General hypothesis

Each cell contains millions of copies of different messenger RNAs (mRNAs) whose levels must be changed in response to growth or stressing conditions, and sometimes up- and down-regulation must be achieved in a very short time. The levels of mRNAs depend, from one side on the speed and efficiency of their synthesis and splicing, and, from the other side on the efficiency and speed of their degradation. In our published studies of cells exposed to ionizing radiation those transcripts whose levels were up-regulated were enriched in target motifs for microRNAs (miRNAs), and at the same time increased levels of reactive oxygen species (ROS) and oxidative damage to RNA were observed in cells. We have created a mathematical model of these processes which suggests the existence of a class of mRNAs whose change after irradiation depends mainly on interactions with miRNAs. On the basis of these observations we propose that:

The key step in the modulation of mRNA levels in cells exposed to stressing conditions or during proliferation is based on changes of the level of ROS that modify RNAs and consequently influence crucial and specific interactions between mRNAs and miRNAs.

Aim

The aim of this project is to study novel elements of the regulation of gene expression in human cells. These elements are interactions between mRNAs and microRNAs and their changes induced by RNA oxidation. Using bioinformatics tools we will identify which mRNAs and miRNAs are influenced by this mechanism and verify this by experiments with mRNAs for reporter genes. A further aim is to construct the next version of our mathematical model which will include new experimental observations and be able to better predict the mRNA changes in different conditions. Characteristics of other elements of this hypothetical regulatory system (effects on the RISC complex, detection of nucleotides in miRNAs and mRNAs that are subjected to oxidative modification, sources of cellular ROS) are also planned.

Methodology

Studies of the intracellular levels of ROS will be performed using fluorescent probes and cytofluorimetric or microscopic assays. Oxidative changes to RNA will be studied by HPLC with mass spectrometry. The changes of mRNA and miRNA levels in cells with different ROS levels will be quantitated with the Agilent microarray system. Nucleotide sequences of up- and down-regulated transcripts will be analyzed using bioinformatics tools. The role of particular nucleotide sequence motifs will be studied by construction of luciferase reporter genes containing these motifs and transfection experiments in which the levels of mRNA and proteins will be assessed with RT-qPCR and luciferase activities. All the methods are currently used in our laboratory.

Significance of the project.

Our hypothesis concerning the role of ROS in the modulation of gene expression is novel, and although changes of ROS levels in cells exposed to different stimuli are very common, until now their direct interactions with RNA were not considered as elements of regulatory systems. Creation of an effective model that will allow for simulations of global gene expression changes in cells after irradiation or during intensive proliferation when ROS levels increase, is one of the main objects of this project. A priori knowledge of possible changes of gene expression after exposure of cells to damaging factors will provide new perspectives for individualization of anticancer or other therapies that employ these factors, and could create new targets for therapies.