Summary of Doctoral Dissertation (Doctoral Program (Science))

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論文題名: **Elucidation of the DNA repair mechanisms involved in the repair of DNA damage caused by the Arabinosides and Anti-COVID-19 drugs**
邦題: アラビノシド系抗がん剤と抗 COVID-19 ウィルス治療薬によって引き起こされる DNA 損傷の修復機構の解明（英文）

Introduction

Nucleoside analogs are chemical compounds with structures similar to those of nucleosides and have been used as anti-viral and anti-cancer drugs. Usually, CTNAs interfere with the DNA replication of viruses or cancer cells via two different mechanisms; In the chain termination of replication, nucleoside analogs incorporated at the end of nascent DNA inhibit subsequent polymerization reactions. In the replication fork arrest on the damaged template, nucleoside analogs incorporated into the DNA strand serve as DNA damage and inhibit DNA replication. Nonetheless, significant amounts of antiviral CTNAs may be incorrectly inserted into the host's genomic DNA and thereby interfere with replication. Therefore, those should be efficiently removed for the faithful replication of the host's genomic DNA. In this study, we explored the DNA repair maechanism required for the cellular tolerance to Arabinosides and Anti COVID-19 drugs such as Remdesivir and Molnupiravir.

1: Proofreading exonuclease activity of replicative polymerase epsilon promotes cellular tolerance to arabinosides in CTF18 -dependent and -independent manner

Arabinosides are nucleoside analogs that contain arabinose sugar instead of ribose sugar. Ara-A, Ara-C, Ara-G, and Ara-T are arabinose sugars combined with adenine, cytosine, guanine, and thymine bases, respectively. Ara-C is used in the treatment of acute lymphocytic and myeloid leukemia, Ara-A is used to treat human herpes virus infections, Ara-G is effective against T cell malignancies, and Ara-T is used to treat herpes simplex virus infections Ara- C, an arabinoside, serves as a chain terminator of deoxyribonucleic acid (DNA) replication by interfering with replication after it is incorporated at the 3′end of nascent DNA, thereby restricting the proliferation of viruses and cancer cells. The incorporated Ara-CMP is efficiently removed by the proofreading exonuclease activity of polymerase epsilon (Polε), in which the alternative clamp loader CTF18 plays a pivotal role. However, the requirement of CTF18 for the removal of the other arabinosides from the 3′ end of nascent DNA remains unclear. Here, we explored DNA repair pathways responsible for the cellular tolerance to Ara-A and found that cells deficient in the proofreading exonuclease activity of Polε (*POLE1*$^{exo-/-}$) showed the highest

sensitivity to Ara-A. This activity was also required for cellular tolerance to Ara-G and Ara-T. *CTF18*$^{-/-}$ cells showed higher Ara-A sensitivity than wild-type cells, though it was critically lower than that of *POLE1*$^{exo-/-}$ cells. Similar trends were observed for the sensitivity to Ara-G and Ara-T. These results indicate that these arabinosides are removed by Polε proofreading exonuclease activity, and CTF18 is pivotal for Polε-mediated Ara-C removal but does not play critical roles for Polε-mediated removal of Ara-A, Ara-G, and Ara-T. In this study, we unveiled a difference between Ara-C and the other arabinosides (Ara-A, Ara-G, and Ara-T) in the removal from the 3′end of nascent DNA. This study opens a window for further studies with other arabinosides, in addition to Ara-C, for clinical application to develop an efficient anti-cancer drug.

2: The flap endonuclease-1 mediated maturation of Okazaki fragments is critical for the cellular tolerance to remdesivir

