Original Article

DNA recombinase Rad51 is regulated with UV-induced DNA damage and the DNA mismatch repair inhibitor CdCl₂ in HC11 cells

Hyeong-Ju You[#], Ga-Yeon Kim[#], Seung-Yeon Kim and Man-Jong Kang^{*}

Department of Animal Science, Chonnam National University, Gwangju 61186, Korea

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*Correspondence

Man-Jong Kang

E-mail: mjkang@jnu.ac.kr

ORCID

<https://orcid.org/0000-0002-7628-0614>

[#]Both first authors contributed equally to this work.

ABSTRACT Increasing the efficiency of HR (homologous recombination) is important for a successful knock-in. Rad51 is mainly involved in homologous recombination and is associated with strand invasion. The HR-related mismatch repair system maintains HR fidelity by heteroduplex rejection and repair. Therefore, the purpose of this study is to control Rad51, which plays a critical role in HR, through UV-induced DNA damage. It is also to confirm the effect on the expression of MMR related genes (Msh2, Msh3, Msh6, Mlh1, Pms2) and HR-related genes closely related to HR through treatment with the MMR inhibitor CdCl₂. The mRNA expression of Rad51 gene was confirmed in both HC11 cells and mouse testes, but the mRNA expression of Dmc1 gene was confirmed only in mouse testes. The protein expression of Rad51 and Dmc1 gene increased in UV-irradiated HC11 cells. After 72 hours of treatment with 1 μm of CdCl₂, the mRNA expression level of Msh3, Pms2, and Rad51 decreased, but the mRNA expression level of Msh6 and Mlh1 increased in HC11 cells. There was no significant difference in Msh2 mRNA expression between CdCl₂ untreated-group and the 72 hours treated group. In conclusion, HR-related gene (Rad51) was increased by UV-induced DNA damage. Treatment of the MMR inhibitor CdCl₂ in HC11 cells decreased the mRNA expression of Rad51.

Keywords: cadmium chloride, homologous recombination, knock-in, mismatch repair, Rad51 recombinase

INTRODUCTION

Homologous recombination is a fundamental process that occurs in all species, from bacteria to humans (Vispé et al., 1998). Rad51 and Dmc1, the central homologous pairing and strand exchange proteins, are the main recombinases in the homologous recombination pathway (Hinch et al., 2020). Dmc1 is known to be expressed only in meiosis, whereas Rad51 has been reported to be expressed in both meiosis and non-meiosis (Brown and

Bishop, 2014). In order to confirm whether Rad51 and HR are related, various studies have been carried out to induce artificial double strand breaks (DSBs) with UV irradiation and ionizing-radiation within cells (Elvers et al., 2011; Julien et al., 2013). It has been reported that the expression of Rad51 is increased during the S phase when irradiated with UV in human cells (Tashiro et al., 2000).

The DNA repair pathway HR is also associated with mismatch repair (MMR) (Spies and Fishel, 2015). MMR is a highly preserved post-replication repair system that rec-

recognizes and repairs problems such as base-base replacement and base indels during DNA replication or recombination (Lahue et al., 1989). MMR has been known to maintain HR fidelity (Chakarabarty and Alani, 2016). The bacterial MMR pathway prevents recombination between divergent sequences, called homeologous recombinations, via heteroduplex rejection in the presence of extensive heteroduplexes during the recombination process (Lahue et al., 1989; Tham et al., 2013). The heteroduplex rejection pathway in mammals is not well understood. It has been reported that homeologous recombination increases in MMR defective cells (Elliott and Jasin, 2001). Cadmium chloride has been reported as a carcinogen that inhibits the mismatch repair system (Jin et al., 2003). In addition, it is reported to inhibit the mismatch repair mechanism by inhibiting ATP hydrolysis of the MutS complex and specific binding of mismatched DNA (Clark and Kunkel, 2004). In addition, studies were carried out to inhibit MMR-related genes using CdCl₂, mutations and shRNA (Elliott and Jasin, 2001; Jin et al., 2003; Clark and Kunkel, 2004; Kansikas et al., 2014).

