Molecules and Cells

Cellular Dynamics of Rad51 and Rad54 in Response to Postreplicative Stress and DNA Damage in HeLa Cells

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Homologous recombination (HR) is necessary for maintenance of genomic integrity and prevention of various mutations in tumor suppressor genes and proto-oncogenes. Rad51 and Rad54 are key HR factors that cope with replication stress and DNA breaks in eukaryotes. Rad51 binds to single-stranded DNA (ssDNA) to form the presynaptic filament that promotes a homology search and DNA strand exchange, and Rad54 stimulates the strand-pairing function of Rad51. Here, we studied the molecular dynamics of Rad51 and Rad54 during the cell cycle of HeLa cells. These cells constitutively express Rad51 and Rad54 throughout the entire cell cycle, and the formation of foci immediately increased in response to various types of DNA damage and replication stress, except for caffeine, which suppressed the Rad51-dependent HR pathway. Depletion of Rad51 caused severe defects in response to postreplicative stress. Accordingly, HeLa cells were arrested at the G2-M transition although a small amount of Rad51 was steadily maintained in HeLa cells. Our results suggest that cell cycle progression and proliferation of HeLa cells can be tightly controlled by the abundance of HR proteins, which are essential for the rapid response to postreplicative stress and DNA damage stress.

Keywords: DNA damage, HeLa cell, homologous recombination, Rad51, Rad54

INTRODUCTION

Eukaryotic DNA replication is a highly coordinated process that is a precondition for the separation of chromosomes before cell division (Rothstein et al., 2000). To ensure faithful transmission of genetic information via conserved DNA sequences to daughter cells, various cellular regulatory mechanisms—checkpoint activation, DNA repair, recombination, sister chromatid separation, and cell cycle progression—are involved in the DNA replication process. These processes are necessary for every cell division, and have been well characterized in many organisms, including yeast, plants, and mammals (McGill et al., 1993). During DNA replication, the cell can make errors and ultimately insert incorrect nucleotides into DNA. When the cell divides its DNA without corrections, the paired DNA strands no longer have exactly the same sequence. Therefore, mutations are established and accumulated, and cells cannot recognize the errors during further cell cycle progression. Specifically, if improper DNA replication is allowed to proceed, incorrectly paired nucleotides can become permanent mutations, leading to cell death or abnormal cell growth (Branzei and Foiani., 2010; Merrick et al., 2004).

The sources of DNA damage include endogenous stress such as replication-induced single-stranded DNA (ssDNA) gaps or enzymatic conversions. Furthermore, exogenous chemical agents can induce a variety of DNA lesions such as interstrand crosslinks (ICLs) and DNA breaks. DNA damage

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repair mediated by homologous recombination (HR) is essential for maintenance of genome integrity and for avoidance of cancer in humans (Cejka et al., 2010; Krejci et al., 2012; Nimonkar et al., 2011; Niu et al., 2010). HR serves to eliminate deleterious lesions, such as ICLs, double-strand breaks (DSBs), and DNA gaps. Moreover, HR provides crucial support for the recovery of stalled replication forks, contributing to cellular proliferation (Krejci et al., 2012; Niu et al., 2010; Rothstein et al., 2000). In meiotic cell cycles, HR-mediated exchange of genetic information between allelic sequences is required for genetic variety within the same species (Hong et al., 2013; Kim et al., 2010; Lee et al., 2015; Yoon et al., 2014; 2016). In particular, the Rad51-dependent pathway mediates DNA strand exchange between sister chromatids or homologous chromatids during HR in eukaryotes (Lambert and Lopez, 2000). Furthermore, the Rad51 protein family members form nucleoprotein filaments with ssDNA and promote the homology search and strand transfer, which are key HR processes (Jasin and Rothstein, 2013; Lambert and Lopez, 2000). Rad54 is an accessory factor of Rad51 and has a double-stranded-DNA-dependent ATPase activity, and can stabilize the Rad51-ssDNA filament. It also promotes DNA synthesis via dissociation of Rad51 from heteroduplexed DNA (Li et al., 2007; Mazin et al., 2003). Defects in these controls owing to functional errors in HR proteins induce genomic instability leading to mutation accumulation, cell death, and tumorigenesis.

In this study, we assessed the molecular dynamics of the key HR factors, Rad51 and Rad54, which are intimately involved in strand exchange against various types of DNA damages. We show that both Rad51 and Rad54 are abundantly expressed throughout the cell cycle of HeLa cells, and that these HR factors immediately relocate to chromosome break sites in response to diverse DNA damage. Moreover, we show that depletion of Rad51, but maintenance of a certain amount of Rad51, causes accumulation of cells at the G2-M transition and inhibits cell cycle progression. Our results suggest that constitutive expression of Rad51 and Rad54 helps the cell to handle replication-coupled HR effectively, thereby counteracting replication stress or DNA damage in HeLa cells.

