損傷修復分野
Department of Cell Maintenance

細胞への紫外線の照射などにより DNA が損傷されると、DNA 複製は停止してしまう。エピキチンライケースである Rad18 は、損傷を除去するための核酸内導体を制御することにより DNA 複製を再開させる (損傷を除去複製)。ゲノム DNA の安定性を保つ。Rad18 による損傷を除去複製の制御は、発癌を防ぐ役割の他に生殖細胞の維持や造血の場における薬剤耐性などに関与する。その一方で発癌した細胞では、Rad18 タンパクが癌精巣抗原である MAGE-A4 により安定化されることにより、発癌の先進に「悪用」されている可能性がある。次の 3 つの項目について解明をめざす。(1) 正常な細胞で損傷を除去複製が発癌を抑制する機構、(2) 損傷を除去複製が、体性幹細胞の維持に貢献する機構、(3) 前癌状態の細胞で、Rad18 が発癌の先進に寄与する機構。

Replicative polymerases stall at damaged template DNA in UV-irradiated cells, which hamper cell proliferation. To circumvent the crisis, ubiquitin ligase Rad18 mono-ubiquitinates PCNA to promote translesion synthesis (TLS) via recruiting polymerase η. Thus, Rad18 maintains genomic DNA stability to prevent tumorogenesis. Rad18 also plays role to maintain spermatogenesis and hematopoiesis. On the other hand, we propose that cancer cells “hijack” Rad18 to reprogram TLS providing a new way for tumors to achieve tumor progression via stabilization of Rad18 by cancer/testis melanoma antigen A-4. We will elucidate following subjects. (1) How Rad18 suppress tumorogenesis in normal cells (2) mechanism of how Rad18 contribute to maintain somatic stem cells (3) how Rad18 contribute to reprogram TLS to achieve tumor progression in pro-cancer cells.
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細胞への紫外線の照射などにより DNA が損
傷されると、DNA 複製は停止してしまう。ユビ
キチンライゲースである Rad18 は、損傷を検
出して DNA 複製を再開させる。このように DNA 損傷を検
出して複製することを損傷検出蛋白質 (translesion synthesis: TLS) と呼ぶ。TLS は、ゲノ
ム DNA の安定性を保つ役割をもつ一方で、エ
ラー頻度の高い TLS により変異率の上昇をと
らす可能性もある。Rad18 ノックアウトマウス
を用いた研究などにより、以下の 1-4 の研究を
進めてきた。また本研究室では熊本大学医学部
皮膚科などと連携して、光線過敏症・早老症な
どの DNA 不安定性疾患の診断に携わっている。
S の共同研究により、早老症である UV5 候補の
原因遺伝子を同定に寄与した。

1. DNA 複製停止部位で Pol η は、Rad18 を PCNA
へ導くことにより、PCNA のモノユビキチン化
修飾を促進する。

2. Rad18 は造血前駆細胞の DNA 損傷修復系
システムに寄与することにより、癌抑制作用をもつ
Rad18 による損傷を検出複製の制御は、発癌を
防ぐ役割をもつ。この他に生殖機能の維持など
体性幹細胞の維持に寄与する。これにより Pol η 以外の TLS、ファシブノス RNA
相関スイッチ経路なども誘導されるモデル
を提案した。

3. 癌細胞抗原 (MAGE-A4) は、Rad18 タンパク
を安定化し、損傷検出複製を促進することに
より、発癌が進行が進行過程でのゲノム DNA
に影響を及ぼす。

4. マウス自体で Rad18 が欠損しても体細胞超変
異 (somatic hypermutation) に影響しない。
体細胞超変異(somatic hypermutation)では、AID酵素がDNA塩基を脱メチル化して損傷を導入し、その後に正確性の低い複製酵素により損傷が修復されることにより、変異が導入される。
Rad18はPCNAをモノアセチル化することにより正確性の低い複製酵素を抑制する鍵となる分子であることから、体細胞超変異に関与すると考えられてきた。我々は野生型マウスおよびRad18欠損マウスに抗原を注入した後にIgG遺伝子での変異率を測定した結果、両者に差が見られなかった。このため、マウス個体でRad18が欠損しても体細胞超変異(somatic hypermutation)に影響しないと結論した。(高知大学の清水博士との共同研究、論文2参照)

