-22, we hypothesized that the miR-22 upregulation observed in senescent cells is capable of DSB repair defect as a result of MDC1 down-regulation. As a first attempt to evaluate this hypothesis, we analyzed miR-22 expression and MDC1 expression in replicative senescence and H_2O_2-induced senescence. To do this purpose, we induced senescence in MRC5 cells by serial passaging or by treatment with H_2O_2. The senescent cells displayed a flatted morphology and increased senescence-associated β-galactosidase (SA-β-gal) activity (Fig 4A). Next, using Quantitative(q)RT-PCR analysis, we confirmed that miR-22 expression was increased in replicative senescence and H_2O_2-induced senescence cells. And MDC1 expression was decresed compare with young cells (Fig 4B and 4C). To investigated functional signifi-

cance of upregulated miR-22 in senescent cells, we examined IR-induced MDC1-foci formation. The foci formation of MDC1 markedly was reduced in senescent cells, compare with young cells following exposure to irradiation (Fig 4D). In addition, comet tail was elongated in H_2O_2 -induced senescence cells as well as replicative senescence cells, compare with young cells(Fig 4E). Taken together these results that senescence cells-induced increase of miR-22 expression and decrease MDC1 expression impairs DSBs repair.

Next, we tested whether introducing ASO-miR-22 or MDC1 expression vector into senescent cells cause a functional change in DSB repair. Young and H_2O_2 -induced senescent MRC5 cells transfected ASO-miR- 22(100nM). Western blot analysis of MDC1 was performed. Downregulation of miR-22 rescued the MDC1 protein expression level in senescent cells(Fig 5A). Quantitative(q)RT-PCR analysis for expression of MDC1 mRNA was used to determine whether ASO-miR-22 was rescued MDC1 mRNA expression in

H_2O_2 -induced senescence cells. ASO-miR-22 transfected MRC5 cells decreased miR-22 expression and increased MDC1 expression(Fig 5B). To test, effect of ASO-miR-22 of IR-induced MDC1 foci formation in H_2O_2 -induced senescent cells. IR-induced MDC1 foci formation in senescent cells was rescued by overexpression of ASO-miR-22 (Fig 5C). Furthermore, using comet assay we found that comet tail in senescent cells was shortened by overexpression of ASO-miR-22 (Fig 5D), indicating these results that suppression of miR-22 causes rescue of the MDC1 mediated DNA repair in H_2O_2 -induced senescent cells.

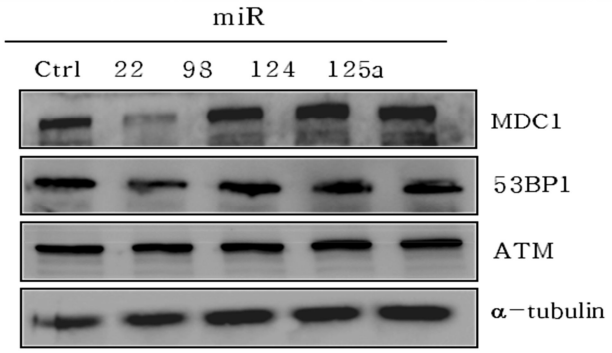
Previous our results show that MDC1 expression was decreased in senescent cells. We hypothesis that MDC1 overexpression was recovered miR-22 mediated MDC1 defect in senescent cells. Young and H_2O_2 -induced senescent MRC5 cells transfected MDC1 cDNA. Immunoblot analysis revealed that the levels of MDC1 protein was rescued in H_2O_2 -senescent cells by over-

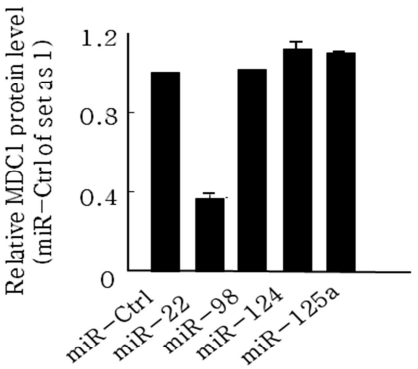
expression of MDC1(Fig 6A). Morever, DNA damage repair was observed by comet assay. MDC1 overexpressed senescent cells decreased comet tail length compare with, H_2 O_2 -induced senescent cells (Fig 6B). Taken together, these results suggest that miR-22 mediated down regulation of MDC1 impedes the DSB repair in senescent cells.

Figure 1

A

miRNA	conserved sites				poorly conserved sites				total context score
	total	8mer	7mer-m8	7mer-1A	total	8mer	7mer-m8	7mer-1A	
miR-124	1	1							-0.51
miR-22					1	1			-0.46
miR-125a					1		1		-0.29
miR-98					1		1		-0.17





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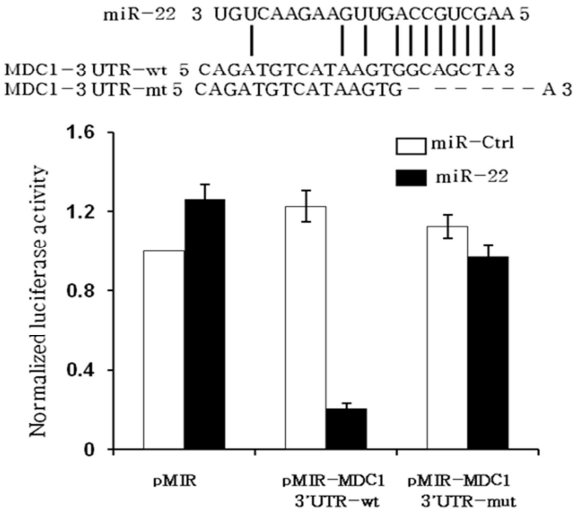