Remdesivir is a 1'-cyano-modified adenine nucleotide analog used for the treatment of COVID-19. This drug was initially developed to inhibit hepatitis C virus and is also effective against Ebola virus, Nipah virus, and Respiratory Syncytial virus infections. This drug inhibits viral RNA-dependent RNA polymerase (RdRp) enzymes and thereby restricts viral proliferation. Moreover, the anti-carcinogenic effect of remdesivir has been identified in human ovarian cancer cells, prostate cancer, hepatocellular carcinoma, and malignant melanoma cells. A prodrug of nucleoside analog, remdesivir is metabolized in cells directly to the remdesivir monophosphate (RMP), bypassing the rate-limiting first phosphorylation step by a series of reactions Recently, the cytotoxicity pathways upon Remdesivir treatment has been elucidated through genome-wide CRISPR-Cas9 screening and transcriptomics, but the mechanisms responsible for the resistance to remdesivir have not been elucidated. Here, we explored DNA repair pathways responsible for the cellular tolerance to remdesivir by monitoring the sensitivity of 24 mutant DT40 cells deficient in various DNA repair pathways. We found that cells deficient in FEN1 displayed the highest sensitivity against remdesivir. Since FEN1 contributes to base excision repair (BER), we measured the cellular sensitivity to remdesivir in mutants deficient in BER and found that other BER mutants are tolerant to remdesivir, indicating that FEN1 contributes to cellular tolerance to remdesivir through roles other than BER. We observed augmented remdesivir-induced DNA damage and acute cell cycle arrest at early S-phase after incorporating remdesivir in the nascent DNA in *FEN1*$^{-/-}$ cells. Moreover, the replication fork progression was significantly slowed by remdesivir in *FEN1*$^{-/-}$ cells, indicating a direct involvement of FEN1 in replication fork progression when replication is challenged by remdesivir. Strikingly, *FEN1*$^{-/-}$ cells exhibited slowed Okazaki fragment maturation (OFM), and remdesivir incorporation critically impaired this process in *FEN1*$^{-/-}$ cells. Taken together, these results indicate that FEN1 plays a critical role in suppressing DNA damage upon remdesivir incorporation by promoting OFM. This work opens a window for further study in remdesivir regarding human cancer cells as well as animal models for efficient clinical

applications.

3: PARP1, TDP2, BRCA2 and ATM are required for the cellular tolerance to molnupiravir

Molnupiravir, a prodrug of the nucleoside analog N^4-hydroxycytidine (NHC) is used to treat COVID 19 although it was initially developed to treat influenza. Molnupiravir exerts its antiviral activity by causing copying errors during the replication of RNA viruses. It is converted into NHC triphosphate (NHC-TP), a ribonucleoside analog that mimics cytidine. Instead of using actual cytidine during replication, the virus's enzyme integrates NHC-TP into newly synthesized RNA. However, the removal mechanism of molnupiravir has not yet been explored. In this study, we investigated the DNA repair mechanisms that give rise to the cellular resistance to molnupiravir by observing the sensitivity of 24 mutant DT40 cells that lacked different DNA repair genes. We observed the sensitivity of *PARP1*$^{-/-}$, *TDP2*$^{-/-}$, *BRCA2*$^{-/-}$ and *ATM*$^{-/-}$ cells against molnupiravir. Since PARP1 plays a pivotal role in base excision repair (BER), we estimated the cellular sensitivity to remdesivir in mutants deficient in BER and found that other BER mutants are tolerant to remdesivir indicating that PARP1 contributes to cellular tolerance to remdesivir through roles other than BER. Moreover, we analyzed different mutants deficient in homologous recombination (HR) because of the involvement of BRCA2 in HR and found that *XRCC2*$^{-/-}$ and *XRCC3*$^{-/-}$ cells showed increased sensitivity to molnupiravir compared with the wild-type cells whereas BRCA1 deficient cells displayed resistance to molnupiravir. This data suggests that BRCA2 contributes to HR independently of BRCA1 in the cellular tolerance to molnupiravir. Furthermore, we identified that only TDP2 but not TDP1 participate in the removal of molnupiravir. This study promotes further study regarding molnupiravir in different cancer cell lines as well as in animal models to clinically establish it for the treatment of cancer.

In this study, we revealed the removal mechanisms for nucleoside anlogs, arabinosides, remdesivir, and molnupiravir. We found that the proofreading exonuclease activity of Polε is the key factor for the removal of all arabinosides, whereas the degree of requirement of CTF18 for the Polε-mediated removal of arabinosides was different in Ara-C and other arabinosides. CTF18 is essential for Ara-C removal. However, this factor plays a minor role in the removal of Ara-A and Ara-G, and Polε excises Ara-T independent of CTF18. Moreover, We identified FEN1 as the essential factor for cellular tolerance to remdesivir and revealed that RMP incorporated in the lagging strand during DNA replication is removed by FEN1 for efficient OFM. Furthermore, we identified PARP1, BRCA2, TDP2 and ATM as important factors for the removal of molnupiravir incorporated in genomic DNA.