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論　文　内　容　の　要　旨

氏　名　（　SIJIA LIU　）	
論文題名	Analysis of Fanconi anemia (FA) associated RAD51 mutation to reveal the role of RAD51 in FA pathway which maintains genome integrity （ファンコニ貧血原因遺伝子変異がもたらすRAD51蛋白質の機能異常とFA経路における働きの解析）

論文内容の要旨

Fanconi anemia (FA) is a human hereditary disease with a defect in the repair process of DNA interstrand crosslink (ICL), which potentially blocks DNA replication and leads to chromosomal instability and a strong predisposition to cancer. FA is a heterogeneous disease with 23 FA-associated genes reported so far. The current model of FA pathway for ICL repair includes a nucleolytic incision step to remove ICL within DNA strands, resulting in DNA double-strand break (DSB) formation, which subsequently restored by homologous recombination (HR). HR is a central process to repair DNA double strand breaks called DSBs. DSB is the most dangerous DNA damage among many types of DNA damages. If DSB can not be repair properly, it will cause genomic instability, which potentially leads to cell death. In human cell, during mitosis, DSBs are accidently formed by exogeneous DNA damages, such as irradiation, active oxygen and so on. During HR process, RAD51 forms a nucleoprotein filament on the DNA, which is facilitated by RAD51 mediators, such as BRCA2. This process is also negatively regulated by anti-recombinases, including FIGNL1. Once RAD51 forms a nucleoprotein filament on ssDNA, it works as a key recombinase; it can search for and invade into a homologous double-stranded DNA (dsDNA), and carried out the strand exchange, which is the central process of HR. Human RAD51 has four unique motifs: Walker A and B motifs for ATP binding and hydrolysis, and L1 and L2 loop for DNA binding. When RAD51 binds to the DNA, RAD51 protomers interact with each other and form a nucleoprotein filament on the DNA. Between two RAD51 protomers, an ATP binding site exists where ATP binds, which serves as a glue to connect two RAD51 protomers. Therefore, ATP binding can stabilize the RAD51 nucleoprotein filament, while ATP hydrolysis will stimulate RAD51 to dissociate from the DNA. In addition, proper ATP binding is essential to induce the structural change of RAD51 nucleoprotein filament. When the structural change of RAD51 is properly induced, RAD51 filament stretches itself while bindng the DNAinside the filament, so DNA is also streached to generate more space for subsequent HR process. Recently, RAD51 was identified as an FA-associated gene. So far, four types of heterozygous *RAD51* mutations are reported to cause an FA-like phenotype. Initial hypothsis about RAD51's function in FA pathway is that it may work in later part of FA pathway as a recombinase fpr carried out HR process. However, *in vivo* analysis revealed that patient cells with a FA-associated *RAD51* mutation showed hypersensitivity to DNA crosslinking agents but remained HR activity, which cannot be explained by the initial hypothesis. To elucidate the mechanism how FA-RAD51 mutations leads to FA, two FA-associated RAD51 mutant proteins, RAD51-T131P and RAD51-A293T, were biochemically analyzed in detail. It was hypothesized

that these mutations resulted in defects in the protection of stalled replication fork, which is caused by their unstable nucleoprotein filament upon defective ATP binding and hydrolysis. However, the special function of RAD51 in FA pathway distinct from that in HR is still not fully clarified.

