Studies on the 2-thiouridine-tRNA damage induced in the cells under oxidative stress

Transfer RNA (tRNA) is a type of low molecular weight RNAs in the cells, which plays an important role of supplying the amino acids to the ribosome, venue of protein biosynthesis. Therefore, tRNA is a basic and a necessary component of intracellular translational machinery. The tRNA molecules adopt a characteristic secondary structure resembling a clover leaf, with the individual sections of the structure called arms and loops. Among them are, acceptor arm, which binds the specific amino acid, and anticodon loop comprising an anticodon sequence, recognizing the specific three-nucleotide-genetic-code referred to codons in mRNA sequence.

After synthesis of pre-tRNA in the nucleus, its maturation and transport into cytoplasm, part of the tRNA nucleotides undergoes chemical modifications. There are mainly the methylation, saturation of double bonds, sulfuration or the attachment of specific substituents to the nucleobase or sugar residues. The presence of modified nucleosides in tRNA molecule is very important because modifications enhance an inner stability of tRNA moiety, improve interactions with other biomolecules, facilitate the creation of non-canonical base pairs, and, in general, expand the range of structural features and biological functions of tRNA.

The nucleoside modifications, especially those found in the anticodon sequence, have been the objects of our interest from many years. The 5-substituted 2-thiouridines (X5S2U) are present in a wobble position of selected transfer ribonucleic acids ($tRNA^{Lys}$, $tRNA^{Glu}$, $tRNA^{Gln}$). Nucleosides in this position play an important role in proper reading of genetic information in the process of protein biosynthesis. In our studies we have found that in the oxidizing environment the 2-thiouridine (S2U) alone or built into an RNA chain is desulfured, i.e. the sulfur atom is removed from the molecule, and the products of this reaction are uridine (naturally occurring in RNA) and a deprived of sulfur atom 4-pyrimidinone riboside (H2U), the hydrogen bond acceptors and donors pattern of which is different than that of uridine and 2- thiouridine. The presence of the H2U modification can be considered as damage, since tRNAs containing the H2U unit may do not exert their function properly (e.g. allow for the formation of mutant proteins or inhibit the protein synthesis). H2U-tRNA is also unstable in the cellular environment and can be easily cleaved at the modification site. The resulting tRNA halves can be toxic for the cell, as they may operate as regulatory molecules, driving the cells to abnormal metabolic pathways and leading to the development of cancer.

Recently, we found that damage of S2U-tRNA occurs not only in a test tube (*in vitro*), but also in a living cells, e.g. yeast cells. Experiments carried out in *Saccharomyces cerevisiae* yeast cells cultured under oxidative stress conditions confirmed the presence of products of S2U-tRNA desulfuration: the mcm5H2U and mcm5U in the mixture of tRNA-derived nucleosides. However these interesting results, require further verification in the other types eukaryotic cells.

The presented Project is aimed at verification in the biological systems our test results obtained by the chemical synthesis pathway, This verification should give the answers to the following questions:

- Is desulfuration of S2U the natural process taking place in cells subjected to oxidative stress?
- What is the ratio of S2U desulfuration products (H2U vs U) in cells?
- What is the mechanism of 2-thiouridine-tRNA desulfuration in the cellular conditions?
- Is decreased level of nucleoside type X5S2U in cells, subjected to oxidative stress, due to suggested altered expression of the specific X5S2U-tRNA metabolizing enzymes, or rather due to the effect of S2U-tRNA desulfuration in cells?
- What are the consequences of S2U-tRNA desulfuration for the cell metabolism?

To obtain answers to these questions and prove the research hypothesis, we plan to conduct the comprehensive research on the 2-thiouridine-tRNA desulfuration process occurring in the selected yeast and mammalian cells, subjected to exo- and endogenous oxidative stress. Basic research undertaken to identify desulfuration nucleoside products will be carried out by LC-MS/MS mass spectrometry - these studies will be performed in collaboration with our consortium Partner (University of Lodz). Additionally, we designed the proteomic studies.

The results of our studies will help us to address the issue of how the specific tRNAs desulfuration translates to the specific cellular response, that is the regulation of gene expression at the level of RNA transcriptomics and protein synthesis.