Unknown areas of activity of human ribonuclease Dicer

Ribonuclease Dicer is one of the key enzymes involved in the biogenesis of small regulatory RNAs (srRNAs) in humans, such as microRNAs (miRNAs) and small interfering RNAs (siRNAs). This ribonuclease processes 50-70 nucleotide (nt) stem-loop precursors (pre-miRNAs) as well as double-stranded RNAs (dsRNAs) into short RNA duplexes containing functional 21-23 nt miRNAs or siRNAs, respectively. SrRNAs play essential roles in many important biological processes, including developmental timing, growth control, differentiation and apoptosis. In humans, the vast majority of small regulatory RNAs are miRNAs. They have been found to control the expression of most human protein-coding genes through the miRNA pathway. Moreover, it has been demonstrated that miRNAs play a very important role in host-virus interactions in mammals. Therefore, the cellular levels of miRNAs and other components of miRNA pathways must be tightly controlled, both spatially and temporally. Aberrant regulation of miRNA levels can initiate pathological processes, including carcinogenesis as well as neurodegenerative, immune system and rheumatic disorders.

It is clear that due to the Dicer's important functions in organisms, its cellular transcript and protein levels must be strictly controlled, because even small changes in their accumulation can initiate adverse effects in cells. Accordingly, in recent years, a number of studies have been performed to identify the factors regulating Dicer gene expression and protein activity. As a result, a large amount of complex and often contradictory data has been generated. Generally, Dicer abundance, activity and specificity can be regulated by various types of factors and at multiple levels. There is no doubt that the most extensively studied group of Dicer regulators is a group of its protein partners. Among the elements that affect Dicer activity there is also a group of non-protein factors, however this group is still not well recognized.

Interestingly, results of our studies and some literature data imply that RNA molecules can be one of the important factors that regulate the activity of Dicer.

Very recently human transcriptome-wide analysis has identified so-called "passive" Dicer binding sites. These sites are preferentially located in coding sequences and 3′-untranslated regions that adopt stem-loop structures. Dicer has been shown to be capable of binding but not of cutting passive sites. Interactions with Dicer stabilize RNAs carrying passive sites. In addition, passive sites may function as a buffering system to control the catalytic activity of the enzyme by sequestering it from other targets. A similar strategy, based on Dicer sequestering, is utilized by viruses to mislead host defense mechanisms. For example, adenoviruses protect their RNAs by producing high amounts of long self-complementary transcripts that effectively compete for Dicer binding with other endogenous Dicer substrates. As a result, pivotal viral transcripts are not cleaved. Likewise, *in vitro* studies conducted by our group have indicated that the activity of human Dicer (hDicer) can be affected by short RNA molecules that are bound to it. Detailed studies have revealed that short RNAs can not only act as competitive or allosteric inhibitors of Dicer (universal inhibitors) but can also influence this enzyme by base-pairing with its substrates. We have found that RNA oligomers that can simultaneously bind both hDicer and its substrates are selective and effective inhibitors of pre-miRNA processing. Furthermore, results obtained by our research group imply that sequences similar to those of the previously characterized oligomers are encoded in the transcribable portion of the human genome.

Thus, we would like to identify and characterize the pool of endogenous RNA molecules that are capable of regulating the activity of hDicer. Furthermore, we would like to determine specificity of the identified RNA molecules towards individual miRNA precursors.

Recent data also indicate that the activity of ribonuclease Dicer may be associated not only with the RNA interference (RNAi) pathway and production of srRNAs. It has also been found that Dicer may participate in other processes, like chromosome fragmentation during apoptosis, chromatin structure remodeling or inflammation. We also demonstrated that Dicer can support base-pairing of complementary sequences present in nucleic acids (the so called annealing activity), acting as chaperone-like protein [4]. Interestingly, non-RNAi-related functions of Dicer seem to be correlated with truncated forms of this enzyme and occurrence of various Dicer transcript variants, including alternative splicing variants. Literature data and our preliminary results also indicate that occurrence of various transcript variants of Dicer-type proteins may be one of the mechanisms regulating the cellular level and activity of these proteins.

Another problem we would like to investigate is the identification of various hDicer forms in the selected cell types. We would like also to recognize functions of the newly identified variants of hDicer.

We believe that the results of our studies will lead to identification and better understanding of cellular RNA-based mechanisms that regulate Dicer. In addition, the recognition of the molecular mechanisms behind Dicer activity would give a valuable impact towards understanding of the numerous phenomena, including developmental timing, growth, differentiation, apoptosis and even viral infections. On the other hand, the results obtained might be helpful for the further studies on new, specific therapeutic methods that can be used in treatment of diseases caused by changes in the expression levels of specific regulatory molecules, e.g. in the treatment of cancer, neurodegenerative or infectious diseases.