Project number　34

Oxidative stress response of RECQL4 at the crossroads of genomic instability and aging phenotype

［1］Research group

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Consumables 100,000 YEN

［2］Research setup

RECQL4, one of the five RecQ helicases in mammalian cells, is associated with premature aging and cancer prone syndromes . Mutations in human RECQL4 contribute to three autosomal recessive disorders: Rothmund–Thomson Syndrome (RTS), RAPADILINO syndrome and Baller–Gerold Syndrome . Skeletal abnormalities, growth retardation, and in case of RTS and RAPADILINO, predisposition to cancer, are the common clinical features of RECQL4 deficiency. Increased cellular senescence because of the accumulation of DNA damage was also observed in a mouse model of RTS deficient in RECQL4 . Numerous studies have shown that RECQL4 functions in multiple cellular processes, including DNA replication, non-homologous end joining (NHEJ) and homologous recombination (HR) as well as telomere and mitochondrial DNA maintenance.

［3］Research outcomes

While we have identified a number of proteins interacting with RECQL4 including SIRT1, DDB1 and recently OGG1 (1), here we wanted to know which proteins interact with RECQL4 in response to oxidative stress treatment of cell.

（3－1）Results

HEK293 cell stably expressing Flag-tagged RECQL4 (HEK293/RECQI4) was precipitated with anti-Flag antibody and resulting co-precipitated proteins were determined by MS/MS analysis. We identified more than 33 proteins including SIRT1 and DDB1 (Fig. 1).

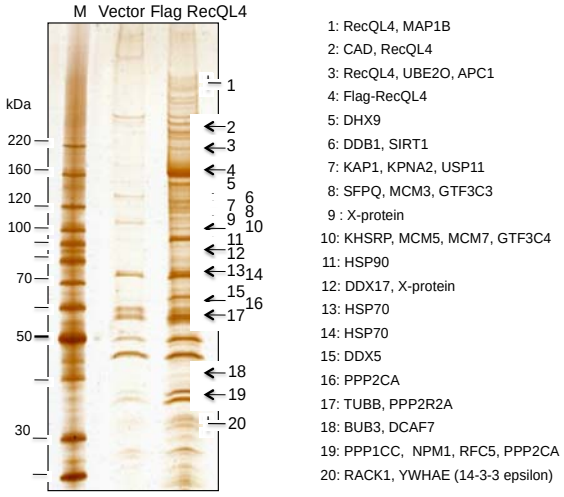

Fig.1 Immuno-precipitation of RECQL4 binding proteins determined by Mass-spectrometry

In order to identify proteins interacting with REXQL4 in response to oxidative stress, we treated HEK293/RECQL4 cell either with H_2O_2 or Arsenite and cell extracts were immune-precipitated with anti-Flag antibody. Precipitants were analyzed with

mass-spectrometry. The results were compared with those of cells obtained without the treatment (Fig. 2).

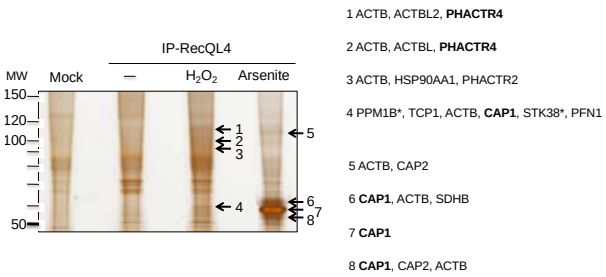

Fig. 2 Determination of proteins bound to RECQL4 after treatment of cells with either H2O2 or Arsenite.

The results showed that RECQL4 interact with PHACTR4 after treatment with H_2O_2, whereas CAP1 was a major interactant of RECQL4 after treatment with Arsenite. CAP1 is related to the S. *cerevisiae* CAP protein, which is involved in the cyclic AMP pathway. The human protein is able to interact with other molecules of the same protein, as well as with CAP2 and actin. PHACTR4 is a member of the phosphatase and actin regulator (PHACTR) family. Other PHACTR family members have been shown to inhibit protein phosphatase 1 (PP1) activity, and the homolog of this gene in the mouse has been shown to interact with actin and PP1. These data suggest that both CAP1 and PHACTR4 are actin-related proteins responding to oxidative stress.

（3－2）Future perspectives

Further analysis are in progress to understand the interaction between RECQL4 and actin in response to oxidative treatment of cell.

［4］List of Papers

none