ABSTRACT

MicroRNAs are noncoding RNAs inducing sequence-specific posttranscriptional inhibition of gene expression and represent the major class of small endogenous RNAs in mammalian cells. Over 2,500 of human microRNAs potentially regulating more than 60% of human protein-coding genes have been identified. MicroRNAs participate in the majority of cellular processes, and their expression changes in various diseases, including cancer.

Currently, there is no efficient small chemical compound available for the modulation of microRNA pathway activity. At the same time, small chemical compounds represent excellent tools for research of processes involving RNA silencing pathways, for biotechnological applications, and would have a considerable therapeutic potential. The presented work represents a part of a broader project, whose ultimate goal is: (i) to find a set of small molecules allowing for stimulation or inhibition of RNA silencing and (ii) to identify crosstalks between RNA silencing and other cellular pathways. This thesis summarizes results from the first two phases of the project, the development of high-throughput screening assays and the high-throughput screening (HTS) of available libraries of small compounds.

To monitor the microRNA pathway activity, we developed and optimized one biochemical fluorescence-based *in vitro* Dicer assay and several cell-based luciferase assays. Our strategy was to generate data allowing sorting HTS results according to different parameters, such as cell-type specific effects, miRNA specific effects, or sorting compounds affecting different steps of RNA silencing pathway. Optimized assays were used for HTS of ~30,000 small chemical compounds. The combination of data from all HTS generated tens of interesting hits that need to be further validated. Selected compounds will undergo series of assays to characterize their pharmacokinetic properties, their mode of action, and their regulatory potential in different model systems.