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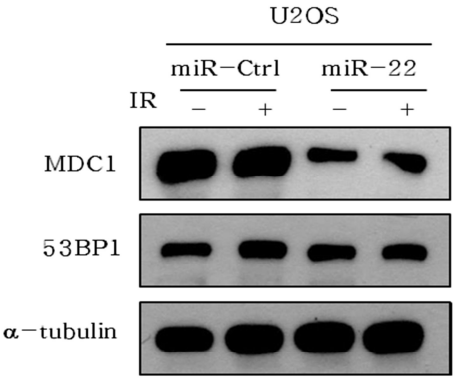

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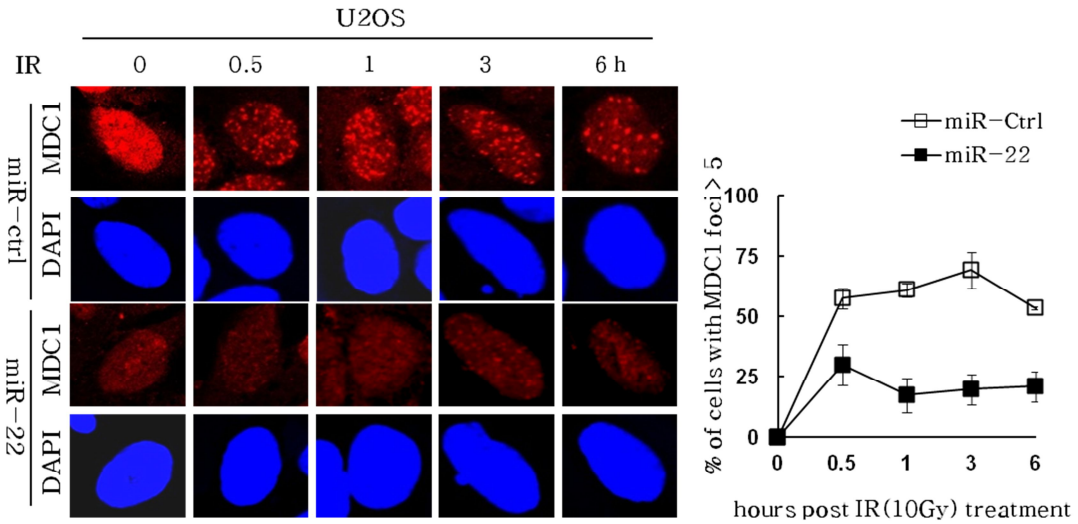

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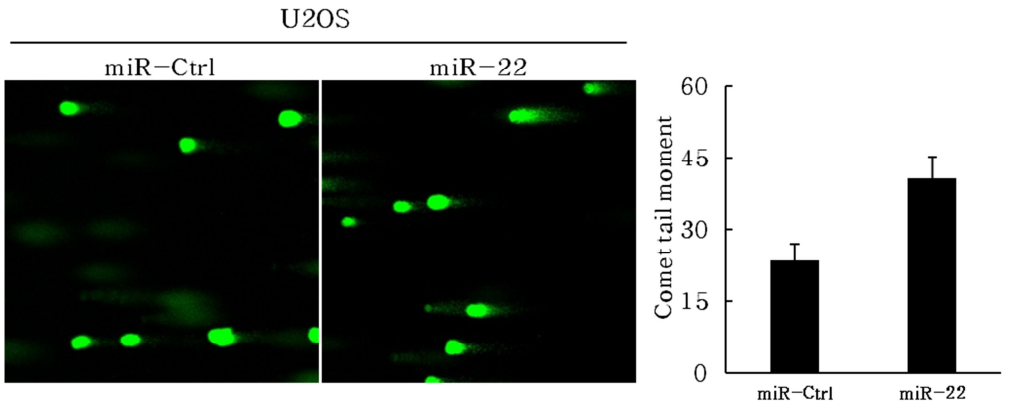


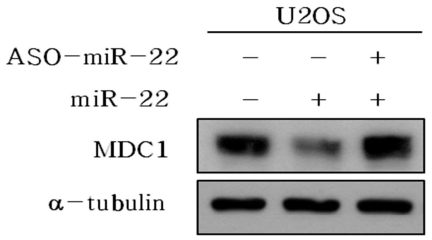
Figure 1. MDC1 is target of miR-22 and impact DNA damage repair.

(A)U2OS cells transiently transfected with control, miR-22, miR98, miR124 and miR-125a, and then harvested after 48hr, and then cell lysates analyzed by western blot using indicated antibodies. (B) miR-22 sequences and recognition site within 3' UTR of MDC1. Hela cells were cotransfected with miR-22 and MDC1 3' UTR luciferase reporter construct containing wild-type(wt), or mutant(mt) MDC1 3' UTR. Firefly luciferase activity of the reporter was normalized to an internal Renilla luciferase control. (C) Control and miR-22 expressed U2OS cells were irradiated at 10Gy for 3hours, and then western blot of MDC1 was performed. (D) Control and miR-22 overexpressed U2OS cells were untreated or treated with 10Gy ionizing radiation(IR) at the indicated times, cells were fixed and immunostained using anti-MDC1 antibody. Nuclei were counter stained with DAPI. Right graph showed percentages of cells, noted above, that contain at least 5 each category of MDC1 foci in 100 cells. (E) Effect of miR-22

on single cell electrophoresis (comet) assay of U2OS cells treated with IR. Representative comet figures for 3hr after IR are shown in the left panels. The comet tail moments of 100 cells were measured, and then values with standard deviations are shown in the right graph.