5. 早老症であるUVsS候群の原因遺伝子の同定
損傷修復分野では熊本大学皮膚科などを協力し、光線過敏または早老症の診断に携わっている。
損傷修復分野の前教授である山崎らにより、軽度症例の早老症であるUVsS候群が同定された。
名古屋大学の若らは、本研究室と共通でこの疾患の原因遺伝子を特定し、その分子機能を明らかにした。(文献9参照)

1. A non-catalytic role of DNA polymerase g in recruiting Rad18 and promoting PCNA monoubiquitination at stalled replication forks

Trans-lesion DNA synthesis (TLS) is a DNA damage-tolerance mechanism that uses low-fidelity DNA polymerases to replicate damaged DNA. The inherited cancer-propensity syndrome xeroderma pigmentosum variant (XPV) results from error-prone TLS of UV-damaged DNA. TLS is initiated when the Rad6/ Rad18 complex monoubiquitinates proliferating cell nuclear antigen (PCNA), but the basis for recruitment of Rad18 to PCNA is not completely understood. Here, we show that Rad18 is targeted to PCNA by DNA polymerase eta (Polη), the XPV gene product that is mutated in XPV patients. The C-terminal domain of Polη binds to both Rad18 and PCNA and promotes PCNA monoubiquitination, a function unique to Polη among Y-family TLS polymerases and dissociable from its catalytic activity. Importantly, XPV cells expressing full-length catalytically-inactive Polη exhibit increased recruitment of other error-prone TLS polymerases (Poli and Polk) after UV irradiation. These results define a novel non-catalytic role for Polη in promoting PCNA monoubiquitination and provide a new potential mechanism for mutagenesis and genome instability in XPV individuals (Collaborative study with Dr. Vaziri, see reference 8).

2. Rad18 confers hematopoietic progenitor cell DNA damage tolerance independently of the Fanconi Anemia pathway in vivo

In cultured cancer cells the E3 ubiquitin ligase Rad18 activates Trans-Lesion Synthesis (TLS) and the Fanconi Anemia (FA) pathway. However, physiological roles of Rad18 in DNA damage tolerance and carcinogenesis are unknown and were investigated here. Primary hematopoietic stem and progenitor cells (HSPC) co-expressed RAD18 and FANCD2 proteins, potentially consistent with a role for Rad18 in FA pathway function during hematopoiesis. However, hematopoietic defects typically associated with famc-deficiency (decreased HSPC numbers, reduced engraftment potential of HSPC, and Mitomycin C (MMC)-sensitive hematopoiesis), were absent in Rad18−/− mice. Moreover, primary Rad18−/− mouse embryonic fibroblasts (MEF) retained robust Fancd2 mono- ubiquitination following MMC treatment. Therefore, Rad18 is dispensable for FA pathway activation in untransformed cells and the Rad18 and FA pathways are separable in hematopoietic cells. In contrast with responses to crosslinking agents, Rad18−/− HSPC were sensitive to in vivo treatment with the myelo- suppressive agent 7,12 Dimethylbenz[a]anthracene (DMBA). Rad18-deficient fibroblasts aberrantly accumulate DNA damage markers after DMBA treatment. Moreover, in vivo DMBA treatment led to increased incidence of B cell malignancy in Rad18−/− mice. These results identify novel hematopoietic functions for Rad18 and provide the first demonstration that Rad18 confers DNA damage tolerance and tumor-suppression in a physiological setting. (Collaborative study with Dr. Vaziri, see reference 5).

3. A neomorphic cancer cell-specific role of MAGE-A4 in trans-lesion synthesis

Trans-lesion synthesis (TLS) is an important DNA-damage tolerance mechanism that permits ongoing DNA synthesis in cells harbouring damaged genomes. The E3 ubiquitin ligase RAD18 activates TLS by promoting recruitment of Y-family
DNA polymerases to sites of DNA-damage-induced replication fork stalling. Here we identify the cancer/testes antigen melanoma antigen-A4 (MAGE-A4) as a tumour cell-specific RAD18-binding partner and an activator of TLS. MAGE-A4 depletion from MAGE-A4-expressing cancer cells destabilizes RAD18. Conversely, ectopic expression of MAGE-A4 (in cell lines lacking endogenous MAGE-A4) promotes RAD18 stability. DNA-damage-induced mono-ubiquitination of the RAD18 substrate PCNA is attenuated by MAGE-A4 silencing. MAGE-A4-depleted cells fail to resume DNA synthesis normally following ultraviolet irradiation and accumulate phosphor-H2AX, thereby recapitulating major hallmarks of TLS deficiency. Taken together, these results demonstrate a mechanism by which reprogramming of ubiquitin signalling in cancer cells can influence DNA damage tolerance and probably contribute to an altered genomic landscape. (Collaborative study with Dr. Vaziri, see reference 4).

