

Project number 19

Proteomic pursuit of the causes for premature aging, Werner and Rothmund Thomson syndromes

[1] Research group

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Consumables 200,000 YEN

[2] Research setup

RECQL4 is a human RecQ helicase which is mutated in approximately two-thirds of individuals with Rothmund-Thomson syndrome (RTS), a disease characterized at the cellular level by chromosomal instability. BLM and WRN are also human RecQ helicases, which are mutated in Bloom and Werner's syndrome, respectively, and associated with chromosomal instability as well as premature aging. We showed previously that primary RTS and RECQL4 siRNA knockdown human fibroblasts accumulate more H₂O₂-induced DNA strand breaks than control cells, suggesting that RECQL4 may stimulate repair of H₂O₂-induced DNA damage. In order to understand the role of RECQL4 under oxidative stress condition, we performed proteome analysis of RECQL4 in response to the treatment of cells with hydrogen peroxide or with Arsenite.

[3] Research outcomes

(3 - 1) Results

GFP-tagged RECQL4 was expressed in U2OS cell with or without Arsenite treatment and the localization of the fusion protein was observed under microscope. Arsenite treatment made foci of GFP-RECQL4 within the nucleus (Fig. 1). In order to understand the nature of the foci,

protein-protein interaction of RECQL4 was analyzed in cells treated with either H₂O₂ or Arsenite. We expressed FLAG-tagged RECQL4 in 293 cells and immunoprecipitated the tagged protein with anti-FLAG antibody after treatment of the cells with H₂O₂ or with Arsenite and precipitants were compared by gel electrophoresis (Fig. 2). We found treatment-dependent specific interaction. However, interestingly, the interacting proteins in either treatment belong to structural proteins of similar type. Therefore, this type of interaction of RECQL4 may be one major response of the protein to oxidative stress.

Fig. 1

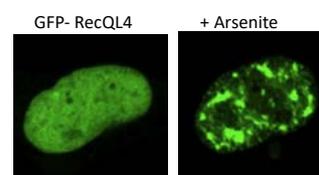
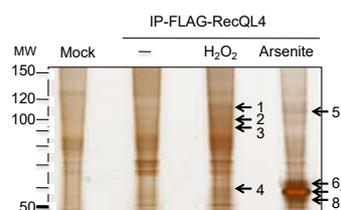


Fig. 2



(3 - 2) Future perspectives

Our results suggest a novel mechanisms of the stress response of RECQL4. Since cellular structural proteins have recently been shown to play important roles in replication and DNA damage response as well as DNA repair, our results may suggest how the mutation in RECQL4 gene contributes to cellular defect and possibly the phenotype of RTS.

[4] List of Papers None yet.