課題番号（Project number）25

Mouse model for nucleolus instability leading to early aging: RACK1-APNX-PARP1 complex

［1］組織（Research group）
代表者（Principal Investigator (PI)）：
Gijbertus T.J. van der Horst
（Erasmus University Medical Center）
対応者（Host researcher at IDAC）：
Akira Yasui
（IDAC, Tohoku University）
分担者（Co-investigator）：
Shin-ichiro Kanno（IDAC, Tohoku University）

研究費（Expenditure report of research funds）：
物件費 200,000YEN，旅費 0 YEN

［2］研究経過（Research setup）

Human *APNX* gene has been isolated, but unpublished yet, by Shinichiro Kanno using bioinformatics of PARP1-binding DNA repair protein PALF (Kanno et al. *EMBO J.* 26, 2094-2103, 2007). By immune-precipitation experiments it turned out that APNX builds a heterodimer with PARP1 and knock down of APNX expression provided cells with sensitivity to MMS, suggesting its role in the response to oxidative DNA damage. PARP1 is activated by DNA single-strand break (SSB) or DNA double-strand break (DSB) and poly(ADP-ribosyl)ated itself and its surrounding proteins near the DNA damage. Poly(ADP-ribosyl)ation acts as a signal for DNA damage and many DNA repair proteins as well as RNA-relared proteins accumulate at the poly(ADP-ribose). There are many proteins with RNA interacting domain designated as intrinsically disorder proteins (IDPs) with low complexity domains like Fus, EWS, TARF15 and RBM14. These proteins form foci, called as stress granule in response to oxidative stress and are believed to function as RNA maintenance and possibly in DNA repair. We have previously identified RACK1 protein as an interacting protein of RECQL4, mutations in *which* are associated with the autosomal recessive disease Rothmund-Thomson Syndrome, a disorder that has features of premature aging. RACK1 interacts with IDPs and forms stress granule in response to H_2O_2 treatment of cell. We here analyzed interaction between RACK1 and APNX, both of which respond to DNA damage and PARP1 dependent poly(ADP-ribosyl)ation.

Akira Yasui visited Erasmus University Medical Center Rotterdam twice in 2016, February and September, and we discussed this subject thoroughly.

Because of the preparation for AAALAC application, mouse model has not yet been created. Instead we have analyzed and identified the interaction between APNX and RACK1 shown below.

［3］成果（Research outcomes）

（3－1）研究成果（Results）

We have first characterized GFP-tagged RACK1 expressed in U2OS cell with laser micro-irradiation (Fig. 1). GFP-tagged RACK1 accumulated to laser-irradiated site and indicated its response to DNA damage. We then established a HEK293 cell line having integrated and constitutively expressing FLAG-tagged RACK1 gene. By immune-precipitation using FLAG antibody we pulled down TRACK1 interacting proteins (Fig. 2). We identified APNX interacting with RACK1 in oxidative stress-dependent manner. Thus, we identified RACK1, which contributes to DNA repair by interacting with DNA repair protein and endonuclease APNX in response to oxidative DNA damage.

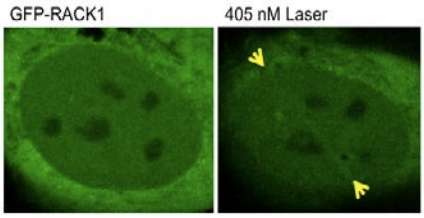

Figure 1. Accumulation of GFP-Tagged RACK1 at laser-irradiated site.

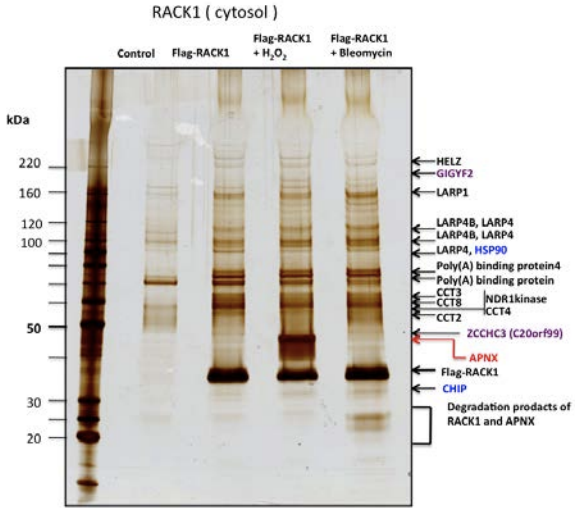

Figure 2. RACK1 interacting proteins i

（３－２）波及効果と発展性など（Future perspectives）

APNX and PARP1 are found in the nucleolus and interact to each other. RACK1, however, is present in the nucleus but not in the nucleolus. Therefore, in response to oxidative stress, RACK1 may build a complex with APNX and PARP1 enabling DNA damage repair. Since oxidative DNA damage plays the most important role in genome integrity, APNX-PARP1-RACK1 complex may be a core complex for anti-cancer and anti-aging.

［4］成果資料（List of Papers）

Manuscript is in preparation.