4. Somatic hypermutation of immunoglobulin genes in Rad18 knockout mice

Somatic hypermutation (SHM) of immunoglobulin (Ig) genes is triggered by the activity of activation-induced cytidine deaminase (AID). AID induces DNA lesions in variable regions of Ig genes, and error-prone DNA repair mechanisms initiated in response to these lesions introduce the mutations that characterize SHM. Error-prone DNA repair in SHM is proposed to be mediated by low-fidelity DNA polymerases such as those that mediate trans-lesion synthesis (TLS); however, the mechanism by which these enzymes are recruited to AID-induced lesions remains unclear. Proliferating cell nuclear antigen (PCNA), the sliding clamp for multiple DNA polymerases, undergoes Rad6/Rad18-dependent ubiquitination in response to DNA damage. Ubiquitinated PCNA promotes the replacement of the replicative DNA polymerase stalled at the site of a DNA lesion with a TLS polymerase. To examine the potential role of Rad18-dependent PCNA ubiquitination in SHM, we analyzed Ig gene mutations in Rad18 knockout (KO) mice immunized with T cell-dependent antigens. We found that SHM in Rad18 KO mice was similar to wild-type mice, suggesting that Rad18 is dispensable for SHM. However, residual levels of ubiquitinated PCNA were observed in Rad18 KO cells, indicating that Rad18-independent PCNA ubiquitination might play a role in SHM. (Collaborative study with Dr. Shimizu, see reference 2).

5. Mutations in UVSSA cause UV-sensitive syndrome and impair RNA polymerase II processing in transcription-coupled nucleotide-excision repair

UV-sensitive syndrome (UVSS) is a genodermatosis characterized by cutaneous photosensitivity without skin carcinoma. Despite mild clinical features, cells from individuals with UVSS, like Cockayne syndrome cells, are very UV sensitive and are deficient in transcription-coupled nucleotide-excision repair (TC-NER), which removes DNA damage in actively transcribed genes. Three of the seven known UVSS cases carry mutations in the Cockayne syndrome genes ERCC8 or ERCC6 (also known as CSA and CSB, respectively). The remaining four individuals with UVSS, one of whom is described for the first time here, formed a separate UVSS-A complementation group; however, the responsible gene was unknown. Using exome sequencing, we determine that mutations in the UVSSA gene (formerly known as KIAA1530) cause UVSS-A. The UVSSA protein interacts with TC-NER machinery and stabilizes the ERCC6 complex; it also facilitates ubiquitination of RNA polymerase II stalled at DNA damage sites. Our findings provide mechanistic insights into the processing of stalled RNA polymerase and explain the different clinical features across these TC-NER-deficient disorders. (Collaborative study with Dr. Ogi, see reference 9).


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**著書・総説目録**


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**Meeting Presentations**


2. 田上友貴、立石智. Chk2 による、UV 誘発性皮膚がんの防御機構. 日本分子生物学会年会 第40回大会、12月6日-9日 2017, 神戸市.


5. 田上友貴、立石智. Chk2 に依存しておこる分裂期細胞死による、発がん防御機構. がん支援活動公開シンポジウム、1月27-28日 2015, 東京.

6. 田上友貴、立石智. Rad18 と Chk2 は Gapped DNA の形成を抑制し、ゲノスを安定化する. 第23回 DNA 複製・組換え修復ワークショップ研究会、10月19日-21日 2015, 静岡県焼津市.


8. 田上友貴、立石智. Rad18 と Chk2 は Gapped DNA の形成を抑制し、ゲノスを安定化する. 第38回日本分子生物学会年会 (BMB2015)，12月1日-3日 2015, 神戸.


10. Tateishi, S. Chk2 and Rad18 concertedely maintain genomic integrity to prevent tumor formation. International Conference Replication, repair and transcription; coupling mechanisms and chromatin dynamics for genome integrity, 4-5 February 2014, Kyoto, Japan.

11. 田上友貴、立石智. Chk2 による、分裂期細胞死の誘導機構. 日本生化学学会年会、10月14日-17日 2014, 京都.


13. 田上友貴、立石智. Chk2 による、分裂期細胞死の誘導機構. 日本放射線影響学会 第57回大会、9月30日-10月2日 2014, 鹿児島.

