News Release

Title

Regulatory mechanisms of risk management for cellular response to DNA damage
–Novel mechanisms of DNA damage tolerance pathway choice regulated by ubiquitin ligases–

Key Points

○ DNA damage tolerance is a mechanism to complete DNA replication of genomic DNA containing DNA damage.

○ DNA damage tolerance is involved in mutagenesis and cell death of cancer cells promoted by the anti-cancer drugs, which induce DNA damage.

○ Two sub-pathways of DNA damage tolerance are determined, and the pathway choice between the sub-pathways is one of key steps to determine the fate of damage cells.

○ The finding of molecular mechanism of DNA damage tolerance pathway choice sheds light on the understanding of cellular response to DNA damage, such as mutagenesis.

Summary

An associate professor Yuji Masuda, an assistant professor Rie Kanao and an undergraduate student Satoshi Mitsuyuki, members of the research group led by Prof. Chikahide Masutani in Research Institute of Environmental Medicine, Nagoya University (Director, Koji Yamanaka, MD, PhD) and Nagoya University Graduate School of Medicine (Dean, Kenji Kadomatsu, MD, PhD), and Prof. Hiroshi Hashimoto and an assistant professor Asami Hishiki in School of Pharmaceutical Sciences, University of Shizuoka demonstrated molecular mechanism of DNA damage tolerance pathway choice regulated by ubiquitin ligases.

DNA damage tolerance is a mechanism to complete DNA replication of genomic DNA containing DNA damage, and involved in mutagenesis and cell death of not only normal cells but also cancer cells exposed with the anti-cancer drugs, which induce DNA damage as well as ultraviolet light. So far two sub-pathways of DNA damage tolerance are recognized. One is translesion DNA synthesis, in which DNA synthesis is performed using the damaged base as template, thus it is suggested that the process could induce the point mutation. The other is template switch, in which DNA synthesis is performed using the corresponding region of the sister chromatid as template. Since the process does not use the damaged base as template, it is error free in principle, however it could induce the chromosomal rearrangement. Although the mechanism of the pathway choice between the two sub-pathways is critical to regulate the risk of mutagenesis and survival or death of cells exposed with DNA damage agents such as anti-cancer drugs and ultraviolet light, it remains to be elucidated.

Masuda et al. established an *in vitro* reconstituted system using a model DNA mimicking stalled replication ends and enzymes that are assumed to exist around there, and examined the biochemical reactions, especially focused on two ubiquitin ligases, RAD18 and

HLTF. Consequently, Masuda et al. discovered that the ligase activity of HLTF is sophisticatedly regulated at the stalled replication ends and demonstrated biochemical evidence that translesion DNA synthesis and template switch are independently selected, and template switch is directed after once translesion DNA synthesis is selected. These findings of molecular mechanisms of DNA damage tolerance pathway choice shed light on the understanding of cellular response to DNA damage, such as mutagenesis. This work was published online in Nucleic Acids Research on October 18, 2018.

Research Background

DNA damage tolerance is a mechanism to complete DNA replication of genomic DNA containing DNA damage, and involved in mutagenesis and cell death of not only normal cells but also cancer cells exposed with the anti-cancer drugs, which induce DNA damage as well as ultraviolet light (Figure 1). So far two sub-pathways of DNA damage tolerance are recognized. One is translesion DNA synthesis, in which DNA synthesis is performed using the damaged base as template, thus it is suggested that the process could induce the point mutation. The other is template switch, in which DNA synthesis is performed using the corresponding region of the sister chromatid as template. Since the process does not use the damaged base as template, it is error free in principle, however it could induce the chromosomal rearrangement. The mechanism of the pathway choice between the two sub-pathways is critical to regulate the risk of mutagenesis and the survival or death of cells exposed with DNA damage agents such as anti-cancer drugs and ultraviolet light.

It has been established that the level of ubiquitination of PCNA plays a key role of damage pathway choice (Figure 2). PCNA, a ring-shaped homo-trimer, is one of the essential factors of DNA replication. The subunit has one ubiquitination site; therefore, PCNA molecule has three sites for ubiquitination. The PCNA ring encircles DNA for its binding to DNA, thereby hardly dissociating from DNA and has a potential for sliding along DNA. Because of the properties, PCNA can locate always at the growing end of DNA replication and function with other replication factors as a kind of platform. When PCNA is modified with one ubiquitin molecule (monoubiquitination) at the stalled replication end, the resultant monoubiquitinated PCNA promotes translesion DNA synthesis. Since the PCNA molecule is a homo-trimer, it has a potential that the all three subunits are simultaneously monoubiquitinated (multi-monoubiquitination), and the resultant multi-monoubiquitinated PCNA also promotes translesion DNA synthesis. On the other hand, when PCNA is modified with a ubiquitin chain, it stimulates template switch (Figure 2). Results from experiments using yeast and human cells indicated that, in certain situations, the selection of translesion DNA synthesis or template switch is defined at the time of onset and the pathways are not interchangeable, whereas under specific conditions, translesion DNA synthesis and template switch are interchangeable. Despite these extensive analyses, the molecular basis determining the choice between translesion DNA synthesis and template switch remains to be determined.