MATERIALS AND METHODS

Cell culture

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM; cat. # 11995-073, Gibco), which was supplemented with 10% (v/v) of fetal bovine serum (FBS) (16000-044, Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin (15140-122, Gibco). The cells were cultured at 37°C in a humidified atmosphere containing 5% of CO_2.

Western blot analysis

Cell samples were washed twice with PBS and lysed in cell lysis buffer supplemented with a protease inhibitor cocktail (Calbiochem) containing 1 mM PMSF. Protein samples (50 μg) were resolved by SDS-PAGE in a 9% gel. The following antibodies were used: anti-Rad51 (cat. # sc-8349, Santa Cruz Biotechnology), anti-Rad54 (sc-374598, Santa Cruz Biotechnology), anti-β-actin (A5316, Sigma), and anti-α-tubulin antibodies (sc-8035, Santa Cruz Biotechnology). Immunoreactivity was detected using a Power Opti-ECL solution (Animal Genetics, Inc.). Rad51 and Rad54 expression levels were quantified using the Quantity One software.

Cell cycle synchronization and fluorescence-activated cell sorting (FACS) analysis

HeLa cells were synchronized at the G1-S checkpoint by treatment with 2 mM thymidine (T1895, Sigma) for 16 h, after which they were washed briefly with Dulbecco's phosphate-buffered saline (DPBS) (14190, Gibco) and supplied with a fresh medium. For FACS analysis, the cells were harvested, fixed overnight in 70% ethanol, and stained with propidium iodide (PI; P4179, Sigma) for 30 min in the dark. The samples were analyzed using a FACSCalibur flow cytometer (Becton Dickinson).

Induction of DNA damage

HeLa cells were treated with the following: 4 mM hydroxy urea (HU) for 16 h or 10 μM etoposide (ETP) or 30 mM caffeine for 4 h. The cells were then analyzed by western blotting, immunofluorescence, and FACS.

RNA interference

A commercially available siGENOME siRNA SMART pool against the Rad51 gene (siRad51) was used to knock down the endogenous expression of Rad51 in HeLa cells (M-062730-01-005, Dharmacon). This siRNA pool consisted of a mixture of four targeting oligonucleotides with the following sequences: 5′-CAUCAUCGCUCAUGCGUCA-3′, 5′-UGUCAUACGUUGGCUGUUA-3′, 5′-GGUAAUCACCAACCAGGUA-3′, and 5′-GAGAUCAUACAGAUAACUA-3′. siRNA transfection was performed using DharmaFECT-1 (T-2001, Dharmacon). A nontargeting siRNA (siCtrl) served as a negative control (ON-TARGETplus non-targeting pool, Dharmacon).

Immunofluorescence analysis

Cell were treated with 1% paraformaldehyde for 15 min and permeabilized with 0.1% Triton X-100 for 15 min. Cells were then blocked with 3% bovine serum albumin (BSA) in PBS with 0.1% Tween 20 (PBST) for 30 min and incubated for 1 h with the following primary antibodies, diluted at the indicated ratios in 3% BSA in PBST: anti-Rad51 (sc-8349, Santa Cruz) and anti-Rad54 (sc-374598, Santa Cruz). After three washes with PBST, the cells were incubated for 1 h with appropriate Alexa 488-, fluorescein isothiocyanate (FITC)-, Cy3-, or Cy5-conjugated secondary antibodies and then mounted by means of a mounting solution containing 4′,6-diamidino-2-phenylindole (DAPI). Images were captured using the Eclipse Ti-E fluorescence microscope (Nikon, Tokyo, Japan) with a 100× objective lens (NA 1.40).

Homologous recombination assay

HeLa cells were transfected with pDR-GFP and selected using puromycin at 1.5 μg/ml to obtain cells with stable transfection. The clones that contained pDR-GFP were verified by

determining the frequency of the I-SceI-inducible HR, which produces green fluorescent protein (GFP)-positive signals.

Cell death analysis

To analyze cell death, thiazole orange (TO; 349483, BD) and PI (349483, BD) were added into the cell suspension. The cell samples were incubated for 5 min at 25°C, then analyzed on the FACSCalibur flow cytometer (Becton Dickinson).

