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Abstract S1.6

DCTPP1 preserves genomic integrity through the modulation of dCTP and dTTP homeostasis

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Objectives: The aim of this work was to gain insight into the role of DCTPP1 in the homeostasis of the dNTP pool and its implication in the preservation of genome integrity.

Methods: We investigated the metabolic function of DCTPP1 by using two different reverse genetics approaches: transiently down-regulating DCTPP1 expression by small interference RNA and using a stable DCTPP1-knockout cell line. The dNTP pool was determined by a DNA polymerase-based assay. Uracil content in the genome was quantified by a qPCR. Phosphorylation of histone H2AX was assessed by immunostaining and cells showing 5 or more foci γH2AX were considered positive. The spontaneous mutation frequency was determined using the gene hypoxanthine phosphoribosyltransferase gene (HPRT) as a marker for nuclear mutagenesis. Resistance to chloramphenicol as a result of mutations in the 16S rRNA gene was used to assess mitochondrial mutagenesis.

Results: The nucleotide pool and the dUTP/dTTP ratio are severely altered in DCTPP1-deficient cells, which exhibit an accumulation of uracil in genomic DNA, an activation of the DNA damage response and a hypermutator phenotype affecting both nuclear and mitochondrial genomes. Notably, DNA damage can be reverted by incubation with thymidine or dUTPase overexpression. Moreover, DCTPP1-deficient cells are highly sensitive to down-regulation of nucleoside salvage.

Conclusions: Our results shed new light on the mechanisms that regulate dNTP homeostasis. We have identified DCTPP1 as a crucial element in de novo synthesis of pyrimidines, providing a novel pathway for the conversion of dCTP into dTTP. In addition we have establshed that a defect in DCTPP1 causes disturbed nucleotide pools, replication stress and genomic instability.

Relevant references:

- Requena, C.E., Perez-Moreno, G., Ruiz-Perez, L.M., Vidal, A.E., Gonzalez-Pacanowska, D. (2014). “The NTP pyrophosphatase DCTPP1 contributes to the homoeostasis and cleansing of the dNTP pool in human cells”. Biochem J **459**: 171-180
- Requena, C.E., Perez-Moreno, G., Horvath, A., Vertessy, B.G., Ruiz-Perez, L.M., Gonzalez-Pacanowska, D., Vidal, A.E., (2016). “The nucleotidohydrolases DCTPP1 and dUTPase are involved in the cellular response to decitabine.” Biochem J **473**: 2635-2643
- Llona-Minguez, S., Hoglund, A., Jacques, S.A., Johansson, L., Calderon-Montano, J.M., Claesson, M., Loseva, O., Valerie, N.C.K., Lundback, T., Piedrafita, J., et al. (2016) Discovery of the First Potent and Selective Inhibitors of Human dCTP Pyrophosphatase 1. J Med Chem **59**: 1140-1148

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