Figure 2

A



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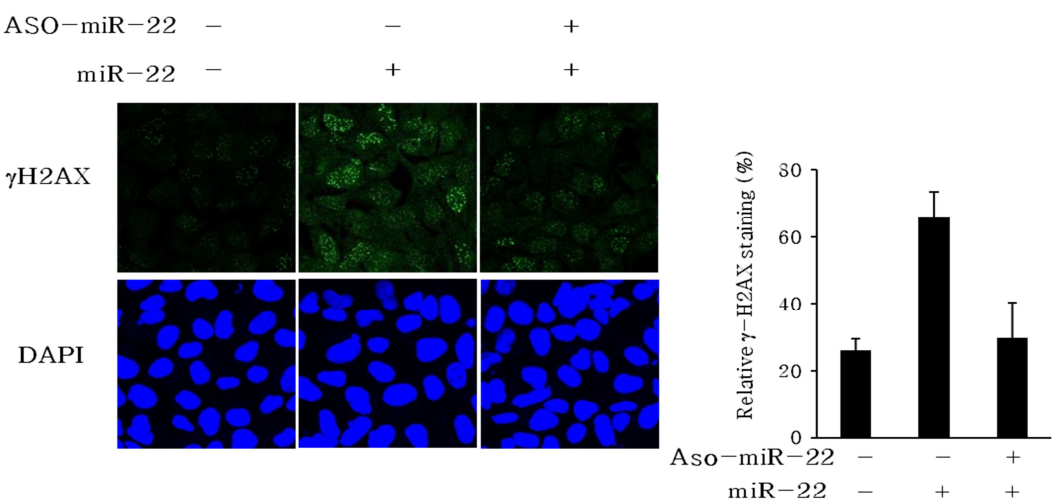

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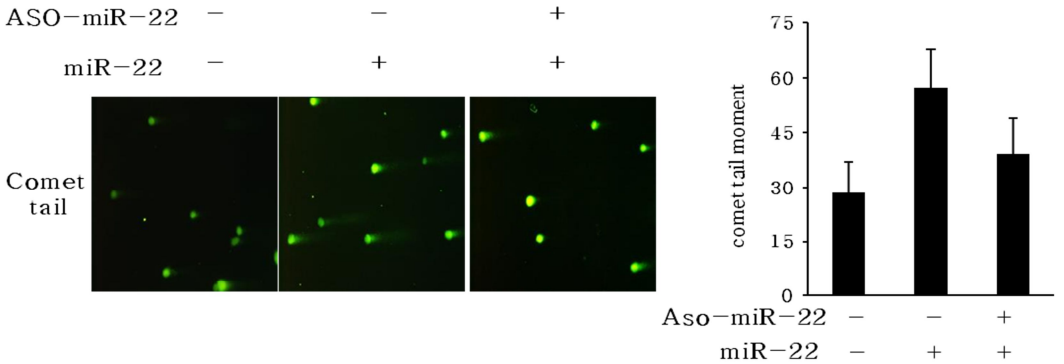
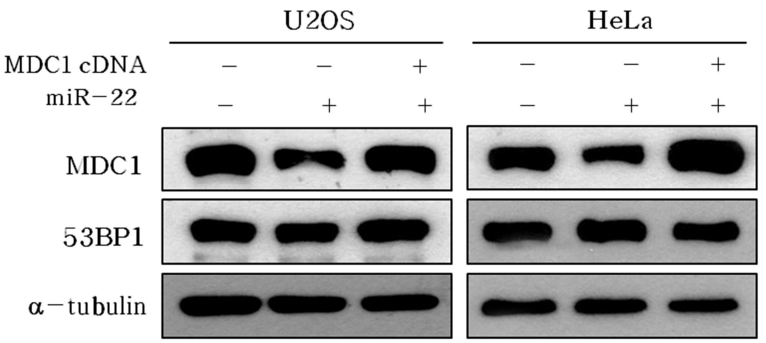


Figure 2. ASO-miR22 overexpression causes rescue of the MDC1-mediated DNA repair. miR-22 cells treated with or without ASO-22. (A) immunoblot analysis of MDC1 in indicated U2OS cell. (B) Cells were treated IR and immunostained using anti-γ-H2AX antibody. Counts of γ-H2AX foci au 16hours after irradiated with 10Gy of IR in indicated cells. Right graphs show percentages of cells containing at least 10 foci per cells in 100 cells. (C) Influence of DSB repair of miR-22 rescue. U2OS cells were treated with IR(10Gy) for 3hours, and analysed by single-cell gel electrophoresis.

