ABSTRACT

Dicer ribonucleases are mostly known from their important role in microRNA (miRNA) and small interfering RNA (siRNA) biogenesis. Their substrates in this process are: precursors of miRNAs (pre-miRNAs) and long double stranded RNAs (dsRNAs), respectively. However, Dicers have also been reported to be involved in other processes, like chromatin structure remodeling, apoptotic DNA degradation or DNA damage repair. Contribution of Dicer to these processes implies that this ribonuclease can interact with many different RNA- and DNA-type substrates. Most Dicer enzymes are multi-domain proteins, comprising an amino-terminal helicase domain, a domain of unknown function (DUF283), Piwi–Argonaute–Zwille (PAZ) domain, two RNase III domains (RNase IIIa and RNase IIIb), and a dsRNA-binding domain. The research presented in this doctoral dissertation focuses on the helicase domain of human Dicer (hDicer).

Currently it is known that the helicase domain of hDicer contributes to recognizing the precursors of miRNAs from siRNAs, presumably through the interactions with the apical loop of pre-miRNAs. In comparison to the full-length hDicer, hDicer variants lacking the helicase domain bind and cleave long dsRNA substrates more efficiently. The hDicer helicase domain is also suggested to participate in Dicer "passive" binding, i.e. binding without further cleavage of different cellular RNAs. Presumably, the helicase domain of hDicer plays a key role in this passive binding of cellular RNAs. Nevertheless, a comprehensive characterization of the biochemical activities of the hDicer helicase domain and its substrate specificity towards different nucleic acids have never been reported. Consequently, the aim of the research conducted as part of this doctoral dissertation was to expand the state of knowledge about the hDicer helicase domain, in particular to determine what biochemical activities the hDicer helicase domain presents and what kind of nucleic acids can be substrates for the hDicer helicase domain, both *in vitro* and in *in cellulo*.

In *in vitro* studies, the hDicer helicase domain preparation (HEL hDicer), produced in *E. coli* BL21 (DE) system, was used. The obtained preparation displayed ATP-binding and ATP-hydrolysis activities. Importantly, for the first time it was shown that the full-length hDicer can also hydrolyze ATP. The hDicer variant lacking the helicase domain showed no ATPase activity, supporting the hypothesis that the domain responsible for ATP-binding and hydrolysis in hDicer is the helicase domain. Then, by applying the Electrophoretic Mobility Shift Assay (EMSA) and Bio-layer Interferometry (BLI), it was shown that HEL hDicer binds single-stranded RNAs and single-stranded DNAs longer than 20 nucleotides, but it does not bind dsRNA and dsDNA. Binding of nucleic acids by HEL hDicer was ATP-independent. Interestingly, it was noticed that HEL hDicer can induce structural changes within RNA molecules; this activity was also an ATP-independent. It was further shown that HEL hDicer cannot unwind double-stranded nucleic acids, despite the presence of the DExD/H-box motif, which is known from the dsRNA and dsDNA unwinding activity. HEL hDicer also did not support base-pairing of complementary nucleic acids.

To explore the role of the helicase domain in the binding of cellular RNAs by hDicer, the irCLIP-seq strategy (Infrared Crosslinking Immunoprecipitation followed by NGS sequencing) was used. This strategy allows the immunoprecipitation of specific RNA•protein complexes formed in living cells, and then the identification of the protein-bound RNAs. The following were used in the study: (i) HEK 293T 4-25 NoDice cells (hDicer knock-out) transfected with a plasmid encoding for the hDicer variant lacking the helicase domain (hDicer_ΔHEL), and as controls (ii) 4-25 NoDice cells transfected with a plasmid encoding for the wild-type full-length hDicer, WT hDicer (so-called "rescue control") and (iii) HEK 293T cells with endogenous WT hDicer (wild-type line). Preliminary comparative analysis showed that hDicer_ΔHEL interacts with a different pool of RNA molecules or with different fragments of identical RNAs, than WT hDicer. The results from these preliminary studies suggest that the helicase domain of hDicer is involved not only in the differentiation of pre-miRNA substrates from dsRNA, but may also be important in the selection and binding of other types of RNA molecules present in cells.

Understanding the cellular network of interactions between RNA and hDicer would allow to expand our knowledge on hDicer's functions beyond miRNA and siRNA biogenesis pathways. The obtained results may be of interest to a wide range of the researchers focused on the problem of misregulation of cellular processes that may cause the development of many diseases, including cancer. Given the documented importance of the hDicer helicase domain in antiviral defense, the obtained results may also contribute to a better understanding of viral diseases and the role of hDicer in virus-host interactions.