Research Results

The research group previously reported that the ubiquitin ligase, RAD18, efficiently monoubiquitinates the PCNA molecules located at the growing end of the DNA replication and resultant monoubiquitinated PCNA promoted translesion DNA synthesis. The other ubiquitin ligase, HLTF, polyubiquitinates unmodified PCNA in the presence of RAD18, but not already monoubiquitinated PCNA in the absence of RAD18. Therefore, two unanswered questions remain to be elucidated; one is the molecular mechanism for redirection from the pathway of translesion DNA synthesis to the pathway of template switch, the other is where HLTF functions.

Masuda et al. focus on the two unanswered questions. First, Masuda et al. sought the DNA structure required for the stimulation of ubiquitin ligase activity of HLTF, because DNA is absolutely required for the ligase activity of HLTF to exhibit, and consequently, Masuda et al. found that the structure mimicking stalled replication end most efficiently stimulated the ligase activity of HLTF. The finding indicated that HLTF functions at the stalled replication ends. Next Masuda et al. reconstituted the reactions of PCNA-ubiquitination using at maximum 20 proteins, which functions around replication ends and for ubiquitination, with a model DNA mimicking stalled replication end. The excellent reconstitution system revealed an elegant regulatory mechanism for HLTF at the stalled replication ends for polyubiquitination of PCNA. In addition, Masuda et al. serendipitously found that only the three-subunits-monoubiquitinated PCNA is polyubiquitinated by HLTF in the absence of RAD18, indicating that the redirection from the pathway of translesion DNA synthesis to the pathway of template switch specifically occurs on three-subunit-monoubiquitinated PCNA, indicating the redirection reaction requires that all three subunits of PCNA should be monoubiquitinated by RAD18 (Figure 2).

Research Summary and Future Perspective

These findings of molecular mechanisms of DNA damage tolerance pathway choice shed light on the understanding of cellular response to DNA damage, such as mutagenesis. Further studies are needed to elucidate the regulation of the recruitment of HLTF to the stalled replication ends.