Figure 3

A



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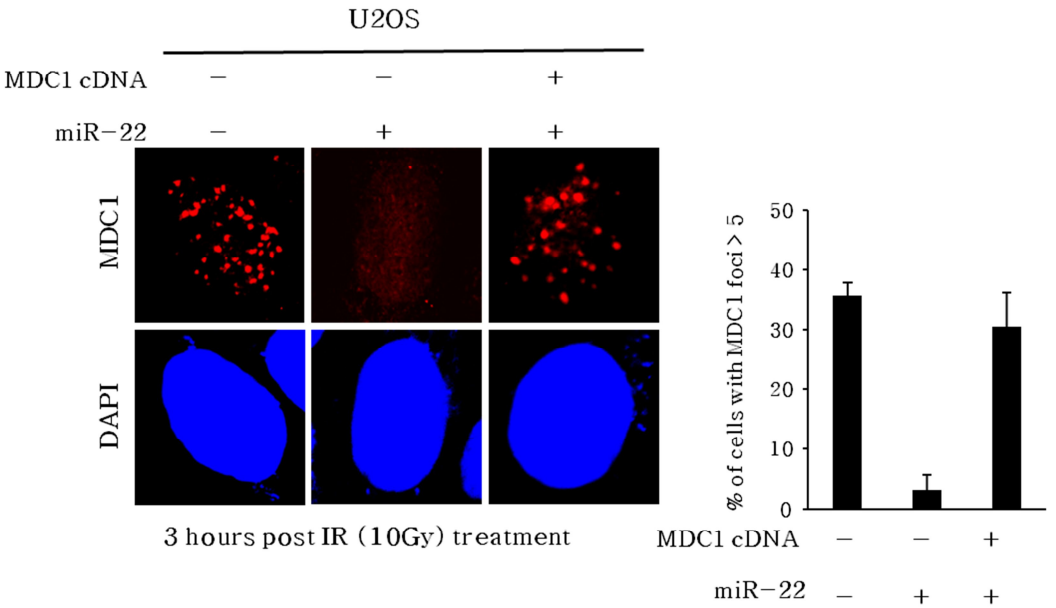

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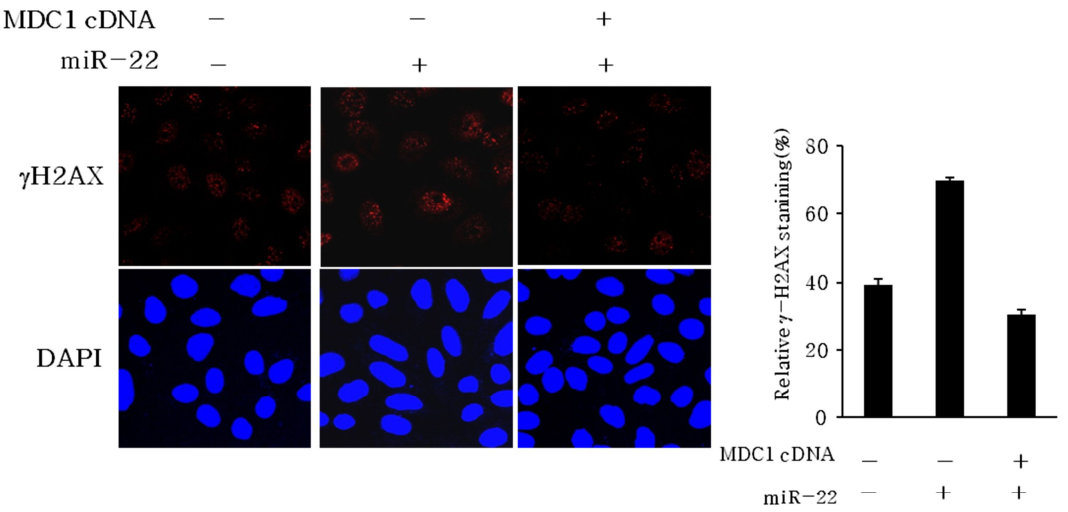