Homologous recombination can precisely knock-in an exogenous gene at a specific gene location by using a gene targeting vector that has a homologous region to a target site (Ceccaldi et al., 2016). NHEJ (non-homologous end joining) occurs throughout the cell cycle and is highly active in the G2/M phase, whereas homology-directed repair (HDR) by homologous recombination is known to occur mainly in the S phase of the cell cycle (Mao et al., 2008). Therefore, the proportion of HR occurrences is significantly lower than that of NHEJ. Research is being carried out on increasing the efficiency of gene targeting through the regulation of HR (Song et al., 2016; Miura et al., 2018; Park et al., 2019).

However, despite much research being done on HR and MMR, the regulation HR related genes or the correlation between HR and MMR related genes do not seem to appear in mouse mammary gland cells. Therefore, the aim of this study is to regulate HR-related genes that play an important role in the DNA repair pathway through UV irradiation in HC11 cells. In addition, the treatment of CdCl₂, a DNA mismatch repair inhibitor, was carried out to identify the expression of MMR and HR related genes in HC11 cells.

MATERIALS AND METHODS

RT-PCR

Total RNA from HC11 cells was isolated using RNeasy mini kit (QIAGEN, Hilden, Germany). For cDNA synthesis, 3 µg of total RNA was used as a template and RT-PCR was performed using random primers and Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Reverse transcription polymerase chain reaction (RT-PCR) was performed using cDNA as a template to confirm the expression of *Dmc1* and *Rad51* genes in HC11 cells and mouse testes. RT-PCR was performed with gene specific primer pairs (Table 1) using KOD Fx neo (Toyobo) under the following conditions: denaturation at 95°C for 15 minutes, followed by 28 cycles of amplification at 98°C for 10 seconds, annealing at 64°C for 30 seconds, and strand extension at 68°C for 1.5 minutes. The PCR fragments were confirmed by electrophoresis on a 2% agarose gel.

Cell culture and transfection

Mouse mammary cells (HC11) were cultured in a growth medium: Roswell Park Memorial Institute (RPMI, Hyclone, Logan, USA) supplemented with 10% fetal bovine serum (FBS, Atlas, FT Collins, CO), 1% penicillin/streptomycin (Hyclone, USA), 0.01% gentamicin (Sigma-Aldrich Inc., St. Louis, MO, USA), 0.01% EGF (Gibco, Thermo Fisher, USA) and 0.05% Insulin (Sigma).

UV irradiation

HC11 cells were seeded at a density of 0.7×10^5 cells per well into 12-well plates (SPL, Gyeonggi-do, Korea). After 24 hours of seeding HC11 cells were irradiated with 312 nm UV light at 10 J/m² by using a BLX-312 UV irradiator (VILBER LOURMAT, Marne La Valle, France) and incubated at 37°C, in 5% CO₂ for 6 hours.

Immunocytochemistry

The cells were fixed for 10 minutes with 4% paraformaldehyde and permeabilized for 10 minutes with 0.25% Triton X-100. The cells were then washed three times with phosphate-buffered saline (PBS), blocked with 1% BSA (Sigma, Bovine Serum Albumin) for 1 hour at room temperature. The cells were probed with mouse monoclonal anti-*Dmc1* antibody (Santa Cruz Biotechnology, Santa Cruz, CA) (sc-53269) or mouse monoclonal anti-*Rad51*