14. 田上友貴、立石智. Chk2 による、分裂期細胞死の誘導機構. 日本放射線影響学会 第58回大会、10月14日-17日 2015, 神戸.
細胞死の誘導機構、日本分子生物学会年会第37回大会、11月25日-27日2014、横浜。

15. 田上友貴、立石智。Rad18とChk2の連携による、発がんの抑制。日本放射線影響学会第56回大会、10月18日-20日2013、青森。

16. 立石 智、田上友貴。Rad18とChk2の連携による、ゲノムを安定に維持する機構の解明。第22回DNA複製・組換え・修復ワークショップ、11月20日-22日2013、仙台。

17. Tanoue, Y., Tateishi, S. Chk2 and Rad18 contribute cooperatively to maintain genomic stability, leading to prevention of tumorigenesus and germ cells maintenance. 29th RBC-NIRS International symposium, 28-29 November 2013, Kyoto, Japan.


20. 田上友貴、高森 秀平、立石 智。Rad18とChk2の連携作用による、発がんの抑制。日本分子生物学会第35回大会、12月11-14日、2012、福岡。

アウトリーチ活動 Outreach activity

1. 立石 智。日本ココイン症候群ネットワーク・患者の集いでの講演。ココイン症候群の治療につながる基礎研究をめざして。10月7日-8日、2017、東京。

2. 立石 智。色素性乾皮症、ココイン症候群などの遺伝性難病の細胞診断（熊本大学医学部皮膚科および名古屋大学との共同作業）。解析中のものを含めて11件、2012-2017、熊本。
DNA repair factor RAD18 and DNA polymerase Polc confers tolerance of oncogenic DNA replication stress

Yang Yang, 1 Yoonhe Gao, 2 Liz Mutter-Rothmayr, 2 Anastasia Zlatanov, 2 Michael Durandao, 2 Yemin Ding, 1, 4 David Wyatt, 1 Dale Ramadard, 3 Yuki Tanoue, 2 Satoshi Tateishi, 1 and Cynis Vaziri 1, 2

1 Department of Biology and Laboratory Medicine, Centre de Recherches sur les Cancers, Centre Communautaire de Santé et de Médicine, and Department of Pathology and Sehnology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
2 Institute of Medical Science, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
3 Department of Molecular Genetics and Genomics, Harvard Medical School, Boston, MA, USA
4 Department of Biochemistry, University of California, San Francisco, CA, USA

The mechanisms by which neoplastic cells tolerate oncogenic-induced DNA replication stress are poorly understood. Cyclin-dependent kinase 2 (CDK2) is a major mediator of oncogenic DNA replication stress. In this study, we show that CDK2-inhibiting stimuli (including Cyclin E overexpression, oncogenic RAS, and WEE1 inhibition) activate the DNA repair protein RAD18. CDK2-induced RAD18 activation required initiation of DNA synthesis and was suppressed by p53. RAD18 and its effector, DNA polymerase ε (Polε), sustained ongoing DNA synthesis in cells harboring elevated CDK2 activity. RAD18-deficient cells aberrantly accumulated single-stranded DNA (ssDNA) after CDK2 activation. In RAD18-depleted cells, the G2/M checkpoint was necessary to prevent mitotic entry with persistent ssDNA. RAD18+/− and Polε−/− cells were highly sensitive to the WEE1 inhibitor MK-1775 (which simultaneously activates CDK2 and abrogates the G2/M checkpoint). Collectively, our results show that the RAD18-Polε signaling axis allows tolerance of CDK2-mediated oncogenic stress and may also nonapoptotic cell death to break homologous carcinogenic.

Introduction

During tumorigenesis, neoplastic cells must endure DNA damage from environmental, metabolic, and other intrinsic sources. (Barvzov et al., 2006; Halazonetis et al., 2008.) Oncogene-induced DNA replication stress can be a major cause of intrinsic DNA damage and represents a potential source of genome instability in cancer cells. Many oncogenes, including v-RAS, cyto-6, and others, induce DNA replication defects that trigger DNA damage signaling (including ATM-CHK2, ATR-CHK1, and p53) and lead to irreparable cell cycle exit or senescence. DNA damage is associated with cell cycle arrest and replication failure. Indeed, oncogene-induced DNA replication stress is often modeled experimentally by overexpression of CDK2 activators (Cyclin E and CDCE3A) or inhibition of the WEE1 kinase to remove negative regulators over CDK2 (Segovia et al., 2002; Barvzov et al., 2006; Beck et al., 2010, 2012; Jones et al., 2013).

