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論文題目	BRCA1 and CtIP Are Both Required to Recruit Dna2 at Double-Strand Breaks in Homologous Recombination （BRCA1 と CtIP は、相同組換えにおいて DNA2 重鎖末端に DNA2 を呼び込むのに必要である）		

（論文内容の要旨）

Homologous recombination plays a key role in the repair of double-strand breaks (DSBs), and thereby significantly contributes to cellular tolerance to radiotherapy and some chemo- therapy. DSB repair by homologous recombination is initiated by 5' to 3' strand resection (DSB resection), with nucleases generating the 3' single-strand DNA (3'ssDNA) at DSB sites. Genetic studies of Saccharomyces cerevisiae demonstrate a two-step DSB resection, wherein CtIP and Mre11 nucleases carry out short-range DSB resection followed by long- range DSB resection done by Dna2 and Exo1 nucleases. Chicken DT40 DNA2$^{-/-}$ as well as DNA2$^{-/D245A}$ (nuclease-dead) cells were conditionally generated using a chicken DNA2 transgene under control of tetracycline-repressible promoter. Measurement of Rad51 focus formation allowed us to find that the inactivation of nuclease activity associated with Dna2 completely inhibited DSB resection, as did the depletion of CtIP in both human cells and chicken DT40 cells [Sartori AA et al., Nature, 2007, Peng G et al., Cancer Research, 2012].

Recent studies indicate that CtIP contributes to DSB resection through its non-catalytic role but not as a nuclease. However, it remains elusive how CtIP contributes to DSB resection. To explore the non-catalytic role, an immuno-cytochemical method was developed for detecting ionizing radiation-induced Dna2-subnuclear-focus formation at DSB sites to examine the dynamics of Dna2 in chicken DT40 and human cell lines. Ionizing radiation induced Dna2 foci only in wild-type cells, but not in Dna2 depleted cells, with the number of foci reaching its maximum at 30 minutes and being hardly detectable at 120 minutes after IR. Induced foci were detectable in cells in the G2 phase but not in the G1 phase. These observations suggest that Dna2 foci represent the recruitment of Dna2 to DSB sites for DSB resection. Importantly, the depletion of CtIP inhibited the recruitment of Dna2 to DSB sites in both human cells and chicken DT40 cells. These observations suggest a previously unappreciated interdependency between CtIP and Dna2, where CtIP recruits Dna2 to DSB sites and the nuclease activity of Dna2 is responsible for DSB resection. Likewise, a defect in breast cancer 1 (BRCA1), which physically interacts with CtIP and contributes to DSB resection, also inhibited the recruitment of Dna2 whereas BRCA1$^{-/-}$/53BP1$^{-/-}$ cells displayed nearly normal Dna2 foci. Moreover, CtIP physically associates with Dna2, and the association is enhanced by ionizing radiation. In summary, BRCA1 and CtIP contribute to DSB resection by recruiting Dna2 to damage sites, thus ensuring the robust DSB resection necessary for efficient homologous recombination.

（論文審査の結果の要旨）

本論文では、ヒト細胞、ニワトリ DT40 細胞を用いて、DNA ヌクレアーゼ Dna2 蛋白質の機能解析を通して、DSB 末端削り込みの分子メカニズム解明を行っている。その結果次のことが明らかになった。

1. ニワトリ DNA2 欠損細胞は致死であること
2. ヒト DNA2(siRNA による)、ニワトリ DNA2 欠損によって、Rad51 蛋白質の局在が低下すること
3. Dna2 と CtIP の作用点が Rad51 Foci 形成において同一経路上に存在すること
4. Dna2 Foci 形成が CtIP や BRCA1 蛋白質に依存すること

これらの結果より、DSB 形成後、BRCA1 蛋白質が CtIP とともに DSB 末端へ結合したのち、その両者の結合に依存して Dna2 が結合すること、また DSB 末端に結合した Dna2 はヌクレアーゼとして DSB 末端の 5'→3'へ削り込みを行うと考えられる。Dna2 による DSB 末端の削り込みは、Rad51 蛋白質が相同鎖検索反応を行うのに十分な一本鎖 DNA を提供すると考えられる。

以上の研究は、DNA ヌクレアーゼである DNA2 蛋白質の機能の解明に貢献し、相同組換えによる DNA 二重鎖切断修復の研究に寄与するところが多い。

したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、平成 27 年 11 月 26 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降