

Horses are important animals in livestock production, recreation, hippotherapy and as working animals in underdeveloped countries. Sarcoid is a significant problem in horse dermatology and breeding. It is a locally malignant skin tumor which may account for as much as 67% of all diagnosed neoplasms. Sarcoids are highly locally invasive and have a high tendency to regrow and progress into a more aggressive form. Moreover, the lesions are usually not amenable to treatment. Sarcoids can significantly threaten the welfare of animals and hamper their use, while unsuccessful, prolonged treatment may lead to euthanasia of the animal, causing economic losses. Bovine Papillomavirus (BPV) infection is generally accepted as the cause of this type of lesions, but it is still vague why BPV infection in equids leads to cancer initiation and what mechanisms underlie this.

Among the sequences that may be involved in such mechanisms are microRNAs (miRNAs). They are short RNA sequences that have the ability to regulate the amount of other sequences in cells. MicroRNAs influence many key biological processes, which makes them important cellular regulators. It has also been established that miRNAs play significant roles in oncogenesis either by contributing to its progression or inhibiting it. Therefore, they are the subject of numerous studies and show a great potential as biomarkers useful in the diagnosis of various types of cancer and the prognosis of the course of a disease. Recently, abnormal levels of another type of small RNAs, namely piRNAs, have also been detected in human tumors. They were first detected in germ cells and considered specific to them. However, an increasing number of reports indicate their role in somatic tissues. So far, it has been shown that they take part in a number of biological processes important from the point of view of cell functioning, related, as in the case of miRNA, with the regulation of the number of other sequences. However, exact roles of individual piRNAs and their mechanisms of action are still under investigation.

In our previous studies, we found abnormal levels of miRNAs in sarcoid tissue samples. Bioinformatics analysis showed that they are involved in cellular processes important for equine sarcoids oncogenesis, related to, inter alia, viral infection and cell invasion. In addition, we detected for the first time the presence of piRNA sequences in equine sarcoid samples. However, the exact functions of the identified miRNAs and piRNAs and their involvement in the neoplastic transformation of this tumor and virus-host interactions have not been characterized. Therefore, we hypothesized in this project that microRNAs and piRNAs identified in our previous research have an impact on the functioning of sarcoid cells by influencing processes involved in viral infection and cellular invasion, and this occurs owing to gene silencing mechanisms, including regulation of DNA methylation.

The aim of the proposed research will be to characterize the roles and mechanisms of action of selected microRNAs and piRNAs in sarcoid carcinogenesis. We will apply a comprehensive analytical approach to realize it, consisting of the following steps. In the first step, we will derive primary sarcoid cell cultures transfected *in vitro* with the tested small RNAs. Next, we will perform the global analyses of the profiles of genes, miRNAs and DNA methylation of these cells using next-generation high-throughput sequencing that will reveal sequences regulated by the examined small RNAs. Thanks to the application of such a multi-level global approach and the integration of the obtained data, it will be possible to identify the most significant candidate sequences with high regulatory potential. The obtained results will be confirmed with the use of additional methods, *i.a.* at the protein level, to assess if the identified changes may have a direct impact on the functioning of the sarcoid cells. Additionally, owing to the luciferase reporter vector technology, it will be possible to characterize the nature of identified interrelations between the investigated small RNAs and the detected regulated sequences, and thus the mechanism of their silencing.

The proposed project will contribute to a better understanding of molecular mechanisms involved in the functioning of small RNAs underlying the carcinogenesis of equine sarcoids. This is particularly important because of the fact that research on piRNA functions in neoplasms, especially in the animal ones, is currently in its infancy. Moreover, the obtained results will bring us closer to determining the influence of BPV on the host organism at the molecular level and strategies used by it in the course of infection, leading to the formation of sarcoid tumors. The obtained results will fill the gap in the knowledge about this type of neoplasm and will provide the basis for the development of a more effective diagnosis, prognosis and treatment of this disease in the future.