Analysis of high-throughput RNA sequencing (RNA-Seq) data

HeLa cell RNA-Seq data were downloaded from the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) web server (https://www.ncbi.nlm.nih.gov/sra). The SRA accession numbers are SRR2002765 and SRR3117568. HISAT2 was used to map the RNA-Seq reads to the human genome assembly hg38 (https://ccb.jhu.edu/software/hisat2/index.shtml) (Kim et al., 2015). Quantitation of transcripts was performed using StringTie (https://ccb.jhu.edu/software/stringtie/).

RESULTS AND DISCUSSION

Rad51 and Rad54 are constitutively expressed in HeLa cells during the cell cycle

Rad51 plays central roles in HR-mediated repair of DNA damage in eukaryotes (Lambert and Lopez, 2000; Subramanyam et al., 2016). Rad54, an accessory factor of Rad51, stabilizes the Rad51-ssDNA filament and stimulates the DNA pairing activity of Rad51. Both Rad51 and Rad54 are cooperatively involved in the search for homologous DNA templates and strand exchange between homologous chromatids (Li et al., 2007; Ristic et al., 2001; Zhdanova and Rubtsov, 2016). Furthermore, the expression of Rad51 in healthy human cells decreases in the G1 phase, increases in the S phase, and reaches the maximum at the G2-M checkpoint (Chen et al., 1997). In this study, expression levels of Rad51, Rad54, and γH2AX in MRC5 cells were 9.3%, 17.8%, and 107%, respectively, compared to Hela cells (Fig. 1A). To further characterize the cellular dynamics of Rad51 and Rad54 in HeLa cells, we analyzed the expression patterns of Rad51 and Rad54 throughout the cell cycle under normal growth conditions. Cells were treated with thymidine, which can inhibit DNA synthesis through an imbalance of the nucleotide pool, causing cell cycle arrest at the G1-S checkpoint (Figs. 1B and 1C). After we released cells from the double-thymidine block, synchronous cells progressed through the cell cycle and underwent mitosis (Figs. 1D and 1E). The synchronized cells at G1 progressed to the S phase, and then gradually returned to an asynchronous state (Fig. 1E). We then analyzed the expression dynamics of Rad51 and Rad54 throughout the cell cycle using western blot analysis of whole-cell lysates of HeLa (Figs. 1F and 1G). As shown in Fig. 1E, Rad51 and Rad54 were consistently expressed, and their expression levels were similar during the entire cell cycle. We speculated that high expression levels of HR proteins, regardless of the cell cycle phase, lead to a rapid response to DNA damage and broken replication forks to promote the HR process.

HR proteins rescue stalled or damaged replication forks including repair of DNA breaks. Therefore, the HR pathway related to the replication is required for rapid resumption of DNA replication when cellar genetic material contains DNA gaps, ICLs, and DSBs. However, whether the expression patterns of multiple genes known as HR factors are critically relevant to the cell cycle has not been investigated in HeLa cells. We conducted a global transcript analysis using RNA-Seq data to measure the gene expression of multiple HR factors in HeLa cells, where cells were cultured under normal conditions. Our fragments per kilobase million (FPKM) analysis from RNA-Seq experiments revealed that the transcripts of genes relevant to prereplication were highly expressed among the numerous genes involved in DNA replication (Fig. 1H). DNA replication errors induced by endogenous or exogenous stressors can lead to a genetic disease or cancer, and several DNA replication proteins including MCMs that function as a helicase are not only required for continuous proliferation but also for cell survival. Moreover, except for *DNA2* involved in DNA replication, the transcripts of genes relevant to synapse formation and DSB processing were maintained at high expression levels (Fig. 1I).

We identified significant gene expression levels for the MCM complex and HR factors that are involved prereplication, synapse formation, and DSB processing in HeLa cells. Therefore, the abundance of HR factors expressed in HeLa cells may induce rapid responses to postreplication repair of ssDNA gaps, fork reversals, and DNA damage via a mechanism that does not affect the DNA replication rate.

The Rad51-mediated HR mechanism is required for cell viability and G2-M transition

