Project number　17

Characterization of a novel “DNA Maintenance Complex (DMC)” for genome integrity

［1］Research group

Principal Investigator (PI)：
Lene Juel Rasmussen
(University of Copenhagen)
Host researcher at IDAC：
Akira Yasui
(IDAC Tohoku University)
Co-investigator：
Shinichiro Kanno
(IDAC Tohoku University)

Expenditure report of research funds：
Consumables 150,000 YEN

［2］Research setup

The goal of the proposed study is to demonstrate that localized deoxyribonucleotide phosphates (dNTP) levels affects repair of nuclear DNA damage and thereby significantly decreases the risk of genomic instability and cellular senescence. Upon DNA damage there is an increased demand *de novo* synthesis of purine and pyrimidines. In case of situations where the cytosolic levels of pyrimidines and purines in the form of dNTPs are decreased and not induced upon DNA damage genomic instability will occur. We hypothesize that this decrease will prevent the formation of a proposed DNA maintenance complex (DMC) and in turn constrict the cells ability to perform nuclear DNA bypass repair. As a result, DNA damages will be sought salvaged by recombination repair, and the risk of chromosomal instability will increase.

Aim 1: To identify and characterize the DMC from human cells in respect to protein components, interaction with DNA and functionality at sites with DNA damage (Akaira Yasui, IDAC).

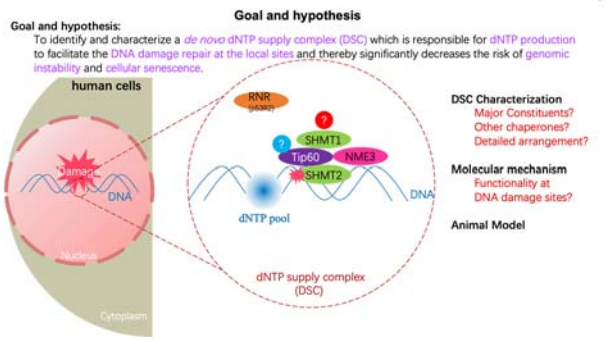
Aim 2: To demonstrate that human cells constricts synthesis of cellular dNTP, which in turn propagates a signal comparable to a cellular starvation signal, which prevents the formation of the DMC and as a result restricts the cells ability to perform DNA bypass repair (Lene Juel Rasmussen, UCPH).

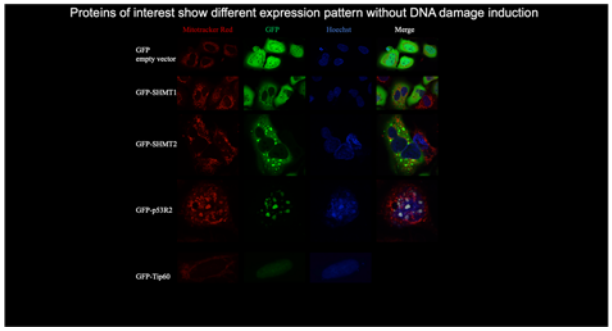
［3］Research outcomes

（3－1）Results

We have established a preliminary DMC containing REV1, SHMT1, SHMT2, and SKAR2. The preliminary results are based on proteomic analysis of proteins interacting with known DMC proteins such as Rev1. These are results from Dr Yasui’s group using affinity chromatography by using GST-tagged SHMT1 purified from insect cells and mass-spectrometry to determine interacting proteins. The results are very promising and the experimental strategy will be continued.

We have confirmed the co-localization of the proteins identified in the preliminary DMC complex using microscopy (Fig 1):





（３－２）Future perspectives

We will continue this approach identifying DMC proteins using proteins extracts from UV-irradiated cells.

［４］List of Papers

118. Drost M, Tiersma Y, Thompson BA, Spurdle AB, Frederiksen JH, Keijzers G, Pappas L, Boucher KM, Molenkamp S, Zonneveld JBM, van Asperen CJ, Sijmons RH, Goldgar DE, Rasmussen LJ†, Greenblatt MS†, de Wind N†, Tavtigian SV†. 2018. An Integrated, Functional Assay-Based Procedure to Classify Mismatch Repair Gene Variants in Lynch Syndrome. Genetics in Med. doi: 10.1038/s41436-018-0372-2.
†Co-corresponding author

117. Hasan-Olive MM, Lauritzen KH, Rasmussen LJ, Storm-Mathisen J, Bergersen LH. 2018. A ketogenic diet improves mitochondrial biogenesis and bioenergetics via the PGC1α-SIRT3-UCP2 axis. Neurochem Res. Jul 19. doi: 10.1007/s11064-018-2588-6.

116. Jakobsen E, Lange SC, Andersen JV, Desler C, Kihl H, Hohnholt MC, Stridh MH, Rasmussen LJ, Waagepetersen HS, Bak L. 2018. The inhibitors of soluble adenylate cyclase 2-OHE, KH7, and bithionol compromise mitochondrial ATP production by distinct mechanisms. Biochem Pharmacol. Jun 22;155:92-101. doi: 10.1016/j.bcp.2018.06.023.