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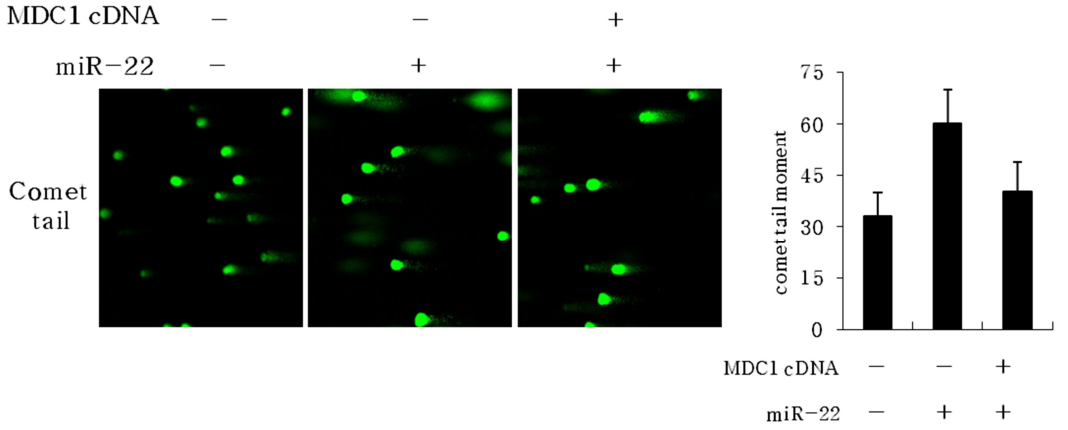
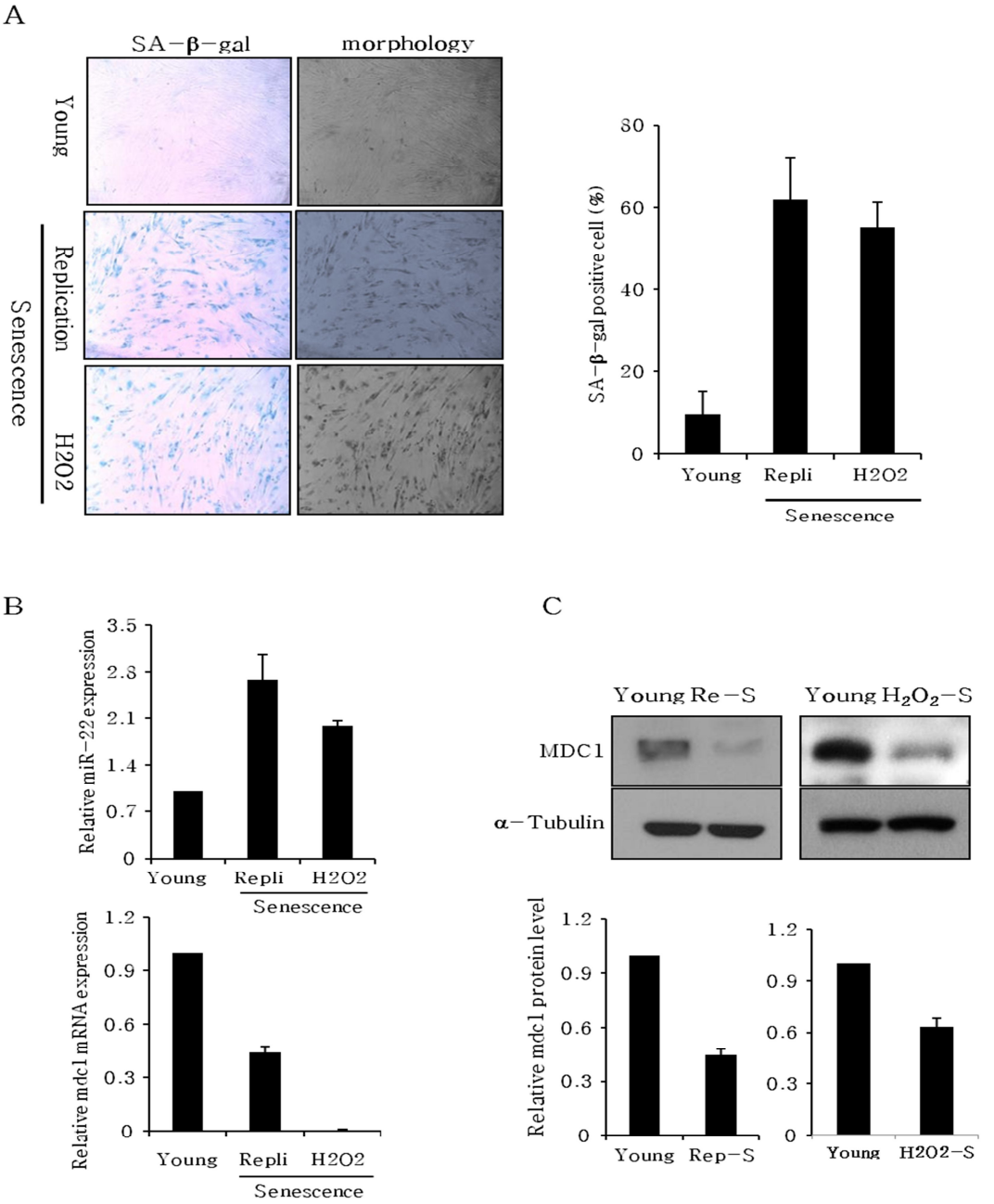


Figure 3. MDC1 overexpression causes rescue of the MDC1-mediated DNA repair.

miR-22 cells treated with or without MDC1 cDNA. (A) immunoblot analysis of MDC1 in indicated U2OS cell. (B)Cells were treated IR and immunistained using anti-MDC1 antibody. Nuclei were counter stained with DAPI. Right graph showed percentages of cells, noted above, that contain at least 5 each category of MDC1 foci in 100 cells. (C) Cells were treated IR and immunostained using anti-γ-H2AX antibody. Counts of γ-H2AX foci au 16hours after irradiated with 10Gy of IR in indicated cells. Right graphs show percentages of cells containing at least 10 foci per cells in 100 cells. (D) Influence of DSB repair of MDC1 rescue. U2OS cells were treated with IR(10Gy) for 3hours, and analysed by single-cell gel electrophoresis.