Rad51 and Rad54 pair with ssDNA to form nucleofilaments that mediate the processes of DSB repair and recovery of replication fork collapse that spontaneously arises during the cell cycle (Blow and Gillespiel, 2008; Puchta et al., 1993; Rouet et al., 1994; Sieber et al., 2003). HeLa cells constitutively express HR proteins throughout the cell cycle (Fig. 1G). Therefore, the HR mechanism could actively rescue diverse DNA lesions induced by exogenous DNA damage. To study the response of Rad51 and Rad54 in HeLa cells growing in an abnormal environment, we induced DNA damage using chemical reagents with different targeting mechanisms. HeLa cells were cultured in a medium containing ETP, the most selective topoisomerase II inhibitor that prevents religation of the DNA strands; HU, which blocks nucleotide synthesis by acting as a ribonucleotide reductase inhibitor; cisplatin, which induces inter-strand crosslinks; caffeine, which blocks activation of ATM or ATR leading to the G2-M cell cycle arrest (Zelensky et al., 2013). We found that ETP, cisplatin, and caffeine induced cell cycle arrest at the S-G2 transition, and approximately 88.1% of the cells were arrested at the G1-S checkpoint after treatment with HU (Fig. 2A). Therefore, we concluded that the damaged cells could not complete DNA replication and G2-M transition.

To investigate the expression patterns of the HR factors in HeLa cells, we performed western blot analysis of DNA

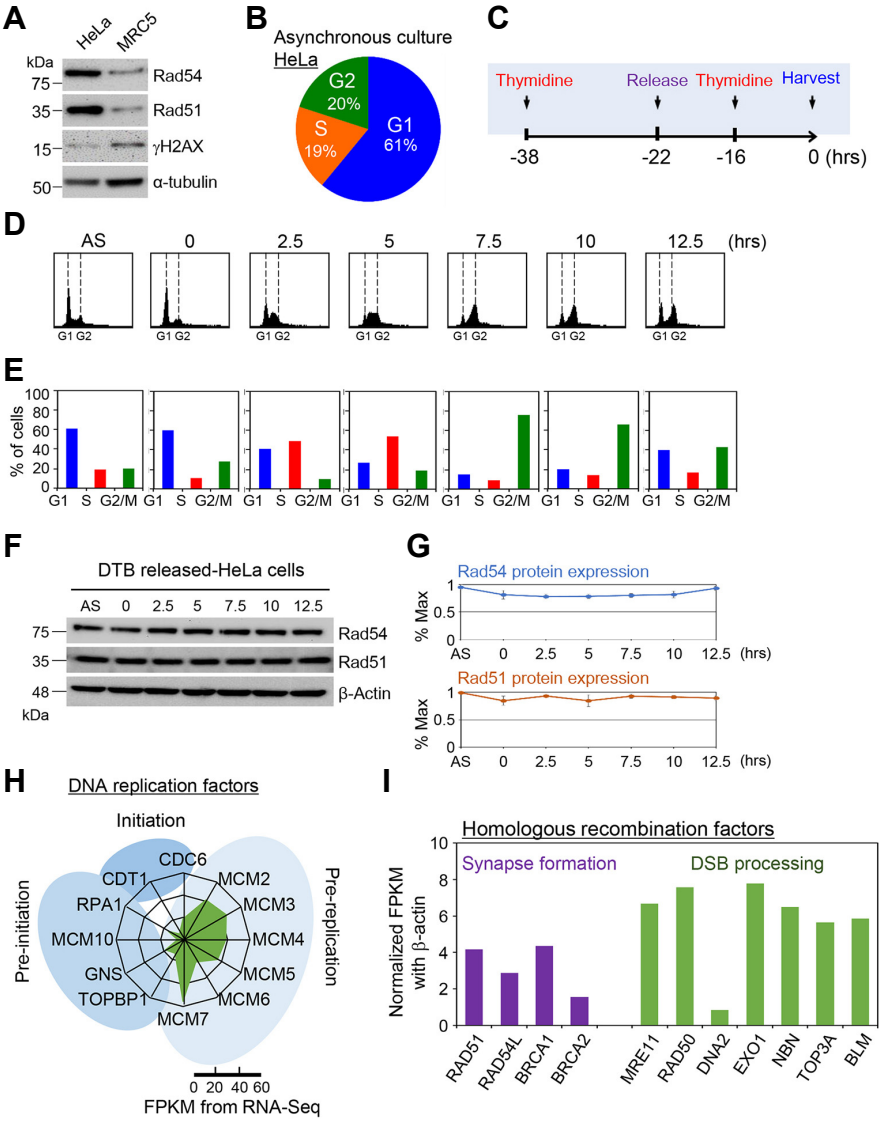

Fig. 1. Expression patterns of HR factors in HeLa cells. (A) Expression analysis of Rad51, Rad54, and γH2AX in MRC5 and HeLa cells. (B) Quantification of cell cycle profiles in asynchronous (AS) HeLa cells. (C) A schematic of the mitotic time-course experiment (see Methods section). (D) FACS analysis showing cell cycle progression after G1-S phase synchronization in Fig. 1C. The HeLa cells were harvested at 2.5 h intervals, as indicated by the time points, and the proportion of HeLa cell population in each cell cycle phase is shown. (E) The proportions of each cell cycle phase in (D) were quantified in plots. (F) Western blot analysis showing the protein levels of Rad51 and Rad54. The β-Actin served as a loading control. DTB, double-thymidine block. (G) Each protein in (F) was quantified, and the amounts relative to β-actin were determined for each time point. (H) FPKM values for the gene expression of DNA replication in asynchronous cultured cells. The scale bar represents the absolute FPKM value from an RNA-Seq run. (I) Normalization of FPKM values of HR factors under normal growth conditions. The FPKM values were normalized to the β-actin level.