Despite our limited mechanistic understanding of how oncogenes dysregulate DNA synthesis and cause DNA damage, there is general consensus that OSS poses a barrier to tumorigenesis. Clearly, however, the OSS barrier is imperfect and can be breached. The precise mechanisms by which oncogenes expressing cells withstand replication stress and DNA damage are poorly understood. DNA repair and DNA damage
A neomorphic cancer cell-specific role of MAGE-A4 in trans-lesion synthesis

Yanzhe Gao1,*, Elizabeth Mutter-Rottmayer1,2,*, Alicia M. Greenwall1,3,*, Dennis Goldfarb4, Peng Yan3, Yang Yang3, Raquel C. Martinez-Chacin1,6, Kenneth H. Pearce1, Satoshi Tateishi6, Michael B. Major1,5,6 & Cyrus Vaziri1,2,3

Trans-lesion synthesis (TLS) is an important DNA-damage tolerance mechanism that permits ongoing DNA synthesis in cells harboring damaged genomes. The E3 ubiquitin ligase RAD18 activates TLS by promoting recruitment of Y-family DNA polymerases to sites of DNA-damage-induced replication fork stalling. Here we identify the cancer/testes antigen melanoma antigen-A4 (MAGE-A4) as a tumour cell-specific RAD18-binding partner and an activator of TLS. MAGE-A4 depletion from MAGE-A4-expressing cancer cells destabilizes RAD18. Conversely, ectopic expression of MAGE-A4 (in cell lines lacking endogenous MAGE-A4) promotes RAD18 stability. DNA-damage-induced mono-ubiquitination of the RAD18 substrate PCNA is attenuated by MAGE-A4 silencing. MAGE-A4-depleted cells fail to resume DNA synthesis normally following ultraviolet irradiation and accumulate γH2AX, thereby recapitulating major hallmarks of TLS deficiency. Taken together, these results demonstrate a mechanism by which reprogramming of ubiquitin signalling in cancer cells can influence DNA damage tolerance and probably contribute to an altered genomic landscape.

ABSTRACT

In cultured cancer cells the E3 ubiquitin ligase RAD18 activates Trans-Lesion Synthesis (TLS) and the Fanconi Anemia (FA) pathway. However, physiological roles of RAD18 in DNA damage tolerance and carcinogenesis are unknown and were investigated here. Primary hematopoietic stem and progenitor cells (HSPC) co-expressed RAD18 and FANC-D2 proteins, potentially consistent with a role for RAD18 in FA pathway function during hematopoiesis. However, hematopoietic defects typically associated with fanconi-deficiency (decreased HSPC numbers, reduced engraftment potential of HSPC, and Mitomycin C (MMC) -sensitive hematopoiesis), were absent in Rad18−/− mice. Moreover, primary Rad18−/− mouse embryonic fibroblasts (MEF) retained robust Fancd2 mono-ubiquitination following MMC treatment. Therefore, RAD18 is dispensable for FA pathway activation in untransformed cells and the Rad18 and FA pathways are separable in hematopoietic cells. In contrast with responses to crosslinking agents, Rad18−/− HSPC were sensitive to in vivo treatment with the myelosuppressive agent 7,12-Dimethylbenz[a]anthracene (DMBA). Rad18-deficient fibroblasts aberrantly accumulated DNA damage markers after O6MeA treatment. Moreover, in vivo DMBA treatment led to increased incidence of B cell malignancy in Rad18−/− mice.

These results identify novel hematopoietic functions for Rad18 and provide the first demonstration that Rad18 confers DNA damage tolerance and tumor-suppression in a physiological setting.

INTRODUCTION

Cells are frequently subject to DNA damage from environmental, intrinsic and therapeutic sources. Failure to tolerate and accurately repair DNA damage can lead to loss of cell viability or genome instability, enabling characteristic of cancer cells (1). The E3 ubiquitin ligase RAD18 plays key roles in Trans-Lesion Synthesis (TLS), a DNA damage tolerance mechanism that allows cells to replicate genomes harboring bulky DNA lesions including poly(ADP-ribose) (PAR) adducts (2). In response to DNA damage, RAD18 monoubiquitates stalled DNA replication forks (3,4) and mono-ubiquitinates the DNA polymerase γ (RPCN6) (5). DNA damage tolerance mechanisms (6) are critical in many malignant cells (7,8). Indeed, DALB (9) and its effectors TLS polymerases can confer viability, but also have the potential to compromise genomic stability (7). Indeed, Rad18-deficient cells are prone to tumorigenic and hypermutagenic bypass of various DNA lesions, including PAR (10). The RAD18-mediated TLS pathway has been studied in...
Two replication fork maintenance pathways fuse inverted repeats to rearrange chromosomes

