課題番号（Project number） 58

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

［1］組織（Research group）

代表者（Principal Investigator）：
Lene Juel Rasmussen
(University of Vopenhagen)
対応者（Host researcher at IDAC）：
Akira Yasui
(IDAC, Tohoku University)

研究費（Expenditure report of research funds）：
consumable goods 100,000 Yen

［2］研究経過（Research setup）

The goal of the proposed study is to demonstrate that mitochondrial dysfunction affects nuclear DNA damage responses and thereby significantly increases the risk of genomic instability and cellular senescence.

Mitochondria houses rate-limiting enzymes essential for *de novo* synthesis of pyrimidines. In case of dysfunction of the mitochondrial respiration, the cytosolic levels of especially pyrimidines, but also purines in the form of deoxyribonucleotide phosphates (dNTPs) are decreased. 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.

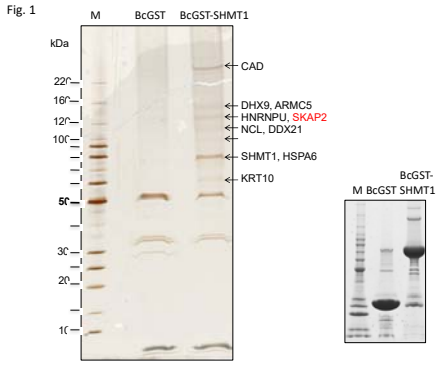
Aim 1: To identify and characterize the DMC from human cells in respect to protein components, interaction with DNA and functionality at sites with DNA damage (Akaira Yasui, IDAC).

Aim 2: To demonstrate that dysfunction of the mitochondrial respiration, in 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］成果（Research outcomes）

（3－1）研究成果（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. Fig. 1 depicts the results of an 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.



（3－2）波及効果と発展性など（Future perspectives）

If mitochondrial dysfunction is linked to dNTP anabolism via the DMC, it could have implications for understanding many aspects of human aging and age-associated human disease. The results of the proposed studies will also contribute to basic understanding of DNA repair, carcinogenesis, nucleotide metabolism, and mitochondrial dysfunction. In the long term, the proposed studies could also stimulate development of novel diagnostic and therapeutic agents for treating human disease.

［4］成果資料（List of Papers）

1. Fakouri NB, Durhuus JA, Regnell CE, Angleys M, Desler C, Hasan- Olive MDM, Martín-Pardillos A, Tsaalbi-Shtylik A, Thomsen K, Lauritzen M, Bohr VA, de Wind N, Bergersen LH, **Rasmussen LJ**. **2017**. Rev1 contributes to proper mitochondrial function via the PARP-NAD^+-SIRT1-PGC1α axis. **Sci Rep.** Oct 2;7(1):12480. doi: 10.1038/s41598-017-12662-3.

2. Martín-Pardillos A, Tsaalbi-Shtylik A, Chen S, Lazare S, van Os RP, Dethmers-Ausema A, Fakouri NB, Bosshard M, Aprigliano R, van Loon B, Salvatori DCF, Hashimoto K, Dingemanse-van der Spek C, Moriya M, **Rasmussen LJ**, de Haan G, Raaijmakers MHG, de Wind N. **2017**. Genomic and functional integrity of the hematopoietic system requires tolerance of oxidative DNA lesions. **Blood**. Sep 28;130(13):1523-1534. doi: 10.1182/blood-2017-01-764274.

3. Thomsen K, Sherazi N, Yokota T, Hasan-Olive M, Fakouri NB, Desler C, Regnell C, **Rasmussen LJ**, Dela F, Bergersen LH, Lauritzen M. Initial brain aging: heterogeneity of mitochondrial size is associated with respiratory dysfunction in cortex and hippocampus. **2017**. **Neurobiol Aging**. Aug 12. pii: S0197-4580(17)30259-2.
doi: 10.1016/j.neurobiolaging.2017.08.004.

4. Strickertsson J, Desler C, **Rasmussen LJ**. **2017**. Bacterial infection increases risk of carcinogenesis by targeting mitochondria. **Semin Cancer Biol**. Jul 25. pii: S1044-579X(17)30192-X.
doi: 10.1016/j.semcancer.2017.07.003.

5. Liu D, Keijzers G, **Rasmussen LJ**. **2017**. DNA mismatch repair and its many roles in eukaryotic cells. **Mutat Res Rev**. 773:174-187.
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6. Liu D, Frederiksen JH, Liberti SE, Lützen A, Keijzers G, Pena-Diaz J, **Rasmussen LJ**. **2017**. Human DNA polymerase delta double-mutant D316A E318A interferes with DNA mismatch repair *in vitro*. **Nucleic Acids Res**. Sep 19; 45(16):9427-9440. doi: 10.1093/nar/gkx611.

7. Rudolph C, Nielsen JE, Jensen KV, Liu D, Pena-Diaz J, Rajpert-De Meyts E, **Rasmussen LJ**, Jørgensen A. **2017**. Involvement of the DNA mismatch repair system in the cisplatin sensitivity of testicular germ cell tumours. **Cell Oncol**. 40(4):341-355. doi: 10.1007/s13402-017-0326-8.

8. Nielsen SV, Stein A, Dinitzen AB, Papaleo E, Tatham MH, Poulsen EG, Kassem MM, **Rasmussen LJ**, Lindorff-Larsen K, Hartmann-Petersen R. **2017**. Predicting the impact of Lynch syndrome-causing missense mutations from structural calculations. **PLOS Genet**. 13(4):e1006739.
doi: 10.1371/journal.pgen.1006739. eCollection 2017 Apr.

9. Vohra R, Guruban IS, Henriksen U, Bergersen LH, **Rasmussen LJ**, Desler C, Skytt DM, Kolko M. **2017**. Disturbed mitochondrial function restricts glutamate uptake in the human Müller glia cell line, MIO-M1. **Mitochondrion**. S1567-7249 (17) 30035-1.

10. Desler C, Lillenes MS, Tønjum T, **Rasmussen LJ**. **2017**. The role of mitochondrial oxidative phosphorylation in Alzheimer's disease. **Curr Med Chem**. doi: 10.2174/0929867324666170616110111.

11. Hopkinson BM, Desler C, Kalisz M, Vestentoft PS, **Rasmussen LJ**, Bisgaard HC. **2017**. Bioenergetic Changes during Differentiation of Human Embryonic Stem Cells along the Hepatic Lineage. **Oxid Med Cell Longev**. 2017;2017:5080128. doi: 10.1155/2017/5080128.

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