Table 1. Primers list

Gene	Primer name	Sequence (5' → 3')	Size (bp)
Primers used for reverse-transcription PCR analysis of specific genes			
<i>Dmc1</i>	<i>mDmc1</i> RT-PCR F	TAGGAGGTGGCATTGAAAGTATGGC	1,023 bp
	<i>mDmc1</i> RT-PCR R	TTGGAGTCGTGACAACATCTGGG	
<i>Rad51</i>	<i>mRad51</i> RT-PCR F	TAACTACTGGCTCCAAAGAGCTGGA	1,019 bp
	<i>mRad51</i> RT-PCR R	TTGGTGATTACCACTGCGACACC	
Primers used for quantitative real-time PCR analysis of specific genes			
<i>Rplp0</i>	<i>mRplp0</i> F	CGACCTGGAAGTCCAATA	109 bp
	<i>mRplp0</i> R	ATCTGCTGCATCTGCTTG	
<i>Rad51</i>	<i>mRad51</i> CDS S	AATTCGAACTGGGAAGACAC	79 bp
	<i>mRad51</i> CDS AS	TCACCTCCACCACGGTCAAT	
<i>Msh2</i>	<i>mMsh2</i> CDS S	TGAAGTTGGACATGGCAGCA	89 bp
	<i>mMsh2</i> CDS AS	TAATGCGCCAGAGACTGAG	
<i>Msh3</i>	<i>mMsh3</i> CDS S	CCATCGCCTATGCAACTCTA	85 bp
	<i>mMsh3</i> CDS AS	TCACAGACTGGTGGATAGTG	
<i>Msh6</i>	<i>mMsh6</i> CDS S	ATACTCAGGCATGCAACAGC	71 bp
	<i>mMsh6</i> CDS AS	TCAAAAGTTGCGGTGCCTCT	
<i>Mlh1</i>	<i>mMlh1</i> CDS S	AGATTAGTGAGCGGTGCCAT	78 bp
	<i>mMlh1</i> CDS AS	ACTGAGGATTACACAGCCC	
<i>Pms2</i>	<i>mPms2</i> CDS S	ACTTCCAGGACGCCACAAAA	92 bp
	<i>mPms2</i> CDS AS	AACTGCCTGTCTGTTGCACT	

antibody (sc-398587) at a dilution of 1:50 in 1% BSA at 4°C for 1.5 hours. For the secondary antibody, the cells were washed with PBS three times every 5 minutes and incubated with mouse IgG BP-FITC (sc-516140) at a dilution of 1:50 in 1% BSA at room temperature for 2 hours. Nuclei were counterstained with 10 mg/mL DAPI (4', 6-diamidino-2-phenylindole) in PBS at a dilution of 1:50. The slides were then mounted with UltraCruz® Aqueous Mounting Medium (sc-24941) at room temperature for 16 hours without lights. Foci were viewed with a Laser Confocal Scanning Microscope system (TCS SP5 AOBS/TANDEM, Leica microscopesystem, Wetzlar, Germany).

CdCl₂ treatment

HC11 cells were cultured by the method described above. They were seeded in a 6-well plate (SPL) at a density of 2.5×10^5 cells per well, and incubated for 24 hours in a 5% CO₂ incubator at 37°C. After that, 1 µM of CdCl₂ was treated for 72 hours, and the cells were harvested at intervals of 24 hours, 48 hours and 72 hours for 24 hours.

RT-qPCR analysis

RT-qPCR was performed to analyze the mRNA expression level of MutS, MutL (DNA MMR-related gene) and Rad51 (HR-related gene) with 1 µM of CdCl₂ treatment.

Total RNA was extracted from HC11 cells using RNeasy mini kit (QIAGEN, Hilden, Germany). To synthesize cDNA from 5 µg of total RNA, RT-PCR (reverse transcription reaction) was performed using a random primer and Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The following quantitative real-time PCR was performed to measure the relative mRNA level of Rad51 (HR-related gene) and the MutS and the MutL (MMR-related gene) of DNA. We prepared and used 20 ng of cDNA as a template, and qPCR reaction was performed using TOPreal™ qPCR 2X PreMIX (Enzynomics, Daejeon, Korea) and 10 pmol of real-time PCR primer. The primer is indicated in Table 1. All RT-qPCR was performed in triplicate, and Mx3000p (Agilent Technologies, Santa Clara, CA, USA) was used. Conditions were denatured at 95°C for 10 minutes, 95°C for 10 seconds, 60°C for 15 seconds, 72°C 15 seconds for a total of 40 times, and it was confirmed that a specific amplification reaction occurred through a melting curve analysis. For qPCR comparative quantification, mouse Rplp0 was used as a housekeeping gene, and mRNA expression levels were compared and analyzed.

Statistical analysis

Statistical analysis of the mRNA expression level was

performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, DA, USA). This data was analyzed by the Dunnett test of One-way ANOVA. The confidence interval was set to 95%.

RESULTS

The mRNA expression of Dmc1 and Rad51 in the HC11 cell and testes

To determine whether the Dmc1 and Rad51 genes are specifically involved in mitosis and meiosis, the mRNA expression of the Dmc1 and Rad51 genes were identified through RT-PCR in HC11 cells and mouse testes. As a result, the Dmc1 gene, which is involved in HR during meiosis, was identified only in mouse testes. However, the Rad51 gene, which is involved in meiotic and non-meiotic

ic cells, was identified in both testes and in the HC11 cells (Fig. 1).

