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PLK1 is a Novel Partner for DNA Endonuclease CtIP

Dear Editor,

DNA double-strand breaks caused by ionizing radiation are the most severe DNA damage types. If not being timely or correctly repaired, cells will undergo apoptosis or gene mutation and subsequent genomic instability[1-2]. For double-strand breaks, there are two main repair pathway in cells, namely homologous recombination and non-homologous end-joining [3-4]. Homologous recombination repair utilizes homologous fragments of sister chromosomes, so that damaged DNA can be repaired more precisely. Homologous recombination repair mainly acts during the S/G2 phase of the cell cycle.

A large number of proteins are involved in the HR repair process, in which CtIP is the core protein. CtIP plays its endonuclease activity coupling with BRCA1 and MRN complex in the cleavage of DNA ends to facilitate the end resection completion of homologous recombination repair[5-8]. And mutations in CtIP are associated with Seckel and Jawad syndrome[9] as well as endometrial, colorectal, breast, ovarian, and myeloid cancers. Though CtIP's role as an endonuclease in HR repair has been well established, it is still not clear how it coordinates with other proteins in HR. Therefore, we attempted to investigate the novel partners for CtIP.

To better understand the molecular networks of CtIP, CtIP interaction proteins were predicted with online tool PrePPI (http://bhapp.c2b2.columbia.edu/PrePPI). PrePPI utilizes the Bayesian framework to predict protein interactions, which integrates structural, functional, evolutionary and expression information [10]. By searching for the CtIP interaction proteins in the PrePPI database, we obtained a list of potential or verified CtIP interaction proteins (Figure 1a). BRCA1, a well-documented CtIP-interacting protein was on the top of the list. We also noted that the PLK1 was the novel CtIP interaction protein.

Immunoprecipitation approach was used to validate the interaction between CtIP and PLK1. Strong interactions between these two proteins were observed in both immunoprecipitation sides (Figure 1b and 1c). In order to show which part of PLK1 mediates its interaction with CtIP, we used different truncations of PLK1, which were named as PBD (polo-box domain), KD (kinase domain), middle domain as well as the PLK1 shRNA plasmid as negative control. The results of immunoprecipitation showed that both the KD and middle truncations had a strong interaction with CtIP, suggesting that this part plays an important role in mediating interaction between the two proteins (Figure 1d).

Furthermore, Frodock 2.0 was used to dock the interaction mode between these two proteins[11-12]. The PDB structure of full length PLK1, aa 93–293 of PLK1, aa 407–508 of PLK1 and aa 495–603 of PLK1 and PDB structural file of CtIP were obtained from the PrePPI database. The structure docking results suggested that PLK1 protein may act on the N-terminus of CtIP (Figure 1e). The N-terminus of CtIP contains a domain that mediates protein-protein interactions (pfam03962) and is implicated to be involved in promote homologous chromosome pairing and meiotic double-strand break repair. To validate the docking results, we confirmed that the N-terminus of CtIP is important in mediating the interaction between CtIP and PLK1 by immunoprecipitation with different deletion mutants of CtIP (Figure 1f).

To test if the interaction was related to DNA damage, we performed immunoprecipitation assay in IR treated cells and found that the PLK1-CtIP interaction increased slightly (Figure 2a). Meanwhile, we used immunofluorescence staining to detect the subcellular localization of PLK1 and CtIP proteins. After ionizing radiation, CtIP formed discrete foci in the nucleus, which was consistent with its reported role

(a)

Interaction proteins	Gene name	Prediction score
P06400	RB1	34903.8
P28749	RBL1	7261.2
P53350	PLK1	1796.9
P38398	BRCA1	1730.6
O60934	NBN	1710.6
Q99728	BARD1	1437.1
Q13315	ATM	860.3
P12004	PCNA	441.2
Q00987	MDM2	1517.8