damaged-cells after treatment with a chemical reagent: HU, ETP, cisplatin, or caffeine (Fig. 2B). The overall amounts of HR proteins were unaffected by the chemical reagents because HR proteins were already sufficiently expressed prior to exposure to DNA-damaging stress (Fig. 2B). As shown in Figs. 1 and 2, we observed that the expression levels of HR factors in HeLa cells did not change significantly during the cell cycle or because of the collapse of replication forks induced by DNA-damaging agents. Additionally, we assessed cell viability by FACS analysis after inducing DNA damage (Supplementary Fig. S1). The number of damaged cells was approximately 2-fold higher among cells with DNA damage than among normal cells (Fig. 2C). Furthermore, these DNA damage-inducing reagents blocked DNA replication and induced cell death via apoptosis.

Considering the abundance of HR proteins and their functions in HeLa cell cycle progression, we propose that the abundance of Rad51 and Rad54 rapidly participates in cell cycle progression, DNA repair, and cell viability. The HR-mediated cell cycle is an essential mechanism that maintains genomic integrity by dealing with stalled DNA replication and G2-M transition of HeLa cells. HeLa cells require a high level of HR activity even during normal cell cycle progression. Therefore, these results imply that HeLa cells require high levels of HR activity even during normal cell cycle progression, and that HeLa cells not only effectively regulate the DNA repair mechanism through HR factors to maintain genomic integrity but also support tolerance of DNA damage and efficient DNA replication in broken replication forks. One study has shown that DNA damage checkpoint proteins and the interaction between DNA damage-sensing proteins can promote cell cycle arrest at specific phases and enable the completion of HR before the cell enters the G2-M transition (Yoon et al., 2014). After exogenous DNA damage, cells in the G1 phase progress through the S phase and accumulate at the G2-M checkpoint. Therefore, our data also show that

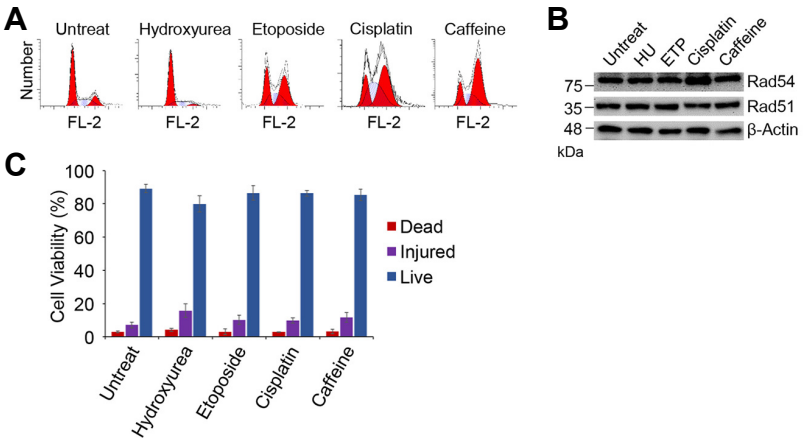

Fig. 2. FACS analysis of cell viability in response to chemical reagents. (A) The cell cycle distribution of HeLa cells in the presence of chemical reagents. (B) The protein levels of each HR factor in response to various DNA damage-inducing agents. (C) Analysis of cell viability in response to DNA damage-inducing agents. The percentages of live, injured, and dead cells were measured after exposure to various chemical treatments (Supplementary Fig. 1).

chemical-reagent-induced DNA damage can stop cell cycle progression in the S phase by preventing general DNA replication, or can induce cell cycle arrest at the late stages of the S or G2 phase. We hypothesized that HR-mediated DSB repair mechanisms are immediately activated in HeLa cells that maintain a high level of HR factors to sustain cell proliferation. This notion points to the possibility that HeLa cells express large amounts of HR proteins to maintain genomic stability, e.g., for the regulation of gene expression related to ssDNA gap repair at postreplication sites and in diverse DNA lesions.