Lingchuan Hu¹, Iae Moori Kims¹, Mi Young Son¹, Sung-A Kim¹, Cory L. Holland¹, Satoshi Tateishi², Dong Hyun Kim³, P. Renee Yew⁴, Cristina Montagnana⁵, Laviatia C. Dumitrache⁶ & Paul Hasty⁷

Replication fork maintenance pathways preserve chromosomes, but their faulty application at nonallelic repeats could generate rearrangements, including cancer, genomic disorders and spasticity. Potential causal mechanisms are homologous recombination and error-free postreplication repair (ERR-PRR). Homologous recombination repair damages induced DNA-strand breaks (DSBs) and single-ended DSBs within replication. To facilitate homologous recombination, the recombinase RAD51 and mediator BRC1 form a filament on the 3' DNA strand at a break to enable annealing to the complementary sister chromatid while the RecQ helicase, BLM ( Bloom syndrome mutant) suppresses crossing over to prevent recombination. Homologous recombination also stabilizes and restarts replication forks without a DSB. ERR-PRR bypasses DNA inaccuracies that impede replication by ubiquitylating PCNA (proliferating cell nuclear antigen) using RAD51-RAD18 and UBC13-MMS2- Rad51-Rad18 ubiquitin ligase complexes. Some components are common to both homologous recombination and ERR-PRR such as RAD51 and RAD18. Here we delineate two pathways that spontaneously fuse inverted repeats to generate unstable chromosomal rearrangements in wild-type mouse embryonic stem cells (ES cells). Genome radiation-induced IFRs regulated fork pathways that selectively fused identical, but not mismatched, repeats. By contrast, ultraviolet light induced a RAD18-dependent pathway that efficiently fused mismatched repeats. Furthermore, TREX2 (a 3'–5' exonuclease) suppressed identical repeat fusion but enhanced mismatched repeat fusion, clearly separating these pathways. TREX2 associated with UBC13 and enhanced PCNA ubiquitination in response to ultraviolet light, consistent with it being a novel member of FF-PRR. RAD18 and TREX2 also suppressed replication fork stalling in response to nucleotide depletion. Interestingly, replication fork stalling induced fusion for identical and mismatched repeats, implying faulty recombination as a causal mechanism for both pathways.

Introduction

The KING-type (ES) ubiquitin ligase RAD18 is a key player in involved post-replication repair (PRR) that regulates ubiquitylation of proliferating cell nuclear antigen (PCNA) in response to DNA double-strand breaks (DSB) sites, where it plays novel functions in the DNA damage response induced by ionizing radiation (IR). This new role is independent of IR-induced cell death, but it is known about how RAD18 functions after IR exposure. Here, we describe a role for RAD18 in the induced DNA damage signaling pathway at the genome scale in the cell cycle. Deducing cells of RAD18 reduced the recruitment of the DNA damage signaling factors ATM, p53 and 53BP1 to foci at the G2/M phase after IR exposure, and attenuated activation of the G2/M checkpoint. Furthermore, depletion of RAD18 induced micronuclei formation and cell death following IR exposure, both in vitro and in vivo. Our data suggest that RAD18 can function as a mediator for DNA damage-responsive signals to activate the G2/M checkpoint in order to maintain genome integrity and cell survival after IR exposure.

Abstract

The ubiquitin ligase RAD18 is involved in post-replication repair pathways via its recruitment to stalled replication forks, and its role in the ubiquitylation of proliferating cell nuclear antigen (PCNA). Recently, it has been reported that RAD18 is also recruited to DNA double strand break (DSB) sites, where it plays novel functions in the DNA damage response induced by ionizing radiation (IR). This new role is independent of IR-induced cell death, but it is known about how RAD18 functions after IR exposure. Here, we describe a role for RAD18 in the induced DNA damage signaling pathway at the genome scale in the cell cycle. Deducing cells of RAD18 reduced the recruitment of the DNA damage signaling factors ATM, p53 and 53BP1 to foci at the G2/M phase after IR exposure, and attenuated activation of the G2/M checkpoint. Furthermore, depletion of RAD18 induced micronuclei formation and cell death following IR exposure, both in vitro and in vivo. Our data suggest that RAD18 can function as a mediator for DNA damage-responsive signals to activate the G2/M checkpoint in order to maintain genome integrity and cell survival after IR exposure.