The protein expression of Dmc1 and Rad51 in the HC11 cells treated with UV

Immunocytochemistry was performed to confirm the expression of Rad51 and Dmc1 proteins by UV irradiation in HC11 cells. As a result, when irradiated with UV, the protein levels of Rad51 and Dmc1 were significantly up-regulated. These results indicate that both Dmc1 and Rad51 are involved in the mechanism of repairing cellular DNA damage by homologous recombination (Fig. 2).

The mRNA expression of DNA MMR related gene and Rad51 in the HC11 cells treated CdCl₂

The following was conducted to investigate the effect

Fig. 1. The mRNA expression of Dmc1 and Rad51 in the HC11 cell and testes. (A) The mRNA expression of Rad51 in the HC11 cell. (B) The mRNA expression of Dmc1 and Rad51 in the HC11 cell and testes. PC, positive control; M, DNA size marker (100 bp ladder); RT-, reverse transcriptase non-treated-, RT+, reverse transcriptase treated.

Fig. 2. Fluorescent immunocytochemistry was conducted on HC11 cells treated with or without UV irradiation by using monoclonal antibodies against Dmc1 and Rad51 coupled with secondary antibodies labeled with fluorescent dyes IgG BP-FITC (green). Nuclear counterstaining was performed using DAPI (blue). Immunostaining showing (A) Rad51, (B) Dmc1. Rad51 and Dmc1 foci are present at comparatively high density in the UV-irradiated HC11 cells.

of 1 μM of CdCl_2 treatment on the mRNA levels of MutS, MutL (DNA MMR-related gene), and Rad51 (HR-related gene) in HC11 cells. Total RNA was extracted from HC11 treated with CdCl_2 at 24-hour intervals, and Fig. 3 shows the results of RT-qPCR. In the case of MutS, the Msh2 and Msh6, the mRNA expression level increased at 24 hours after CdCl_2 treatment and decreased at 48 and 72 hours. However, there was no significant difference in Msh2 at 72 hours after CdCl_2 treatment. In the case of msh6, the mRNA expression level increased by 1.7 times at 72 hours after CdCl_2 treatment compared to the group not treated with CdCl_2 . However, in the case of Msh3, it decreased rapidly at 24 hours after CdCl_2 , and decreased by 4.4 times at 72 hours after CdCl_2 as compared to the group not treated with CdCl_2 . For MutL, Mlh1 increased 1.5 times at 72 hours after CdCl_2 as compared to the group not treated with CdCl_2 . In the case of Pms2 increased at 24 hours after CdCl_2 , decreased at 48 and 72 hours, and decreased by 1.6 times at 72 hours after CdCl_2 . The HR-related gene Rad51 increased 1.7-fold at 24 hours after

CdCl_2 and decreased by 3.4-fold at 72 hours after CdCl_2 .

DISCUSSION

This study investigated whether the DNA recombinase Rad51 is regulated by UV-induced DNA damage and DNA mismatch repair inhibitor CdCl_2 in the HC11 cells.

As stated in the introduction, HR, the repair pathway of DSBs, has a lower incidence than NHEJ. In addition, studies have shown that HR is limited by the NHEJ pathway (Banan, 2020). Therefore, research is being carried out to increase the efficiency of gene targeting by regulating HR-related genes. Numerous studies have attempted to improve the efficiency of homology directed repair by using RS-1, which can activate the Rad51 gene, and SCR7, which can inhibit NHEJ (Maruyama et al., 2015; Song et al., 2016). In addition, studies were carried out to improve the knock-in efficiency of large donor DNA by chemically manipulating the DNA-repair pathway in cells (Quadros et al., 2017). It is important to understand the HR mo-

Fig. 3. Fold changes in mRNA expression of MutS, MutL (DNA MMR-related gene) and Rad51 (HR-related gene) in CdCl_2 -treated HC11 (A) mRNA level of MutS gene in the HC11 cells treated with CdCl_2 . Mouse Msh2 mRNA expression in HC11 no fold change ($p = 0.0062$). Msh3 mRNA expression has 4.4-fold decrease ($p < 0.0001$). Msh6 mRNA expression is increased 1.7-fold ($p < 0.0001$). (B) mRNA level of MutL and Rad51 genes in the HC11 cells treated with CdCl_2 . Mouse Mlh1 mRNA expression in HC11 is increased 1.5-fold ($p < 0.0019$). Pms2 mRNA expression has a 1.6-fold decrease ($p < 0.0012$). mRad51 mRNA expression in HC11 is decreased 3.4-fold ($p < 0.001$). Error bars shows the standard deviation from each sample. NS: no statistical difference. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

lecular mechanisms in order to increase the efficiency of gene targeting.