Figure 4



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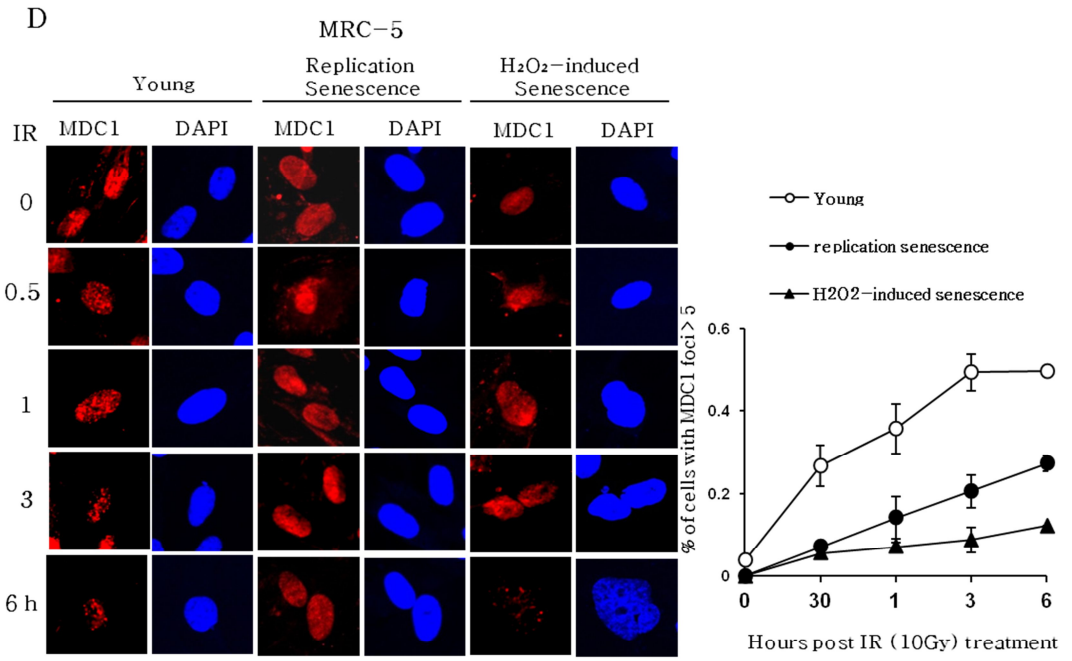

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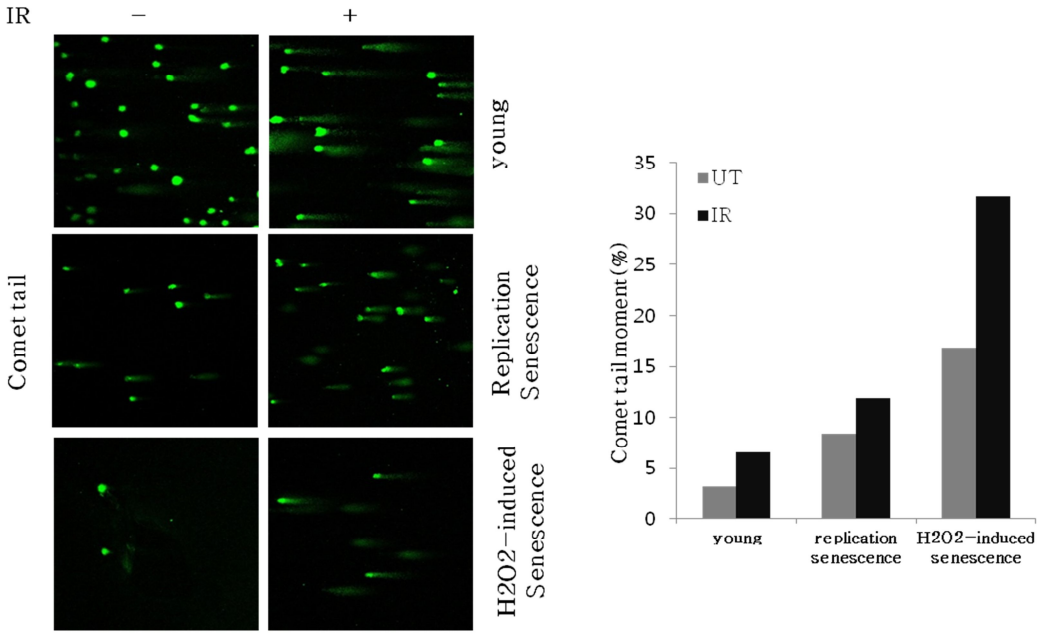
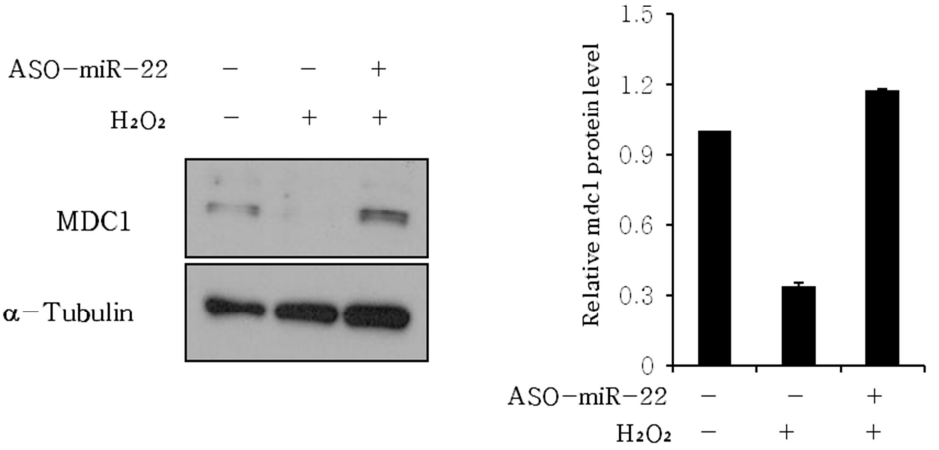


Figure 4. Senescence-induced increase of miR-22 impairs DSB repair through silencing of MDC1. (A) representative pictures of senescence cells showing SA-β-gal positive staining(blue). Right graph was indicated that SA-β-gal activity was presented by the percentage of SA-β-gal positive cells. (B) qRT-PCR analysis of miR-22 and MDC1 mRNA in MRC-5 cells. (C) western blot analysis of MDC1 in indicated senescent cells. (D) Immunofluorscence assay of MDC1 in senescent MRC-5 cells. (E) Comet assay in the senescent MRC-5 cells. Cells were treated with IR(10Gy) for 3hours, and analysed by single-cell gel electro-phoresis.

