Abstract of doctoral dissertation M.Sc. Ewelina Semik entitled:

**"ANALYSIS OF THE METHYLATION STATUS OF CpG ISLANDS WITHIN THE CODING REGIONS IN EQUINE SARCOIDS DNA",**

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Equine sarcoids are the most commonly detected skin tumors in equidae. The Bovine Papillomavirus is considered as the main etiological factor of sarcoid's occurrence and its DNA was detected in almost all studied cases. Currently, histological examination of the equine sarcoids is considered to be the most reliable diagnostic method. However, this method is time-consuming and requires the use of organic solvents, often harmful to human health. Therefore, new diagnostic strategies are being sought that enable rapid and accurate diagnosis of the disease. In the recent years, particular attention was given to the research aimed at optimizing the use of tumor epigenetic markers. The term epigenetics refers to heritable changes in genetic material, other than alteration in the nucleotide sequence of DNA. One of the best known epigenetic changes associated with the process of carcinogenesis is aberrant DNA methylation.

One of the aims of this PhD thesis was to evaluate the methylation profile of genes potentially important in the diagnosis and/or prognosis of the equine sarcoids. The methylation status of nine genes: *APC, CCND2, CDNK2B, DCC, RARB, RASSF1, RASSF5, THBS1* and *TRPM1*, was determined using bisulfite sequencing polymerase chain reaction (BS-PCR). In addition to the analysis of the methylation of individual genes, in the study an attempt was made to identify regions of the genome characterized by a differential DNA methylation pattern in the equine sarcoids, by implementing Reduced Representation Bisulfite Sequencing (RRBS) method in combination with next-generation massively parallel sequencing. In addition, by using cDNA microarrays a comprehensive analysis of transcriptome was performed to identify differences in the level of gene expression in the equine sarcoids compared to healthy tissue and to associate detected DEGs with detected level of DNA methylation.

The results of this study did not reveal any changes in the level of DNA methylation and gene expression in the analyzed group of nine candidate genes between the tumor and healthy tissues. Despite numerous reports describing the aberrant methylation of the promoters of the analyzed genes in human cancers, the data obtained did not confirm the existence of such relationships in the examined tumor tissues, which excludes the possibility of using these genes for the diagnosis of the equine sarcoid.

The comparative analysis of DNA methylation patterns of genome fraction rich in CpG dinucleotides (RRBS technique), allowed identification of 136 regions showing differences in the methylation of CpG sites (DMRs) between tumor and normal tissue. Most of the identified DMRs were small fragments, less than 1 kb in size, located in the intergenic regions, away from the transcription start sites. Among the genes within which DMRs were identified, *SFN locus* was characterized by the presence of the hypermethylation region and decreased mRNA expression in the equine sarcoid. This suggests that methylation may be an element of the mechanism responsible for aberrant expression of this gene in the tumor tissue.

Methylation analysis of selected DMRs in a larger group of samples, revealed significant differences in the level of DNA methylation between healthy and tumor tissues within *RALGPS1, C1orf106* and *EFEMP2* genes. In the tumor tissues, a notably reduced level of methylation was also observed in the DNA fragment located in a predicted promoter of the *SPARC* gene, the mRNA expression of which was found to be increased in tumor tissues. These results may indicate the existence of a relationship between hypomethylation of this region and overexpression of SPARC gene in the equine sarcoids.

Transcriptome analysis of the equine sarcoids and healthy skin revealed 901 differentially expressed genes (DEGs). The expression of 273 genes was up-regulated and expression of 628 genes was down-regulated in the tumor tissue with respect to control tissue. Among detected DEGs, some genes with decreased expression were associated with a suppression of malignant transformation, whereas several overexpressed genes were involved in the processes associated with growth and progression of a tumor, immune functions, oncogenic functions and body's response to viral infection.

Knowledge on the changes taking place both in the process of DNA methylation and gene expression may provide a basis for the development of new alternative diagnostic or therapeutic approaches aimed at restoring the proper functioning of genes whose activity was associated with the development of equine sarcoids.