It is well known that Dmc1 and Rad51, which promote nucleofilaments and play an important role in strand invasion, are the major recombinases of the HR pathway. Dmc1 and Rad51 play similar roles in the HR pathway, but Dmc1 is known to play an important role in the HR pathway in meiosis and Rad51 in both meiotic and non-meiotic cells (Brown and Bishop, 2014). Similarly, in this study, Dmc1 was expressed only in mouse testes, but Rad51 was expressed in both mouse testes and mouse mammary gland HC11 cells. These results confirmed that Dmc1 is specifically involved in the HR of meiosis, and Rad51 is involved in the HR of meiotic and non-meiotic cells.

A recent study reported that the expression of HR-related genes was increased as a result of UV-induced DNA damage in various species such as bacteria, yeast, and human cells (Rapp and Greulich, 2004; McCready et al., 2005; Yin and Petes, 2015). In this study, it was confirmed that when HC11 cells received UV irradiation, the expression of both Dmc1 and Rad51 was elevated as compared to HC11 cells without UV irradiation. These results indicated that the Rad51 and Dmc1 expression can be regulated with UV induced DNA damage, and that Rad51 is closely related to HR.

MMR proteins maintain the fidelity of homologous recombination by blocking divergent sequences with heteroduplex rejection during recombination (Tham et al., 2013). In MMR defective cells, divergent sequence recombination, called homeologous recombination, is increased (Elliott and Jasin, 2001). This suggests that mismatch repair is related to homologous recombination. Therefore, the MMR inhibitor CdCl₂ was used to regulate HR. In 2004, Clark and Kunkel reported that CdCl₂ treatment in eukaryotic cells inhibited ATP hydrolysis of MutS complexes (MSH2-MSH6) and specific binding on mismatched DNA, but did not affect Mlh1-Pms1. In 2003, Jin et al. reported that 3 or 5 µm of cadmium treatment inhibited Msh2/Msh6 and Msh2/Msh3. However, as a result of confirming gene expression level every 24 hours in HC11

cells treated with CdCl₂ for 72 hours, the mRNA expression level of Msh2 and Msh6 increased at 72 hours after CdCl₂, and the mRNA expression level of Msh3 decreased significantly from 24 hours after CdCl₂. The mRNA expression level of Mlh1 increased at 72 hours after CdCl₂, but decreased Pms2. Therefore, 1 µm cadmium treatment of the mouse mammary gland cell HC11 downregulates Msh3 and Pms2. Although studies on MMR inhibition of CdCl₂ treatment in human and yeast are being actively conducted, studies on the MMR system according to CdCl₂ treatment in mouse somatic cells are lacking so far. It has been reported that CdCl₂ treatment in humans and yeast inhibits the MutS complex as ATP activity is reduced (Clark and Kunkel, 2004; Banerjee and Flores-Rozas, 2005). The MutS complex includes MutS α (Msh2-Msh6) and MutS β (Msh2-Msh3) and the roles of MMR-related genes are as indicated in Table 2. However, although CdCl₂ is reported to inhibit the MutS complex, studies on MutS beta (Msh2-Msh3) compared to MutS alpha (Msh2-Msh6) are lacking (Clark and Kunkel, 2004; Banerjee and Flores-Rozas, 2005). Also, comparative studies between MMR-related genes are lacking. It has been reported that 1 µm of CdCl₂ treatment in humans inhibits the endonuclease activity of zinc-dependent MutL α (Mlh1-PMS2) (Sherrer et al., 2018). Taken together, these results suggest that CdCl₂ selectively inhibits the MMR gene by inhibiting the ATP hydrolysis of the Mut S complex and the zinc-dependent enzyme of MuL α. Therefore, it is considered that different mRNA expression levels of mismatch repair genes according to cadmium chloride treatment depend on cell type, cell state, cadmium chloride concentration and treatment time. An enhanced incidence of homeologous recombination was expected to increase the expression level of Rad51, HR strand invasion related gene, in MMR defective cells. However, in this study, the expression level of Rad51 was decreased with CdCl₂ treatment. Therefore, the relationship between MMR and HR related genes is considered to be involved in an additional molecular mechanism.