HR factors in HeLa cells are redistributed to DNA damage sites along with γH2AX

We showed that HR factors are abundantly expressed during the cell cycle, and that the expression patterns of the HR factors are not significantly different in the absence or presence of DNA damage (Figs. 1F and 2B). Furthermore, the relocation of HR factors to DNA breaks is also an important phenomenon in the functions of HR factors in DNA repair. To analyze the redistribution of HR factors in the nucleus of HeLa cells, we assessed the localization of Rad51 and Rad54 on chromatin under DNA-damaging conditions by immunofluorescence image analysis. Cells were incubated with antibodies against Rad51 or Rad54, which binds to ssDNA to drive HR. Moreover, the cells were costained with an anti-γH2AX antibody, which is commonly used to detect DNA DSB sites (Figs. 3A and 3B). In approximately 37% of the cells, the γH2AX staining signal was weak and diffuse, whereas in 34.6% of the cells, we observed a few bright γH2AX foci. When cells were treated with HU, ETP, or cisplatin, the number of Rad51, Rad54, and γH2AX foci per nucleus increased about 2-fold as compared to the control cells (Figs. 3A and 3B). However, when caffeine was added to the culture medium, the number of Rad51 proteins per nucleus decreased from 10.73 ± 0.98 (mean ± SEM) to 7.10 ± 0.71, and the number of Rad54 foci per nucleus decreased from 9.75 ± 0.83 to 6.41 ± 0.62. Moreover, the number of γH2AX foci per nucleus increased from 15.05 ± 1.30 and 14.53 ± 1.15 to 20.11 ± 1.36 and 17.46 ± 1.28 for Rad51 and Rad54, respectively (Figs. 3C and 3D). Some studies showed that caffeine, an independent inhibitor of checkpoint proteins, dissociates previously assembled Rad51-ssDNA filaments but does not inhibit the formation of DNA gaps or breaks (Zelensky et al., 2013). Caffeine directly promotes displacement of Rad51 from ssDNA on chromatin; thus, foci numbers of Rad51 and Rad54 dramatically decreased (Figs. 3C and 3D).

In the present study, we determined whether the levels of Rad51 and/or Rad54 and the frequency of the Rad51 or Rad54 foci are related to the frequency of DSB sites. Under normal conditions, HR and γH2AX foci were present in small numbers in HeLa cells although HR proteins were highly expressed (Figs. 3A-3D). When HeLa cells were treated with chemical reagents, the abundance of both HR and γH2AX foci increased (Figs. 3A-3D). The numbers of colocalized foci between HR proteins and γH2AX were increased after treatment with DNA damage reagents except caffeine treatment (Figs. 3E-3G). These results imply that HR proteins were rapidly accumulated at DNA break sites and may be recruited to ssDNA gaps of the replication fork site, including the replication origin.

Rad51 depletion results in suppression of HR

To test whether Rad51 is directly involved in DNA DSB repair through the HR process, we performed a knockdown experiment using small interfering (siRNA) inhibition of Rad51 (Supplementary Fig. 2). We transfected a GFP-HR substrate plasmid into HeLa cells and selected a clone in a culture medium containing antibiotic puromycin. HeLa cells were transfected with either siRNA specific to Rad51 (siRAD51) or control siRNA (siCtrl). I-SceI expression plasmids were cotransfected after additional culture for 48 h. Cells were then harvested to evaluate Rad51 expression by western blotting (Fig. 4A). We next determined the percentage of GFP-positive cells by flow cytometry for >10,000 cells per sample (Figs. 4B and 4C). Among I-SceI-untransfected cells, no GFP-positive cells were observed. In the presence of I-SceI and siCtrl, ~10% of GFP-positive cells were observed. However, after transfection with siRAD51, the number of cells with recombined GFP alleles was reduced ~7-fold compared to siCtrl cells (Figs. 4C and 4D). We further analyzed cell cycle pro-