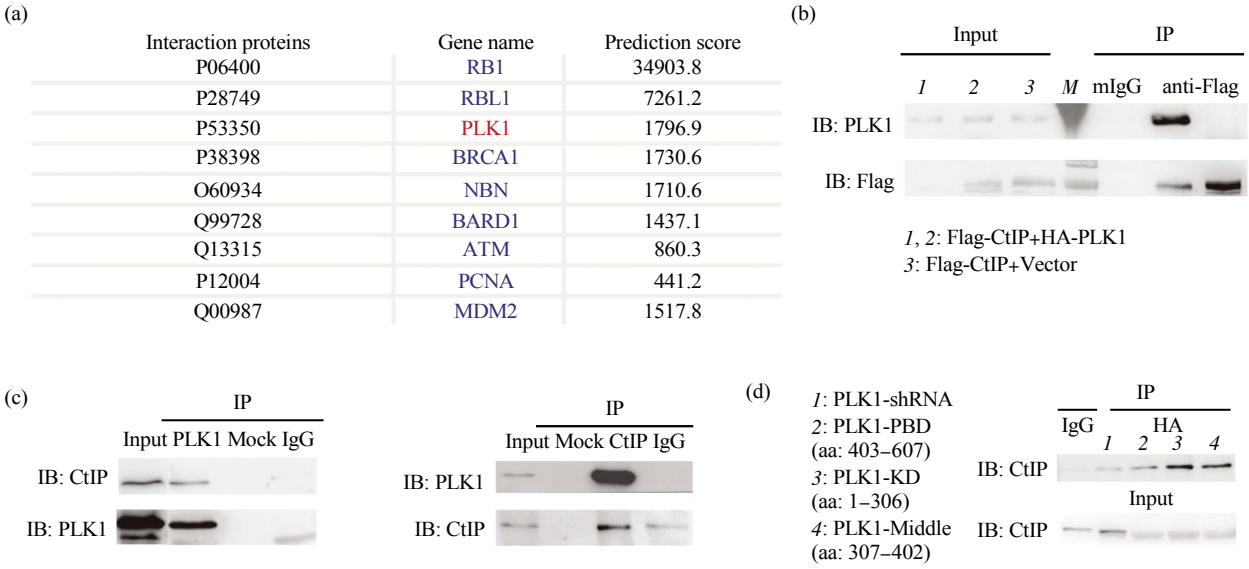

Fig. 1　Prediction and validation of interaction between CtIP and PLK1

(a) Prediction of CtIP interaction proteins. (b) Flag-CtIP interacts with PLK1. (c) Endogenous CtIP interacts with PLK1. (d) Interactions between different truncation mutations of PLK1 and CtIP. (e) Protein docking models for CtIP-PLK1 interaction. (f) Immunoprecipitation to detect the interaction between CtIP deletion mutants and PLK1.

in DNA damage repair. Interestingly we observed PLK1 also formed some discrete foci in the nucleus and showed partial co-localization with CtIP. Furthermore, we used Triton X-100 pretreatment to get rid of the background of cytoplasmic fraction, and the partial co-localization can still be clearly observed (Figure 2b). All the above results suggest that these two proteins are not only functionally related in the DNA damage response but also in other biological processes.

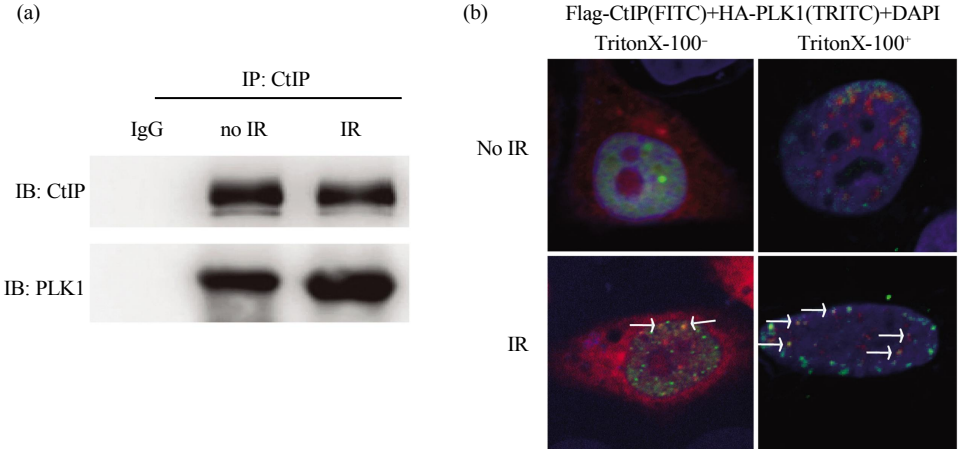

Fig. 2 Interaction and subcellular localization of PLK1 and CtIP after DNA damage

(a) Immunoprecipitation assay to detect the interaction between PLK1 and CtIP after 10 Gy IR treatment. (b) Immnunofluorescence staining with or without Triton X-100 pretreatment to check the subcellular co-localization of PLK1 and CtIP after DNA damage.

PLK1 is the member of the serine protein kinase family and plays an important role in the DNA damage process [13−14]. It recognizes or phosphorylates proteins that have been phosphorylated at a particular site by the Polo box domain. Since PLK1-like protein PLK3 has been reported to phosphorylate CtIP in G1 phase, regulating non-homologous end-joining repair [15], we speculate that PLK1 also plays the similar role in regulation of CtIP.

Above all, our findings reported the novel interactions between CtIP and PLK1, which has important biological and clinical significance for a deeper understanding of homologous recombination repair, genomic instability and human diseases.

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