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Functional characterization and target validation of the bovine foamy virus encoded miRNAs

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Foamy viruses (FVs) are distinct retroviruses with unique features for the development of novel retroviral gene transfer and gene delivery vectors. Recently, a new miRNA expression cassette driven by RNA Pol III has been detected in the long terminal repeats of the bovine foamy virus (BFV). The pri-miRNA has a characteristic dumbbell structure yielding three stable miRNA that may dramatically accumulate in persistently BFV-infected cells. Since RNA therapeutic strategies have a huge clinical potential to knockdown disease related genes efficiently and durably (Kole et al., 2012; Ryther et al., 2005; Wittrup and Lieberman, 2015), the characterization and application of the miRNA cassette and BFV-based delivery vectors carrying therapeutic RNAs (mostly siRNAs) are the overall aims of my basic science research project.

Through *in vitro* DLR analyses and site-directed cloning strategies, I identified significant detrimental effects of flanking sequences on the overall miRNA expression that were not be relieved by the co-expression of the BFV transcriptional transactivator Tas. Furthermore, I also discovered that each single stem-loop is of prime importance to generate functional miRNAs from the unmodified stem-loop while replacement of individual stem-loops by shRNA-encoding hairpins recovered functionality of the whole pri-miRNA opening the avenue to engineer chimeric BFV miRNA cassette-based short therapeutic expression systems for use in molecular medicine.

The persistence of FV infections as well as their long-term apathogenic co-existence with their hosts is an important feature of FV biology and may be influenced and mediated by the virus-encoded miRNAs. MiRNAs have been shown to facilitate virus survival in their host via a plethora of complex mechanisms. Thus, determining and validating the cellular targets of BFV miRNAs and elucidating their functions have the potential to illuminate novel aspects of viral lifecycles and probably leading to discovery of new therapeutic avenues, which is the topic of the second part of my project.

Several potential targets of miR-BF1-5p and miR-BF2-5p were predicted via bioinformatics tools and data base mining. By miRNA target site binding assays and analysis of mRNA and protein levels, the AKNRD17 and Bif1 genes/proteins were verified as two direct cellular targets of the most abundant miRNA miR-BF2-5p. MiR-BF2-5p-mediated suppression of its target gene ANKRD17 may modulate innate immunity response of the host cell towards BFV infections.

All the above results give on one side important information for future manipulation and application of the BFV miRNA expression cassette but also on FV-based vectors for miRNA delivery. On the other side, the data presented here provide new information concerning the biological role of the BFV miRNAs in the context of virus-host co-evolution, and probably adding important implications into FV and vector biology.