CIRCULATING MICRO RNAS AS NOVEL BIOMARKERS OF TRAINING ADAPTATION AND STRESS IN ENDURANCE HORSES

Katia Cappelli (1), Stefano Capomaccio (1), Andrea Viglino (2), Francesca Beccati (1), Maurizio Silvestrelli (1), Livia Moscati (3), Elisabetta Chiaradia (1)

(1) Università degli Studi di Perugia, Dipartimento di Medicina Veterinaria, Italia (2) Università Cattolica del Sacro Cuore di Piacenza, Itituto di Zootecnia, Italia (3) Istituto Zooprofilattico Sperimentale dell'Umbria e della Marche, Italia.

Endurance exercise induces metabolic adaptation involving musculoskeletal, cardiovascular, respiratory, endocrine, immune systems where muscle remodelling, mitochondrial synthesis and angiogenesis occur. Although these changes have been widely investigated, cellular and molecular mechanisms mediating this adaptation are still not completely elucidated [1-2-3]. Physical exercise has been recently associated to the modulation of a peculiar class of small noncoding RNAs (18–22 nucleotides), micro RNAs (miRNA), that act as post-transcriptionally regulators of gene expression. Released also in the body fluids, therefore named circulating miRNAs (ci-miRNAs), they have been recognized as optimal and accurate biomarkers in respect to classical serum/plasma biomarker [4] and highly stability with high resistance to variations in temperature, pH value and multiple thaw and freezing cycles making samples storage and handling an easier task. The aim of this study was to capture the whole picture of plasma circulating miRNAs through massive parallel sequencing in response to prolonged endurance exercise in samples obtained by four (4) trained and performing Arabian horses. Plasma ci-miRNAs were analyzed before (T0) and two hours after the end of competition (T1), when the majority of the significant changes in ci-miRNAs occur. NGS libraries were built from plasma derived RNA and sequenced producing 50 nucleotide Single-End reads. After cleaning procedures, reads were aligned to the reference genome (equcab 2.0). Differential gene expression analysis, assessed with a count based approach using edgeR package, was applied comparing T1 versus T0 samples. Protein-Protein Interaction (PPI) network and significant enriched pathways of target genes were explored with Cytoscape 3.4.0 suite creating cluster of related targets from which Gene Ontology (GO) enrichment was calculated. Our results reveal the modulation of large set of miRNAs (up regulation of miR-1, 133, 206, 208b, 499-5p, down regulation of miR-486) arising from tissues involved in exercise response such as muscle, heart, liver, and blood and activation of correlated processes like inflammatory response, immunity, angiogenesis and cell proliferation. Ci-miRNAs high throughput sequencing is a promising approach for sport medicine itself beside the value of this specific work in horse athletes. Discovery of putative biomarkers for prediction of disease risks related to prolonged activity (i.e. overtraining syndrome) and metabolic adaptations monitoring to ultimately establish efficient training programs, could be transferred to all “sport species”, including humans.

Recent research has demonstrated that circulating miRNAs can be stably detected in peripheral blood and may aid in the detection and diagnosis of various types of disease like e.g. cancer. These findings have opened up the possibility of a new and promising era in the screening and monitoring of human patients. Needless to say, large animal models like horses may be conducive to new advances in different fields of biomedical research, beyond the very interest in veterinary medicine itself. Because of the recognized role of the innate immune system in the response to tissue damage and stress, the

authors should offset their findings against the present knowledge concerning the role of miRNA in the regulation of innate immune responses. This way, they could highlight the relevance of their data to the immunology session.