THE UNIVERSITY OF HONG KONG
SCHOOL OF BIOLOGICAL SCIENCES

Postgraduate Student Public Seminar

"Loss of ultrafine anaphase bridge-binding protein PICH results in cell cycle arrest, p53 activation, and DNA damage in normal human cells"

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Abstract

The even distribution of genetic material during a cell division cycle is vital in circumventing the various high mortality diseases associated with chromosome missegregation events. Emerging evidence has shown that the preservation of genomic integrity during mitosis is challenged by the presence of persistent DNA linkage structures known as ultrafine anaphase bridges (UFBs) that arise from errors in the DNA replication and repair processes. PLK1-interacting checkpoint helicase (PICH) is known for its unique capability in recognizing the UFBs while simultaneously recruiting other UFB-binding proteins, including Bloom's syndrome helicase (BLM), Topoisomerase III-alpha (TOP3A), RecQ-mediated genome instability protein complex 1 and 2 (RMI1/2) and Rap1-interacting factor 1 (RIF1), to aid in the subsequent resolution of the UFBs.

A previous study showed that PICH is essential for mouse embryonic development. To bypass the lethality of PICH knockout, the auxin-degron degradation system was employed to examine the functions of PICH on the cell cycle due to its ability to induce rapid protein degradation and recovery. Through short-homology mediated tagging of the *PICH* gene in the immortalized hTERT-RPE1 parental cell line, $RPE1^{PICH\text{-}mAID\text{-}Clover}$ cell line was generated, in which endogenous PICH is C-terminally tagged with a mini-auxin-inducible degron (mAID) and a GFP tag.

PICH depletion induced by auxin treatment increases the levels of two different DNA damage markers, 53BP1 foci and micronuclei formation, and also induces hypersensitivity to topoisomerase II (TOP2) inhibitor, ICRF-193. Lack of PICH-mediated UFB resolution also leads to a marked increase of both p53 and its downstream target p21, leading to the subsequent G_1 cell cycle arrest. To determine whether the phenotypes observed in PICH-depleted cells are mediated by the p53 pathway, another cell line, $RPE1^{PICH\text{-}mAID\text{-}Clover\ TP53\text{-}/\text{-}}$, was generated by CRISPR/Cas9 technology. Clonogenic survival assays performed on both cell lines demonstrate that loss of p53 rescues the loss of cell viability and cell cycle arrest.

Taken together, we propose that the DNA damage caused by a lack of PICH-mediated UFB resolution triggers G_1 cell cycle arrest in a p53 pathway-dependent manner.

--- ALL ARE WELCOME ---