In summary, DNA recombinase Rad51 is regulated by

Table 2. MMR components and their functions

Prokaryote	Eukaryote	Function
<i>MutS</i>	MutS α (MSH2-MSH6)	Recognition of mismatches and small indels, gene conversion, heteroduplex rejection
	MutS β (MSH2-MSH3)	Recognition of mismatches large indels
<i>MutL</i>	MutL α (MLH1-PMS2)	Endonuclease, gene conversion

UV-induced DNA damage and the MMR inhibitor CdCl₂. The HR pathway can increase gene targeting efficiency. The present study shows an attempt to regulate HR related genes. It was confirmed that the expression of Rad51 was increased with UV irradiation that induces DSBs in HC11 cells. Therefore, this indicates that Rad51 can be regulated by UV-induced DNA damage. These results suggest that Rad51 plays an important role in gene targeting using HR, but it was confirmed that the expression level of Rad51 is reduced by treating treatment with the MMR inhibitor CdCl₂. These results suggest that the relative mRNA level of MMR-related genes is suppressed by CdCl₂ treatment, but this treatment also reduces the expression level of Rad51. This is considered unsuitable to be used as a compound for efficient gene targeting.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Conceptualization: MJK, HJY, GYK
 Data curation: MJK, HJY, GYK
 Funding acquisition: MJK
 Investigation: MJK, HJY, SYK, GYK
 Supervision: MJK
 Writing - original draft: MJK, HJY, GYK

AUTHOR'S POSITION AND ORCID NO.

You HJ, Master's course,
<https://orcid.org/0000-0002-6546-5904>
 Kim GY, Master's course,
<https://orcid.org/0000-0003-1409-432X>
 Kim SY, Doctor's course,
<https://orcid.org/0000-0002-2395-9404>
 Kang MJ, Professor,
<https://orcid.org/0000-0002-7628-0614>

REFERENCES

- Banan M. 2020. Recent advances in CRISPR/Cas9-mediated knock-ins in mammalian cells. *J. Biotechnol.* 308:1-9.
- Banerjee S and Flores-Rozas H. 2005. Cadmium inhibits mismatch repair by blocking the ATPase activity of the MSH2-MSH6 complex. *Nucleic Acids Res.* 33:1410-1419.
- Brown MS and Bishop DK. 2014. DNA strand exchange and RecA homologs in meiosis. *Cold Spring Harb. Perspect. Biol.* 7:a016659.
- Ceccaldi R, Rondinelli B, D'Andrea AD. 2016. Repair pathway choices and consequences at the double-strand break. *Trends Cell Biol.* 26:52-64.
- Chakraborty U and Alani E. 2016. Understanding how mismatch repair proteins participate in the repair/anti-recombination decision. *FEMS Yeast Res.* 16:fow071.
- Clark AB and Kunkel TA. 2004. Cadmium inhibits the functions of eukaryotic MutS complexes. *J. Biol. Chem.* 279:53903-53906.
- Elliott B and Jasin M. 2001. Repair of double-strand breaks by homologous recombination in mismatch repair-defective mammalian cells. *Mol. Cell. Biol.* 21:2671-2682.
- Elvers I, Johansson F, Groth P, Erixon K, Helleday T. 2011. UV stalled replication forks restart by re-priming in human fibroblasts. *Nucleic Acids Res.* 39:7049-7057.
- Hinch AG, Becker PW, Li T, Moralli D, Zhang G, Bycroft C, Green C, Keeney S, Shi Q, Davies B, Donnelly P. 2020. The configuration of RPA, RAD51, and DMC1 binding in meiosis reveals the nature of critical recombination intermediates. *Mol. Cell* 79:689-701.e10.
- Jin YH, Clark AB, Slebos RJ, Al-Refai H, Taylor JA, Kunkel TA, Resnick MA, Gordenin DA. 2003. Cadmium is a mutagen that acts by inhibiting mismatch repair. *Nat. Genet.* 34:326-329.
- Kansikas M, Kasela M, Kantelinen J, Nyström M. 2014. Assessing how reduced expression levels of the mismatch repair genes MLH1, MSH2, and MSH6 affect repair efficiency. *Hum. Mutat.* 35:1123-1127.
- Lahue RS, Au KG, Modrich P. 1989. DNA mismatch correction in a defined system. *Science* 245:160-164.
- Mao Z, Bozzella M, Seluanov A, Gorbunova V. 2008. DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells. *Cell Cycle* 7:2902-2906.
- Maruyama T, Dougan SK, Truttmann MC, Bilate AM, Ingram JR, Ploegh HL. 2015. Increasing the efficiency of precise genome editing with CRISPR-Cas9 by inhibition of nonhomologous end joining. *Nat. Biotechnol.* 33:538-542.
- McCready S, Müller JA, Boubriak I, Berquist BR, Ng WL, Das-Sarma S. 2005. UV irradiation induces homologous recombination genes in the model archaeon, *Halobacterium* sp. NRC-1. *Saline Syst.* 1:3.
- Miura H, Quadros RM, Gurumurthy CB, Ohtsuka M. 2018. Easi-CRISPR for creating knock-in and conditional knock-

