A putative DNase activity and possible biological functions of the selected truncated variants of human ribonuclease Dicer

Summary

A key role in the biogenesis of small regulatory RNAs (srRNAs) is played by the Dicer ribonuclease, which cuts out microRNA (miRNA) and small interfering RNA (siRNA) duplexes from single-stranded hairpin precursors (pre-miRNAs) or long double-stranded RNAs (dsRNAs), i.e., pre-siRNAs, respectively. Dicer-generated srRNAs are involved in the regulation of gene expression. The presented studies focus on human ribonuclease Dicer (hDicer). hDicer is a multi-domain protein composed of an N-terminal helicase domain, a DUF283 domain, Platform, PAZ, and a connector helix, two RNase III domains (RNase IIIa and IIIb) and a C-terminal dsRNA binding domain (dsRBD). Platform, PAZ and connector helix domains (the so-called PPC cassette) play an important role in the binding of pre-miRNA and pre-siRNA substrates. The distance between the PPC cassette and the RNase domains determines the length of the generated products; in the case of hDicer, the length of the produced srRNAs is about 22 nucleotides (nt)

The presence of all hDicer domains is crucial for the efficient and precise cleavage of pre-miRNA substrates, and thus, generating miRNAs of a specific length. Literature data indicate that proteins interacting with Dicer, presumably by affecting the arrangement of its domains, can influence the length of RNAs produced by this ribonuclease. Interestingly, Dicer variants lacking some of the domains, so-called "shortened forms" ("truncated variants") of Dicer, have been identified. Truncated variants of Dicer can arise from alternatively spliced transcripts of the *DICER1* gene, or as a result of a hydrolytic activity of cellular proteases. The biological functions and enzymatic activities of truncated variants of Dicer differ from the functions and activities displayed by the "full-length" proteins. For example, a Dicer variant lacking the helicase domain preferentially binds pre-siRNA substrates. It has also been shown that in nematode *Caenorhabditis elegans*, a truncated form of Dicer (tDCR-1) is produced by the caspases during apoptosis, and this truncated form initiates the chromosomal DNA fragmentation.

The first goal of the PhD studies was to investigate whether hDicer, similarly as *C. elegans* Dicer, has the potential to hydrolyze DNA substrates. In the apoptosis-induced human cell lines, a truncated form of hDicer similar to *C. elegans* tDCR-1 was not identified. Nevertheless, to answer the question whether hDicer, like *C. elegans* Dicer, can display a DNase activity,

a number of truncated hDicer variants were prepared using genetic engineering methods, including a variant corresponding to the tDCR-1 form of *C. elegans*. The hDicer variant corresponding to *C. elegans* tDCR-1 did not exhibit DNase activity. However, the collected data revealed that the hDicer variants lacking the DUF283 domain or the PAZ domain, or the entire PPC cassette, could hydrolyze both single-stranded and double-stranded DNA.

The second part of the research has focused on the analysis of natural hDicer variants and their putative functions. Transcriptomic analyses revealed several truncated transcript variants of the *DICER1* gene. Among them, a variant designated as Dicer1e was identified. This variant is generated by an alternative splicing of the primary transcript of the *DICER1* gene. In comparison to hDicer, the Dicer1e protein has a unique N-terminus and only three domains: RNase IIIa, RNase IIIb and dsRBD. Dicer1e is expressed in healthy (normal) and tumor cells. It is known that the level of Dicer1e expression is tissue-specific and, in the case of cancer cells, it also depends on the type and tumor stage. So far, the biochemical properties of the Dicer1e protein have not been characterized. Consequently, using genetic engineering methods, a protein preparation of Dicer1e was obtained, and its biochemical activities were investigated. The RNase activity assays showed that Dicer1e does not produce miRNAs; however, it can hydrolyze small RNAs. Interestingly, Dicer1e exhibited also the DNase activity.

To investigate the potential biological roles of the Dicer1e variant, HEK293T and HEK293T No Dice cells (a *DICER1* knock out cell line that does not produce the endogenous hDicer) were used. These cells were transfected with a plasmid producing the Dicer1e variant. Next, the RNA-Seq of the total RNA fractions isolated from the used cell lines were performed. The expression levels of selected miRNAs in the cell lines used in the study were also examined. The collected data suggest that the Dicer1e variant may be involved in cellular RNA metabolism and, interestingly, the organization of chromatin structure.

The results obtained in the course of the conducted studies expand our knowledge on the potential role of Dicer-type proteins in cellular processes extending well beyond the srRNA biogenesis pathways.