MiRNAs are short (21-23 nucleotides) non-coding RNA molecules that mediate posttranscriptional silencing of their target genes. MiRNA-dependent inhibition of gene expression is crucial in cellular processes including cell cycle, apoptosis and proliferation. Deregulated miRNA levels have been observed in various pathological conditions including cancer and modulation of miRNA levels have a great potential in anti-cancer therapy.

Exposure of cells to stress conditions such as ionizing radiation induce rapid changes in cell functioning. Ionizing radiation, e.g. X rays, gamma or alpha particles, cause DNA damage, inhibited growth or even cell death. Moreover, cellular effects of ionizing radiation are not restricted to directly irradiated cells, but may also occur in non-irradiated bystander cell. Ionizing radiation is widely used in medicine i.e. to radiotherapy that is used in cancer treatment. The application of ionizing radiation in radiotherapy is based on much faster damage of the fast-growing cancer cells that the slower-growing normal cells. Unfortunately, some cancer types are resistant to radiation. One of the factors that can be used to improve efficiency of radiotherapy are miRNAs, because some miRNAs increase whereas other diminish sensitivity of cells to ionizing radiation treatment. Therefore increase in levels of sensitizing miRNA and/or inhibition of miRNAs that cause resistance of cells may be beneficial for radiotherapy. However, before such interference with cell functioning can be made, we have to understand role of these miRNAs in cells and know the mechanisms that regulate cellular miRNA levels to precisely modulate levels of certain miRNAs.

Proposed project intends elucidate the causes of altered miRNA levels upon stress conditions induced by ionizing radiation. The levels of a mature miRNAs depend on efficiency of pri-miRNA transcription, efficiency of pri-miRNA processing to mature miRNA and miRNA stability. Each of these processed can be regulated. In this project we focus on regulation of pri-mRNA processing. An indication of regulated miRNA processing is a discrepancy between pri-miRNA and mature miRNA levels. We hypothesize that exposure of cells to ionizing radiation modulates maturation of pri-miRNAs. This modulation is manifested in irradiated cells by changes in miRNA/pri-miRNA ratios compared to control cells. **The goal of this project is to determine range of miRNA processing modulation in response to ionizing radiation and to identify underlying molecular mechanism for this modulation.**

To achieve this goal we will analyze miRNA levels and pri-miRNA levels in normal and cellular stress conditions and identify miRNAs that undergo biogenesis modulated by cellular stress. For these miRNAs we will investigate possible mechanisms that may be responsible for the modulated miRNA biogenesis. We will determine whether pri-miRNA processing can be modulated by proteins that are involved in ionizing radiation response and modifications of pri-miRNA structure mediated by reactive oxygen species. We will also investigate a possibility that miRNAs are exported from the cells to medium in exosomes due to cellular stress.

Obtained results will improve our understanding of a scale of regulated miRNA processing and how ionizing radiation may influence this regulation. This will enable not only identification of miRNAs that show altered levels upon cellular stress, but also provide information on the underlying mechanisms of these alterations. Studies regarding miRNA biology will surely contribute to a better prediction of therapeutic outcome of radiotherapy and understanding of the pathogenetic mechanisms involved in therapy resistance in cancer. This knowledge will also contribute to application of miRNA-based specific manipulation of radiation response. This manipulation would be a useful tool to enhance susceptibility to radiation in cancer cells and/or protect normal cells from the disruption of biological pathways upon radiation. Certainly, thorough understanding the regulatory mechanisms of miRNAs levels will provide new insights for clinical cancer radiotherapy. Tightly controlled induction or inhibition of miRNA levels in combination with predictable outcome of such modulations will enable the application of miRNA modulating agents in anti-cancer therapy, including radiotherapy, in future studies.