RAD18 Activates the G2/M Checkpoint through DNA Damage Signaling to Maintain Genome Integrity after Ionizing Radiation Exposure

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| DOI: 10.1038/s12255-016-0946-3 |
| February 12, 2016 |

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A non-catalytic role of DNA polymerase η in recruiting Rad18 and promoting PCNA monoubiquitination at stalled replication forks

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Received August 28, 2012; Revised December 13, 2012; Accepted December 24, 2012

ABSTRACT

Trans-information synthesis (TLS) is a DNA damage-tolerance mechanism that uses low-fidelity DNA polymerases to replicate damaged DNA. The inherited cancer-predisposition syndrome xeroderma pigmentosum variant (XPV) results from error-prone TLS of UV-damaged DNA. TLS is initiated when the Rad18/ Rad1 complex monoubiquitinates proliferating cell nuclear antigen (PCNA), but the basis for recruitment of Rad18/PCNA to damage sites is not clearly understood. Here, we show that Rad18 is targeted to PCNA by DNA polymerase η, the XPF-XPV product that is mutated in XPV patients. The XPF-XPV domain of Polθ binds to both Rad18 and PCNA and promotes PCNA monoubiquitination, a function unique to Polθ among X-family TLS polymerases and dissociable from its catalytic activity. Importantly, XPV cells express Polθ exclusively and lack the full-length catalytically-inactive Polθ. We observed increased recruitment of other error-prone TLS polymerases (Polξ and Polη) after UV irradiation. These results define a novel non-catalytic role for Polθ in promoting PCNA monoubiquitination and provide a new potential mechanism for genotoxic stress damage in XPV individuals.

INTRODUCTION

Living organisms are constantly exposed to genotoxic assaults from endogenous and external sources (1,2). However, cells have evolved numerous DNA damage response (DDR) pathways that protect genic DNA and prevent genotoxic stress (3,4). Trans-information synthesis (TLS) is a DDR mechanism involving specialized DNA polymerases that can replicate damaged DNA templates (3,4).

TLS relies on inherently error-prone DNA polymerases of the η family to replicate damaged DNA (5). TLS by η-family polymerases (Pο, Πο, Φο and Φε1) maintains replication in cells harbouring damaged DNA, albeit at the cost of reduced fidelity. Each TLS polymerase performs relatively error-free replication past a pre-attached cognate lesion; in the absence of the appropriate TLS polymerase for the prior lesion, mutagenic replication by error-prone polymerases predisposes to genetic instability (6). Polη is unique among η-family polymerases in its ability to perform accurate replication past UV-damaged DNA (6,7). Lack of Polη in the inherited cancer-predisposition syndrome xeroderma pigmentosum variant (XPV) (8) results in error-prone replication by other η-family polymerases in sunlight-exposed cells (8,9). Thus, UV-induced mutagenesis due to Polη deficiency compromises genetic integrity in mammalian systems early in skin carcinogenesis and early skin cancer progression. A prerequisite for error-prone replication in TLS is the Rad18/Rad1 complex monoubiquitinates proliferating cell nuclear antigen (PCNA) at the highly conserved lysine 164 (11,12). η-family polymerases contain ubiquitin-binding (UBD) domains that confer affinity to monoubiquitinated PCNA (13,14). Failure to monoubiquitinate PCNA at K164 promotes η-mediated TLS by compromising TLS and sensitizing cells to UV light and other ubiquitous genotoxins (15–18). Several other DDR pathways also depend on PCNA monoubiquitination, including CHIPHR/HTLF-mediated template switching (19), ZRANB-dependent replication fork restart (20), SMV-A-dependent interstrand cross-link repair (21) and the Fancos Aneurisms pathway activation (22). Despite its pivotal role in the DDR, the molecular mechanisms regulating Rad18-mediated PCNA monoubiquitination are incompletely understood. The Rad18/ Rad1 complex is thought to be recruited to the vicinity of damaged DNA via direct interactions with RPA-coated replication forks and the C-terminus of PCNA. 

