Abstract

MicroRNAs (miRNAs) are small, non-coding RNAs that act as post-transcriptional regulators of gene expression. Through their interactions with target transcripts, miRNAs direct them for degradation or inhibit the translation of the encoded proteins. The biogenesis of miRNAs is a tightly controlled process wherein the ribonuclease Dicer plays a fundamental role, cleaving mature miRNAs from their hairpin precursors (pre-miRNAs).

Viral miRNAs have been identified as a class of crucial regulators of virus-host crosstalk, modulating processes such as viral replication, antiviral immune response, viral latency, and pathogenesis. For over a decade, there has been a steady increase in the number of reports on the identification of other miRNAs encoded in the genomes of alphaherpesviruses, including pseudorabies virus (PRV), which is a swine pathogen and a model for research on herpesvirus biology. PRV encodes eleven distinct miRNAs clustered within an intron of a Large Latency Transcript (LLT), but their role in the viral replication cycle has not been thoroughly investigated.

This PhD thesis aimed to provide a more detailed characteristic of the functions of the first three miRNAs located within the LLT intron of PRV. The research was divided into two parts: an analysis of the impact of the miRNA cluster (LLT[1-3]) on the course of infection and studies on the selective regulation of LLT[1-3] miRNA biogenesis using inhibitors to observe the resultant phenotypic effects associated with the loss of specific miRNA function.

In the first part of the project, I generated a research model based on the constitutive expression of viral miRNAs in swine testis cell line, named ST_LLT[1-3]. Using a cell culture system providing a stable production of heterologous miRNAs at high levels, I demonstrated that the LLT[1-3] miRNA cluster downregulated viral transactivators and the glycoprotein gE at the early stages of PRV infection. Analysis of the viral growth kinetics in the ST_LLT[1-3] cell line indicated the potential of the analyzed miRNA cluster to regulate the infection process by slight distortion in transmission and proliferation abilities.

The second part of my PhD project was related to research on the suppression of Dicer activity and the control of PRV miRNA biogenesis by employing oligomers with inhibitory capabilities. The initial part of the work concerned the development of a production and purification system for recombinant Dicer ribonuclease using a baculovirus expression system. The biochemical examination of the obtained Dicer preparation demonstrated its strong and highly specific activity and also confirmed its high degree of homogeneity. The aforementioned properties allowed the use of Dicer preparation, among others, for research concerning the interactions of this ribonuclease with RNA

inhibitors of various lengths, consequently enhancing our comprehension of the mechanisms governing Dicer activity.

Research on the regulation of PRV miRNA maturation was conducted using two types of miRNA biogenesis inhibitors: 2'-*O*-methylated oligonucleotides designed in the EvOligo software, and commercially available Morpholino oligomers. *In vitro* analysis of their activity showed that both types of oligomers efficiently inhibited the processing of synthetic PRV pre-miRNAs carried out by Dicer. The next step involved examining the activity of 2'-*O*-methylated and Morpholino oligomers in a cellular system. However, preliminary results of experiments investigating the regulatory potential of the aforementioned inhibitors introduced into ST_LLT[1-3] cells indicated that they inhibited PRV miRNA maturation in a non-selective manner, which precludes their utilization in more detailed experiments exploring the role of individual miRNAs from the LLT[1-3] cluster. To confirm the involvement of the selected miRNA in PRV-infected cells, additional optimization of the working conditions using miRNA biogenesis inhibitors is required. Modifying the experimental model could potentially facilitate the observation of the phenotypic effect linked to the reduction in the selected miRNA level.

Overall, the conducted research has contributed to a better understanding of the function of miRNAs in PRV biology and has expanded knowledge regarding the potential use of inhibitors of miRNA maturation in a cellular system.