News Release

Title

RFWD3 and translesion DNA polymerases contribute to PCNA-modification dependent DNA damage tolerance

Key Points

- **Cellular sensitivity to illudin S could also be a hallmark of deficiency in DNA damage tolerance, as well as TC-NER (transcription-coupled nucleotide excision repair).**
- **RFWD3 and DNA damage-specific TLS polymerases Pol κ and Pol η, for illudin S and UV DNA lesions, respectively, contribute to PCNA ubiquitination-dependent DNA damage tolerance pathway.**
- **RFWD3, encoded by the gene responsible for *FANCW*, has a function in damage tolerance independent from the FANC pathway.**

Summary

DNA replication blockage at DNA damage could cause genomic instability resulting in cancer and cell death. Cells have some mechanisms to overcome replication blockage at DNA damage sites, called DNA damage tolerance. PCNA modifications at lysine 164 are important to regulate DNA damage tolerance. Mono-ubiquitinated PCNA activates translesion DNA synthesis. However, precise molecular mechanisms other than translesion DNA synthesis are still unclear. In this study, the research group found that human cells have two PCNA ubiquitination-dependent DNA damage tolerance pathways; one is translesion DNA synthesis mediated by damage-specific DNA polymerases, and the other is RING finger WD repeat 3 (RFWD3)-mediated pathway. RFWD3 is a Fanconi anemia-responsible gene product and plays an essential role in interstrand crosslink repair (FANC pathway). However, RFWD3 has an outside role in DNA damage tolerance from the FANC pathway.

This study was published in the open access journal Life Science Alliance on 29 Jul 2022.

This work was supported by JSPS KAKENHI (21K12238 to RK and 20H04335, 21K19843 to CM) and the Takeda Science Foundation (to RK and CM).

Research Background

DNA damage disrupts transcription and DNA replication that induces genomic instability resulting in cancer and cell death. Cells have various types of “DNA repair” mechanisms for repairing DNA damage and “DNA damage tolerance” pathways to overcome DNA replication blockage. Modifications of proliferating cell nuclear antigen (PCNA) on lysine 164 (K164) play important roles in controlling DNA damage tolerance pathways. Translesion DNA synthesis

(TLS) is one of the DNA damage tolerance pathways catalyzed by special DNA polymerases (TLS polymerases) which can incorporate nucleotides opposite damaged DNA. Mono-ubiquitinated PCNA facilitates TLS. However, precise mechanisms of DNA damage tolerance mechanisms other than TLS are still under investigation. UV-induced replication block is mainly overcome by DNA polymerase eta (Polη), the responsible gene product of the Xeroderma Pigmentosum Variant.

Research Results

To identify Polη independent DNA damage tolerance pathways, we used a natural compound illudin S and its derivative irofulven for DNA damaging agents. Polη is not involved in cellular survival of these compounds. Illudin S and irofulven-induced DNA damage are repaired by transcription-coupled nucleotide excision repair (TC-NER), thus, cellular sensitivity of these compounds had been thought to be a hallmark of TC-NER deficiency. However, we showed that illudin S or irofulven sensitivity can be a hallmark of DNA damage tolerance defect as well as TC-NER.

We found two pathways of PCNA ubiquitination-dependent DNA damage tolerance to illudin S or irofulven induced damage; DNA polymerase kappa (Polκ) mediated TLS, and RING finger and WD40 repeat 3 (RFWD3) mediated pathway.

Mutations in the *RFWD3* gene resulted in an inherited disorder Fanconi anemia, and RFWD3 is involved in FANC-mediated interstrand crosslink (ICL) repair. Importantly, however, we found that the function of RFWD3 in DNA damage tolerance is independent of the FANC pathway.

RFWD3 function in PCNA ubiquitination-dependent DNA damage tolerance is not only for specific DNA damage but also for UV-induced damage. RFWD3 and Polη had an independent role in UV damage tolerance. Our results indicate that PCNA ubiquitination-dependent DNA damage tolerance is consist of an RFWD3-dependent pathway and TLS mediated by DNA damage-specific polymerases.

Research Summary and Future Perspective

DNA damage tolerance is an important mechanism for maintaining genomic stability, however, instead, it could make cells resistant to anti-cancer drugs such as Cisplatin that create DNA damage to disrupt DNA replication resulting in cell death. It is expected to be understood not only how genomic stability is maintained in human cells but also the mechanisms of chemoresistance by exploring the precise mechanism of RFWD3-induced DNA damage tolerance.

Publication

RFWD3 and translesion DNA polymerases contribute to PCNA modification-dependent DNA damage tolerance. ***Life Sci Alliance*** (2022)

Rie Kanao[1,2], Hidehiko Kawai[3], Toshiyasu Taniguchi[4], Minoru Takata[5], and Chikahide Masutani[1,2]*

1 Department of Genome Dynamics, Research Institute of Environmental Medicine, Nagoya University

2 Department of Molecular Pharmaco-Biology, Nagoya University Graduate School of Medicine,

3 Department of Nucleic Acids Biochemistry, Graduate School of Biomedical and Health Sciences, Hiroshima University

4 Department of Molecular Life Science, Tokai University School of Medicine

5 Laboratory of DNA Damage Signaling, Department of Late Effects Studies, Radiation Biology Center, Graduate School of Biostudies, Kyoto University

DOI: 10.26508/lsa.202201584

Japanese ver.

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