

Title	Analysis of Fanconi anemia (FA) associated RAD51 mutation to reveal the role of RAD51 in FA pathway which maintains genome integrity
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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coni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	
<p>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 accidentally 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 FIGL1. 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 binding the DNA inside the filament, so DNA is also stretched to generate more space for subsequent HR process. Recently, RAD51 was identified as an FA-associated gene. So far, four types of heterozygous <i>RAD51</i> mutations are reported to cause an FA-like phenotype. Initial hypothesis about RAD51's function in FA pathway is that it may work in later part of FA pathway as a recombinase for carried out HR process. However, <i>in vivo</i> analysis revealed that patient cells with a FA-associated <i>RAD51</i> 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</p>	

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.

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。

筆頭著者論文: Liu, S., Shinohara, A., and Furukohri, A. A Fanconi anemia-associated mutation in *RAD51* compromises the coupling of DNA binding and ATPase activities. *J. Biol. Chem.* 299. 105424. 2023. Online, DOI: [10.1016/j.jbc.2023.105424](https://doi.org/10.1016/j.jbc.2023.105424)