Methods in UVSSA cause UV-sensitive syndrome and impair RNA polymerase II processing in transcription-coupled nucleotide-excision repair

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UVC-sensitive syndrome (UVSSA) characterized by cutaneous phototoxiness without skin canceroma1. Despite mild cutaneous features, cells from individuals with UVSSA, like Cockayne syndrome cells, are very UV sensitive and are defective in transcription-coupled nucleotide-excision repair (TC-NER)2, which removes DNA damage (lesion directly transcribed genome)3. Three of the seven known UVSSA cases carry mutations in the Cockayne syndrome genes ERCC1 or ERCC2 (also known as CSB and CSB, respectively)4. The remaining four individuals with UVSSA, one of whom is described for the first time here, formed a separate UVSSA complementation group (UVSSA1), however, the mechanism responsible for this defect was unknown. Using exon sequencing5, we determined that mutations in the UVSSA gene (formerly known as RASAT1) cause UVSSA. The UVSSA protein interacts with TC-NER machinery and stabilizes the ERCC6 complex; it also facilitates ubiquitination of RNA polymerase II at stalled DNA damage sites. Our findings provide mechanistic insights into the process of stalled RNA polymerase II and explain the different clinical phenotypes in these TC-NER-deficient disorders.

We performed exon sequencing on two cell lines, Kp2 and XFXPAK1, derived from two individuals with UVSSA-A (cell lines described in Table 1, Online Methods, Supplementary Table 2ac and Supplementary Note).

Using a recessive model of inheritance, we directly identified over-lapping mutations in KX443130 (NCBI Gene 90564), a predicted gene at 4p16.3 encoding a 709 amino acid protein of unknown function (Table 1 and Supplementary Table 1). KX443130 was subsequently renamed UVSSA (encoding UV-stimulated scaffold protein A) because of this finding, with support from the Human Gene Nomenclature Committee (HGNC). Affected individuals were homozygous for a c.366T>A mutation, which led to the introduction of a premature stop codon, p.123fsX3. In the UVSSA protein (Fig. 1a), we identified the same homozygous mutation in subject Kp2 (a sibling of Kp3) and a homozygous c.876G>C mutation causing p.118fsX9 frameshift alteration in Israeli subject UVSSA/1 (Fig. 1b, Supplementary Fig. 1c and Supplementary Note). The identified mutations are summarized (Fig. 1d). We did not detect the 80 kb UVSSA transcript in any of the individuals with UVSSA-A (Fig. 1e). We also examined several mild xeroderma pigmentosum cases; in one case, XPD501 (Supplementary Table 1), we identified a homozygous nonsense mutation (encoding C375X) in UVSSA (Fig. 1c), which implies that XPDI501 is also in the UVSSA complementation group. The mutant protein was stably expressed in XPDI501 cells, although band intensity was lower (Fig. 1f, and Supplementary Fig. 2a–d).

The mutated amino acid (p.123) was observed in the heterozygous state in 1% of control individuals (allele frequency of 0.09%) evaluated by direct sequencing or high-resolution melting

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Received 26 August 2011; accepted 29 February 2012; published online 1 April 2012 doi:10.1038/ncomms2029
The Vital Role of Polymerase ζ and REV1 in Mutagenic, but Not Correct, DNA Synthesis across Benzo[a]pyrene-dG and Recruitment of Polymerase ζ by REV1 to Replication-stalled Site

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Background: DNA damage caused by various factors, such as ultraviolet light, ionizing radiation, and chemical carcinogens, is repaired by the DNA damage response (DDR) pathway. The DDR pathway includes both the direct repair of DNA damage and the activation of genes that respond to DNA damage, which can include the repair of DNA damage. The DDR pathway is essential for the maintenance of genome integrity and the prevention of cancer.

Results: The authors investigated the role of polymerase ζ and REV1 in the repair of DNA damage caused by benzo[a]pyrene-dG, a common environmental carcinogen. They found that polymerase ζ and REV1 are essential for the repair of DNA damage caused by benzo[a]pyrene-dG, but not for the repair of DNA damage caused by other types of DNA damage.

Conclusion: The results of this study suggest that polymerase ζ and REV1 play a critical role in the repair of DNA damage caused by benzo[a]pyrene-dG, which is a common environmental carcinogen. This study also highlights the importance of understanding the role of polymerase ζ and REV1 in the repair of DNA damage caused by different types of DNA damage.

Significance: This study provides new insights into the role of polymerase ζ and REV1 in the repair of DNA damage caused by benzo[a]pyrene-dG, which is a common environmental carcinogen. These findings have important implications for the development of new therapies for cancer and other diseases caused by DNA damage.