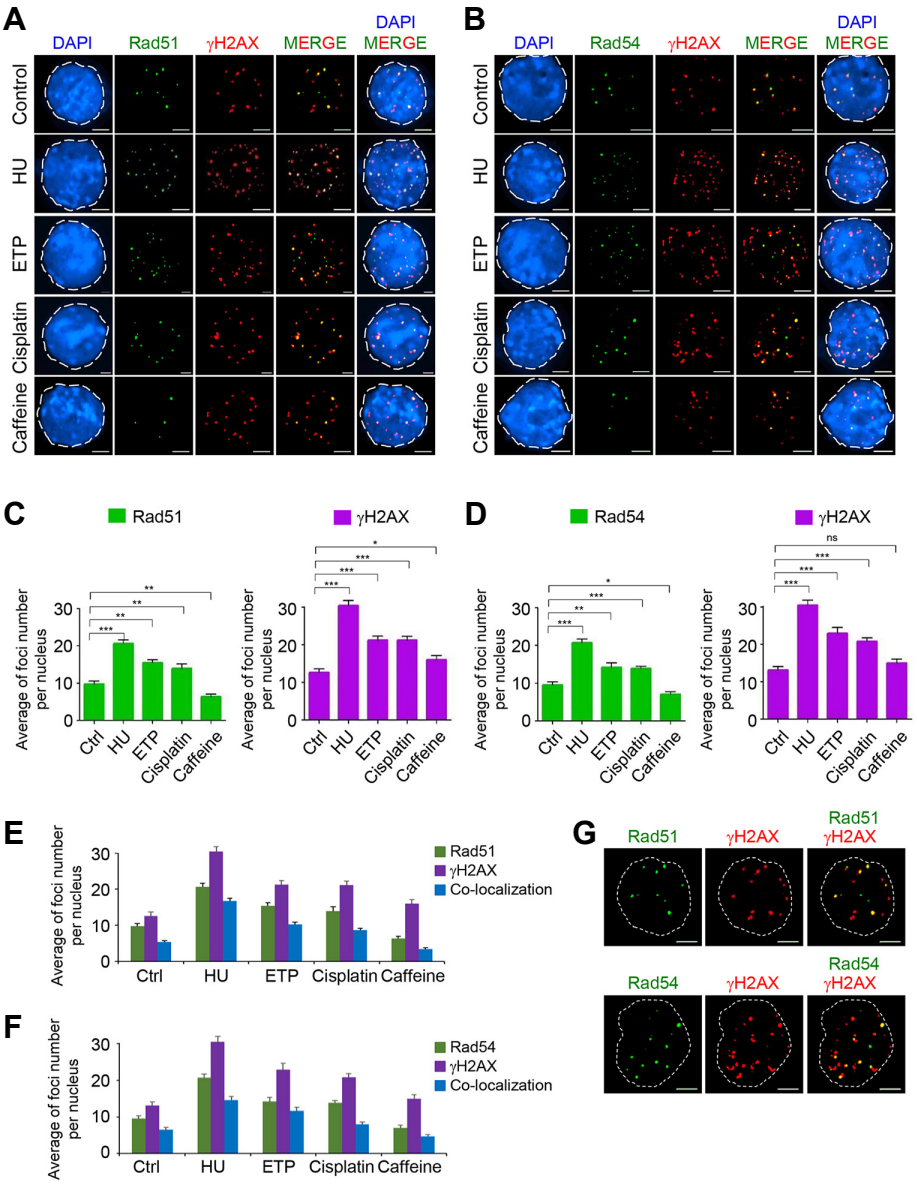

Fig. 3. Redistribution of HR factors on chromatin in response to DNA damaging conditions in HeLa cells. (A, B) Formation of Rad51 and Rad54 foci in HeLa cells in response to DNA damage. HeLa cells were immunostained with antibodies against Rad51 and γH2AX after chemical treatment. Scale bars, 5 μm (A, B). (C, D) Quantification of Rad51, Rad54, and γH2AX foci. Error bars indicate mean ± SEM. $**P \leq 0.01$, $***P \leq 0.001$ (Student's t test). More than 70 cells were counted for each experiment. (E, F) Quantification of colocalization of Rad51/Rad54 and γH2AX after DNA damage reagent treatment. Error bars indicate mean ± SEM. (G) Representative images for colocalization of Rad51/Rad54 and γH2AX. Scale bars, 5 μm.

gression in siRAD51-treated cells to characterize the effect of Rad51 activity on the proliferation of HeLa cells. The cell cycle showed a phase similar to the asynchronous state, regardless of the absence or presence of I-SceI. However, when the cells were treated with siRAD51, the cell population at the G2-M checkpoint increased from 22.27% to 41.03% (Fig. 4B). It should be noted that there is still approximately 29.5% Rad51 after siRAD51 treatment; therefore, this state of affairs may imply that HeLa cells are highly sensitive to the amounts of Rad51 protein under normal growth conditions. Rad51 plays crucial roles through homology-directed repair (HDR) and regulation of the HR process, which is essential for maintenance of genomic integrity and for cell cycle progression, specifically at the G2-M transition. Therefore, the results presented here suggest that depletion of Rad51 by siRNA reduced the recruitment of HR factors to DNA break sites, and then prevented cells from efficient replication in the S phase and during the postreplication process. In addition, disruption of the cell cycle caused by replication stress not only delayed proliferation of HeLa cells but also led to cell growth impairment and apoptosis (Fig. 4E). Taken together, these findings support the hypothesis that the abundantly expressed Rad51 in HeLa cells is subjected to rapid recruitment to replication forks or DNA-damage sites, and Rad51-dependent HR is passively involved in cell cycle progression, cell proliferation, and cell viability. Cell type-specific maintenance of HR may be crucial for overcoming genomic-instability-related DNA lesions in the replication fork and DNA breaks.