Third *de novo* FA-associated *RAD51* mutation, *RAD51-Q242R* was recently identified, but resulting mutant protein has not been characterized in detail yet. Q242 is highly conserve among species and it locates nearby L1 loop, which is the DNA binding site. Interestingly, Q242 locates the opposite site of the protein compared to other FA-associated RAD51 mutations. Therefore, I speculated that *RAD51-Q242R* might have a different mechanism to cause FA symptoms from other FA-RAD51 mutations. To study how RAD51 works in FA pathway, I purified wild-type RAD51 and RAD51-Q242R proteins and investigated what defect by *RAD51-Q242R* mutation causes on RAD51's function. By comparing RAD51-Q242R protein's defect with other FA-RAD51 mutant proteins, I wanted to know if the mechanism causing FA is the same or not in *RAD51-Q242R*. To achieve it, biochemical analysis of RAD51-Q242R protein was carried out. As a result, RAD51-Q242R retains ATP hydrolysis and DNA binding activities comparable to those of wild-type protein. However, the addition of single-stranded DNA (ssDNA), which is known to stimulate RAD51's ATPase activity, did not stimulate RAD51-Q242R's ATPase activity. Similarly, the prevention of ATP hydrolysis, which is known to stabilize RAD51's nucleoprotein filament on ssDNA, weakly stabilized RAD51-Q242R-DNA complex compared to wild-type RAD51. These results suggest that RAD51-Q242R has an impaired DNA-dependent ATPase activity, which may disturbs the coordination between it's DNA binding and ATPase activity. In addition, I found that RAD51-Q242R could protect dsDNA from MRN nuclease as well as wild-type RAD51 does. Therefore, RAD51-Q242R maybe proficient in the fork protection process when the replication fork stalls, which is different from other FA-associated RAD51 mutations. On the other hand, unlike wild-type RAD51, I found that RAD51-Q242R quickly dissociates from ssDNA in the presence of competitor dsDNA, which potentially causes a disability of the nucleoprotein filament to form the key intermediate of HR, D-loop. Interestingly, although RAD51-Q242R cannot form D-loop by itself, RAD51-Q242R retains the ability to help wild-type RAD51 to form D-loop, suggesting that a mixed filament with both wild-type RAD51 and RAD51-Q242R proteins might be formed *in vivo*. Combine with all these results, I speculated that RAD51-Q242R may be proficient in both HR and the fork protection process. If so, RAD51-Q242R should have an another defect which leads to FA symptoms. Since RAD51-Q242R showed the defective ATP-dependent RAD51 dissociation from ssDNA in my nuclease protection assay's result, I wonder if this defective dissociation could be a possible defect of RAD51-Q242R causing FA. To elucidate why RAD51-Q242R has the dissociation problem, both RAD51 dismantle assay and topology assay were carried out. These findings may help us to understand the molecular mechanism of how *RAD51-Q242R* causes a defect in FA pathway but is still proficient in HR in FA patient cells. Besides, how Q242R substitution affects the function of RAD51 maybe elucidated, which help us to better understand the function of RAD51 in structural level.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

ヒトゲノムDNAは様々な内的・外的要因によって恒常的に損傷されており、その修復機構の異常は種々の疾患の原因となる。Fanconi anemia（Fanconi貧血）はヒト遺伝性疾患であり、染色体の不安定性を誘導し早期の発癌や神経系異常などの重篤な症状を患者に引き起こす。これまでにFanconi anemiaの原因遺伝子として23個の遺伝子が報告されているが、その中に相同組換えで重要な働きを行う *RAD51* がある。RAD51はATPase活性を持つDNA結合蛋白質であり、一本鎖DNAに結合して相同鎖検索や鎖交換反応を促進するリコンビナーゼとしての酵素活性を持つことが知られている。またRAD51は停止した複製フォークの保護に働くなど、DNA複製期に相同組換え以外の役割をもつことも報告されている。Fanconi anemia関連 *RAD51* 変異では、特にこの複製フォーク保護能の異常が染色体不安定性に関連していると考えられてきた。

Liu Sijia氏は近年新たにFanconi anemiaに関連するとして報告があった *RAD51-Q242R* 変異の遺伝子産物であるRAD51-Q242R蛋白質の機能を解析し、野生型RAD51と比較した。その結果、(1) RAD51-Q242RはDNA結合能やATPase活性を維持していること、(2) 野生型RAD51で見られるATP加水分解によって誘導されるDNAからの解離、およびDNA依存的ATPase活性に異常があること、(3) 相同組換えに必須のD-loop形成能を失っていることを明らかにした。RAD51-Q242Rがリコンビナーゼとしての機能を失っていることが示されたが、その一方、RAD51-Q242Rの添加により野生型RAD51のD-loop形成が促進することを見出した。これらの結果から、RAD51-Q242RはDNAに結合し野生型RAD51と協調して相同組換えで働くことが示唆された。またLiu Sijia氏はRAD51-Q242RがDNAをヌクレアーゼから保護する機能を維持していることを見出し、RAD51-Q242R変異におけるFanconi anemiaの発症機構が従来の定説である複製フォーク保護能の異常以外でも引き起こされる可能性を見出した（Liu et al., JBC, 2023)。

Liu Sijia氏は多くの実験に基づき、*RAD51-Q242R* 変異がRAD51の機能に与える影響を明らかにする結果を得た。これらは将来的にFanconi anemiaの病態の理解へとつながる重要な知見であると考えられる。よって本博士論文内容を学位論文として合格と判定する。

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