Figure 5

A



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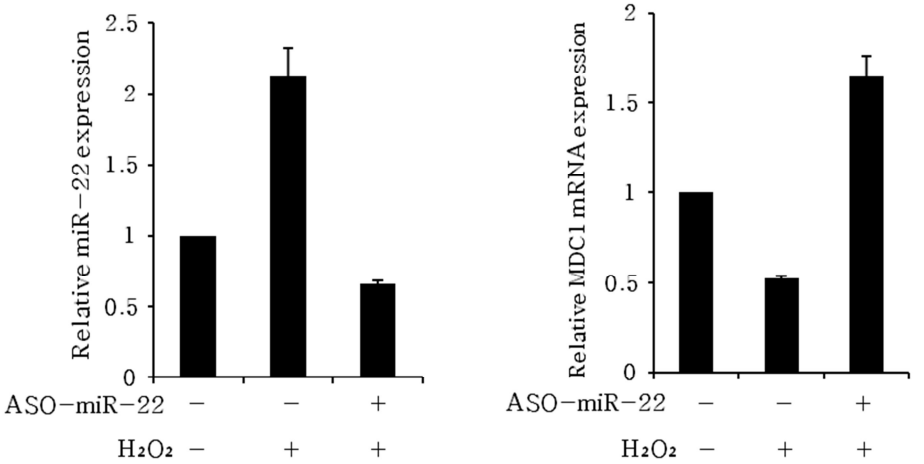

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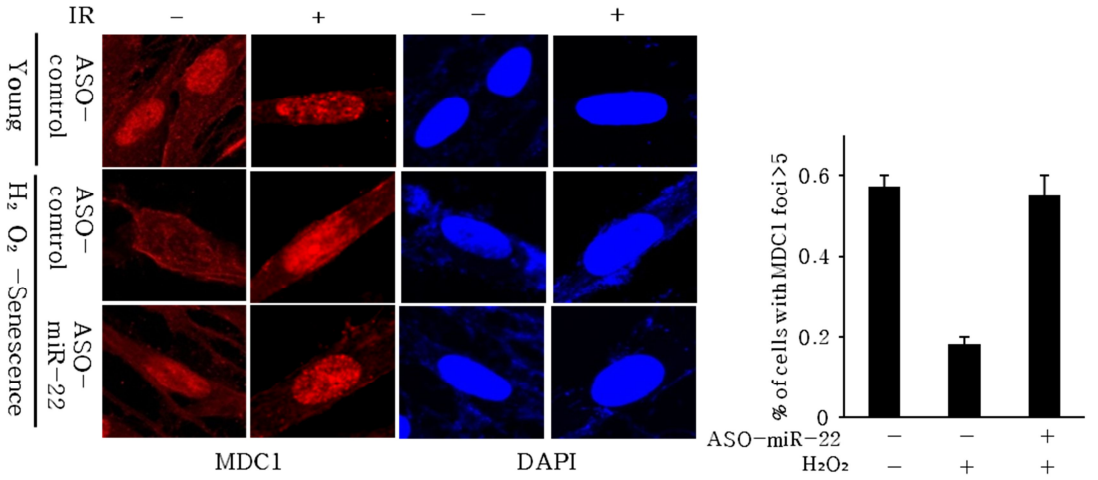

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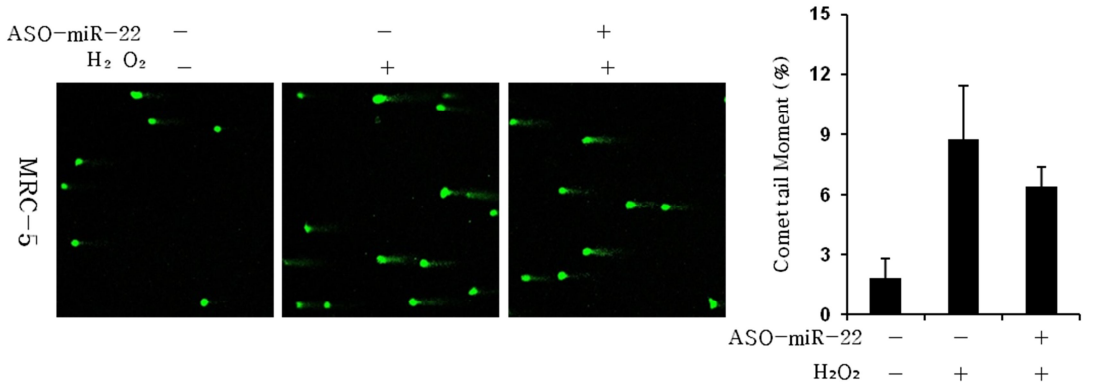
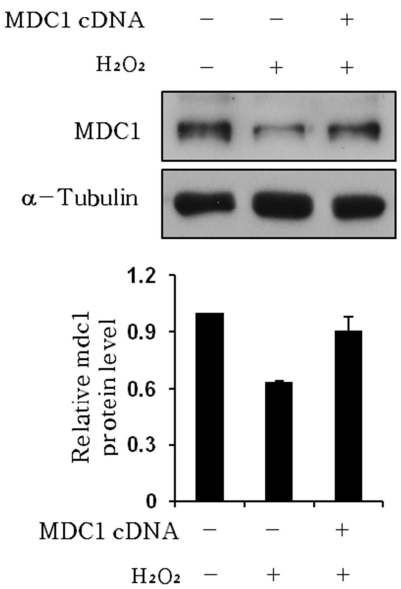