In conclusion, the abundant key HR proteins, Rad51 and

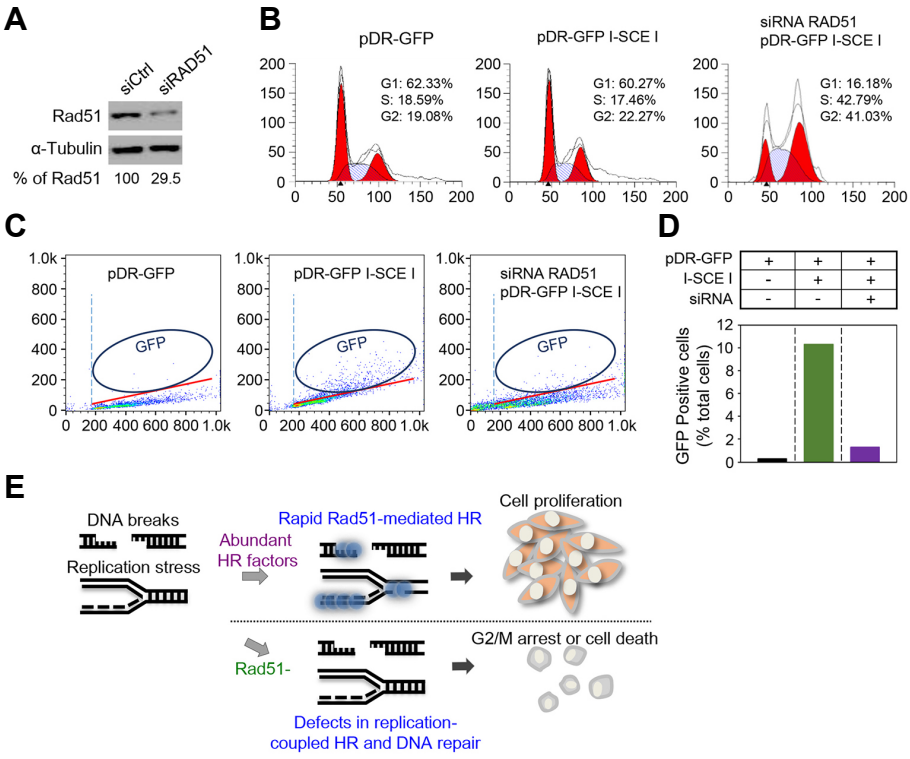

Fig. 4. Depletion of *RAD51* causes defects in HR-mediated DNA repair. (A) The expression level of Rad51 in HeLa cells transfected with siRNA against Rad51. HeLa cells were transfected with siCtrl and the siRAD51 pool and incubated for 48 h. Protein levels were analyzed by western blot analysis. (B) Analysis of cell cycle profiles of HeLa cells transfected with pDR-GFP (left), ISce-I in the presence of pDR-GFP (middle), or ISce-I in the presence of pDR-GFP after a Rad51 knockdown (right). (C) HeLa cells carrying the pDR-GFP were transfected with I-SceI after treatment with 200 nM siRNA for 48 h post-transfection. (D) The proportions of each GFP-positive cell type in (C) were quantified. (E) The proposed model of HR-mediated maintenance of genome integrity in HeLa cells. When HR factors are abundantly expressed, the HR-mediated repair pathway can respond rapidly to induce DNA repair. However, defective HR factors can cause accumulation of ssDNA gaps at the S-G2 transition or reversed forks; this situation induces G2-M arrest or cell death.

Rad54, dynamically counteract postreplication stress and DNA breaks. Downregulation of HR proteins may cause severe defects in cell cycle progression and postreplicative gap repair in HeLa cells. Therefore, an abundant amount of HR proteins is steadily maintained in HeLa cells, and these proteins can rapidly respond to DSBs, thereby promoting cellular proliferation, cell cycle progression, and cell viability. We propose that expression dynamics of HR proteins is a cell-specific phenomenon. In the future, it will be necessary to study HR protein expression regulatory pathway according to cell type.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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