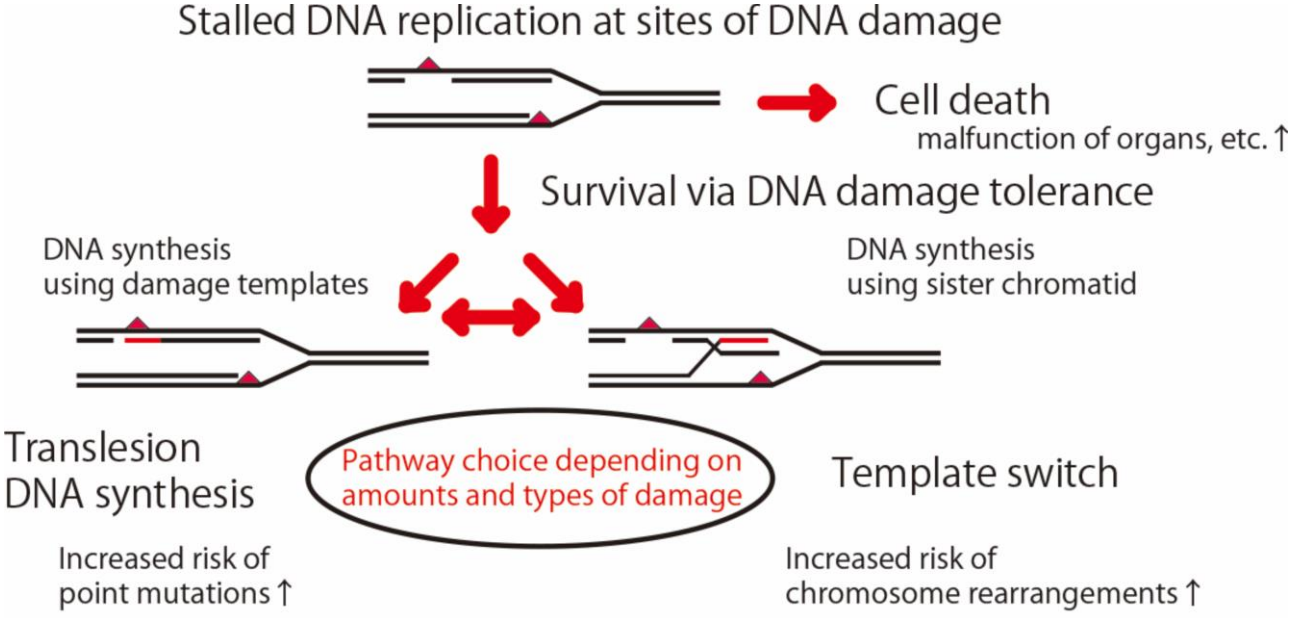

Figure 1 Function of DNA damage tolerance

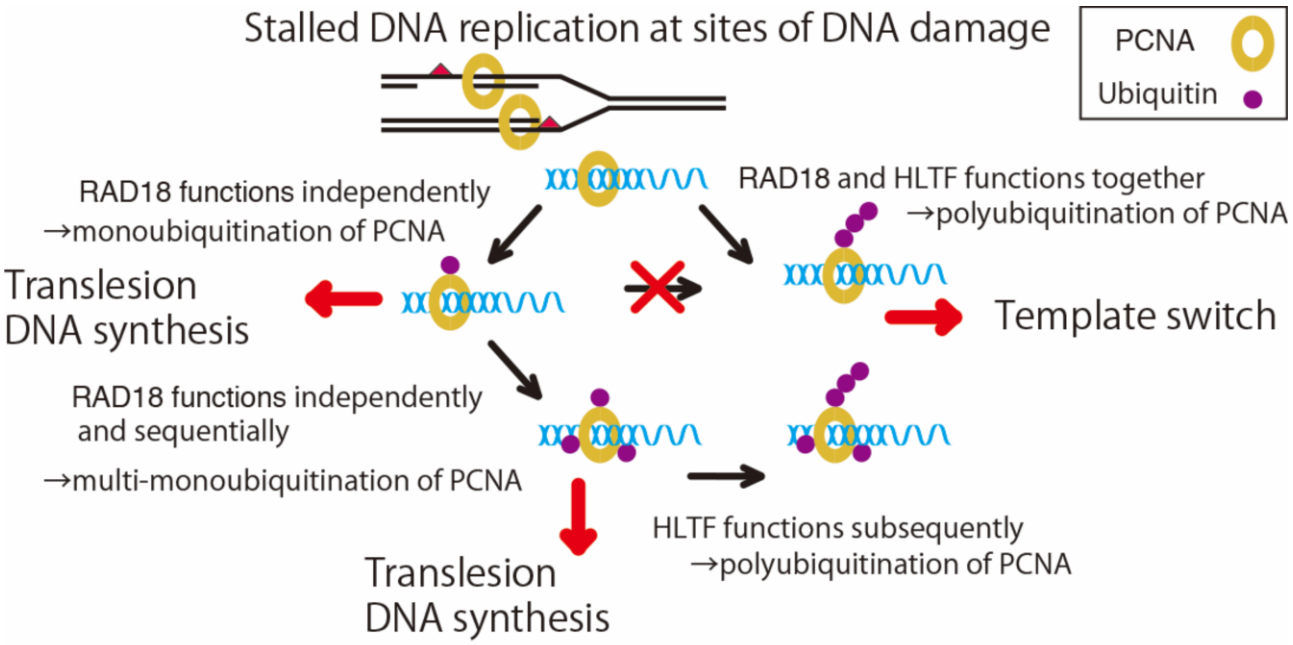

Figure 2 Regulation of pathway choice between DNA damage tolerance pathways

Publication

Regulation of HLTF-mediated PCNA polyubiquitination by RFC and PCNA monoubiquitination levels determines choice of damage tolerance pathway

Yuji Masuda[1,2], Satoshi Mitsuyuki[1,2], Rie Kanao[1,2], Asami Hishiki[3], Hiroshi Hashimoto[3], and Chikahide Masutani[1,2]

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

[2]Nagoya University Graduate School of Medicine.

[3]School of Pharmaceutical Sciences, University of Shizuoka.

Nucleic Acids Research, published online on October 18, 2018

DOI: 10.1093/nar/gky943

Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Nucleic_A_20181022.pdf