

## Project Summary

Ribonucleoside monophosphates (rNMPs), the subunits of RNA, are the most common non-canonical nucleotides found in genomic DNA. Inactivation of ribonuclease (RNase) H2, which is the major player in the removal of rNMPs from nuclear DNA (nDNA), allowed detection of over one million rNMPs in the mouse genome and ~2,400 rNMPs in the budding yeast genome. rNMPs distort the DNA double helix, modulating or altering DNA functions and increasing DNA fragility and instability. There is a need to determine where rNMP sites are in DNA, especially in cells with abnormal genome stability, like cancer cells. We recently developed a method, ribose-seq, to map rNMPs present in genomic DNA (Koh *et al.*, *Nature Methods*, 2015). We applied ribose-seq to yeast *Saccharomyces cerevisiae* RNase H2 deficient cells, and we revealed widespread but not random distribution of rNMPs with several hotspots in nDNA and mitochondrial DNA (mtDNA).

A proven, though poorly explored, cause of rNMP inclusion in DNA is oxidative stress, which, through reactive oxygen species (ROS), converts deoxyribose to ribose both in the deoxyribonucleotide pool and within DNA. Moreover, ROS not only produce abasic DNA, which is repaired via the base excision repair (BER) pathway, but also abasic RNA. Because we recently demonstrated that the BER apurinic/aprimidinic endonuclease Ape1 cleaves also abasic RNA, we aim to determine if BER is involved in removal of rNMPs from DNA. Currently, it is unknown whether and how the profile of rNMP incorporation in genomic DNA changes upon oxidative stress, and whether there is any link with cancer phenotype. Are there genomic sites (i.e. transcriptionally active regions) that are more prone to rNMP formation upon exposure to ROS? Is there a correlation between rNMP and mutation sites occurring in oxidatively stressed and/or cancer cells?

In **Aim 1**, applying ribose-seq, we will reveal for the first time, the spectrum of rNMP incorporation in different conditions of oxidative stress in nDNA and mtDNA of *S. cerevisiae* RNase H2-deficient cells. The rNMP profiles will be analyzed and compared with those of the same yeast cells not exposed to the oxidative stressors, and also with mutation spectra of the same ROS-exposed cells. Because RNase H2 activity for rNMP removal was not found in mitochondria, mtDNA could be particularly sensitive to rNMP incorporation during oxidative stress. Thus, in **Aim 2** we will perform profile and analysis of rNMPs in mtDNA of yeast and normal mammalian RNase H2-proficient cells exposed to oxidative stress and sensitized to it by using mutants and inhibitors of BER factors. rNMP maps will be also compared with mutation maps. In **Aim 3**, we will perform profile and analysis of rNMPs in mtDNA of cancer cells. Cancer cells from different human hepatic cancer cell lines, from a selection of human bioptic hepatocarcinoma samples (tumoral and distal liver tissues) and HeLa cells reconstituted with different functional variants of Ape1, will be processed to obtain purified mtDNA, which will be analyzed for rNMP distribution and hotspots of incorporation to identify significant biomarkers.