Figure 5. ASO-miR22 overexpression causes rescue of the MDC1-mediated DNA repair in H2O2-induced senescence cell. H2O2-induced senescence cells treated with or without ASO-mi22. (A) Immunoblot of MDC1 expression in MRC-5 cells. (B) qRT-PCR analysis of miR-22 and MDC1 mRNA in MRC-5 cells. (C) MRC-5 cells were treated IR10Gy for 3hr, and then fixed. immunostained using anti-MDC1 (D) Comet Assay of DNA Damage Repair. MRC-5 cells were treated with IR(10Gy), incubation for 6hr, and analysed by single-cell gel electrophoresis.

Figure 6

A



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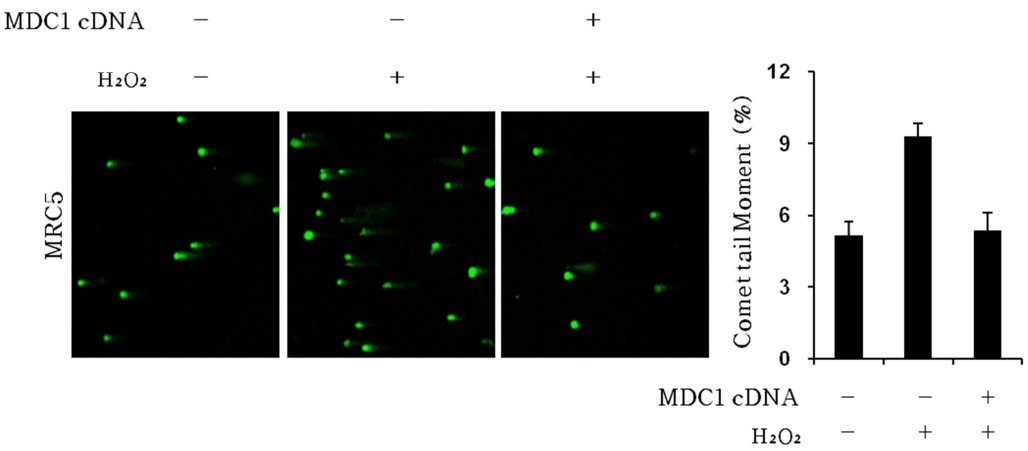

Figure 6. MDC1 overexpression causes rescue of the MDC1-mediated DNA repair in H2O2-induced senescence cell. H2O2-induced senescence cells treated with or without MDC1 (A) Immunoblot of MDC1 expression in MRC5 cell. (B) Comet Assay of DNA Damage Repair. Cells were treated with IR, and an-laysed by single-cell gel electrophoresis.

DISCUSSION

Using TargetScan program, we identified miR-22 as a direct negative regulator of the MDC1 protein. Overexpression of miR-22 reduced both transcriptional and MDC1 3′UTR, impairing DSB repair.

The premature aging and cellular senescence phenotypes in humans and mice with genetic defects in DNA repair factors suggest that genomic instability is a major cause for cellular senescence and aging. Similarly, telomere dysfunction also triggers cellular senescence through the DNA damage response pathway.[20] These processes depend on functional modulation of group of key molecules at transcriptional and post-transcriptional levels. Since miRNAs were found to play an important role in DDR, accumulating evidence has indicated that the expression of miRNAs can be regulated at the transcriptional level and during biogenesis upon DNA damage.[21; 22] However, the mechanisms of senescence-mediated decreases in DSB repair have yet to be elucidated. In the pre-

sent study, we found that MDC1 expression is down-regulated in both replicative and stress-induced senescence due to an increased abundance of miR-22. Our studies reveal an important role of miR-22 in the control of MDC1 function and DSB repair in senescent cells. Our rescue experiments demonstrate that the MDC1 gene is a key target through which miR-22 exerts its inhibitory effect on DDR in senescent cells.

This is an important discovery we have uncovered for the first time. Many genetic studies of DNA repair factors support the important role of DNA repair in the prevention of cellular senescence and premature aging.[23] Accumulation of cellular senescence, perhaps indicate a degree of inevitability in the cellular aging process.[24] Therefore, our results will reveal important insight into cellular senescence and aging. Furthermore, we should expect to have a effective for the therapeutic of senescence-related disease.

ABSTRACT

miR-22 regulates DNA double-strand break repair through silencing of MDC1

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DNA damage responses have been well characterized with regard to their cell-autonomous checkpoint functions leading to cell cycle arrest, senescence and apoptosis. Mediator DNA damage checkpoint protein 1(MDC1) plays check-point activation and DNA repair following DNA damage. MicroRNAs are small non-coding RNA molecules that negatively control the expression of target genes post-transcriptionally. Here we report that MDC1 was identified as one of

the targets of miR-22. miR-22 controls DNA damage repair through regulation of MDC1 expression. Previous studies have shown that miR-22 expression increases in senescent cells. Increase of miR-22 expression in senescent cells exhibits suppression of MDC1 expression and defective DNA repair. ASO-miR22 or MDC1 overexpression causes rescue the MDC1 expression, MDC1 foci recruitment and DNA repairs. Taken together, this study defines a critical role for miR-22 in the senescence-related of DSB repair through targeting MDC1.

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