- out mouse models using long ssDNA donors. *Nat. Protoc.* 13:195-215.
- Park DS, Kim S, Koo DB, Kang MJ. 2019. Current status of production of transgenic livestock by genome editing technology. *J. Anim. Reprod. Biotechnol.* 34:148-156.
- Quadros RM, Miura H, Harms DW, Akatsuka H, Sato T, Aida T, Redder R, Richardson GP, Inagaki Y, Sakai D, Buckley SM, Seshacharyulu P, Batra SK, Behlke MA, Zeiner SA, Jacobi AM, Izu Y, Thoreson WB, Urness LD, Mansour SL, Ohtsuka M, Gurumurthy CB. 2017. Easi-CRISPR: a robust method for one-step generation of mice carrying conditional and insertion alleles using long ssDNA donors and CRISPR ribonucleoproteins. *Genome Biol.* 18:92.
- Rapp A and Greulich KO. 2004. After double-strand break induction by UV-A, homologous recombination and nonhomologous end joining cooperate at the same DSB if both systems are available. *J. Cell Sci.* 117:4935-4945.
- Sherrer SM, Penland E, Modrich P. 2018. The mutagen and carcinogen cadmium is a high-affinity inhibitor of the zinc-dependent MutL α endonuclease. *Proc. Natl. Acad. Sci. U. S. A.* 115:7314-7319.
- Song J, Yang D, Xu J, Zhu T, Chen YE, Zhang J. 2016. RS-1 enhances CRISPR/Cas9- and TALEN-mediated knock-in efficiency. *Nat. Commun.* 7:10548.
- Spies M and Fishel R. 2015. Mismatch repair during homologous and homeologous recombination. *Cold Spring Harb. Perspect. Biol.* 7:a022657.
- Tashiro S, Walter J, Shinohara A, Kamada N, Cremer T. 2000. Rad51 accumulation at sites of DNA damage and in postreplicative chromatin. *J. Cell Biol.* 150:283-292.
- Tham KC, Hermans N, Winterwerp HH, Cox MM, Wyman C, Kanaar R, Lebbink JH. 2013. Mismatch repair inhibits homeologous recombination via coordinated directional unwinding of trapped DNA structures. *Mol. Cell* 51:326-337.
- Vignard J, Mirey G, Salles B. 2013. Ionizing-radiation induced DNA double-strand breaks: a direct and indirect lighting up. *Radiother. Oncol.* 108:362-369.
- Vispé S, Cazaux C, Lesca C, Defais M. 1998. Overexpression of Rad51 protein stimulates homologous recombination and increases resistance of mammalian cells to ionizing radiation. *Nucleic Acids Res.* 26:2859-2864.
- Yin Y and Petes TD. 2015. Recombination between homologous chromosomes induced by unrepaired UV-generated DNA damage requires Mus81p and is suppressed by Mms2p. *PLoS Genet.* 11:e1005026.