RNA-SEQ ANALYSIS OF DIFFERENTIAL GENE EXPRESSION INDUCED BY LYSOZYME TREATMENT IN LYMPHOMA CELLS

1)Bergamo A. 2)Gerdol M. 3)Pallavicini A. 4)Sava G. 5)Allardyce C. 6)Dyson P.

Callerio Foundation Onlus

Lysozyme (LZ) is a natural peptide commonly known for its antimicrobial and immune-modulating activities (Jollés, 1996). Recent findings have shown that LZ, besides its muramidase activity that makes it broadly used as food preservative, is also active in the context of ageing (Yeboah et al., 2003) and to slow down the progression of diabetic complications (Cocchietto et al., 2008; Gallo et al., 2014). We have recently developed and characterized LZ-containing microspheres (MSLZ) as a vehicle to protect and improve the delivery of the bioactive peptide through the gastrointestinal tract (Zorzin et al., 2006). Here we investigate the differential gene expression induced by LZ on monocyte-like cells in vitro, in order to get insights on how these changes might support its pharmacological activities. To this aim we used the human U937 histiocytic lymphoma cells, optionally also differentiated to macrophages. The cells have been exposed for 1 h or 24 h to 7.5, 15 and 30 □g/ml LZ prior to analyse, by RNA-seq, the effects on gene expression immediately after exposure, or 2 h after the 1 h exposure. The effects of pure LZ were compared to those of the LZ extracted from MSLZ solubilized mimicking their passage through the stomach and the gut. A proteomic evaluation was also carried out to know how the changes of gene levels affect their translation products.

The largest number of differentially expressed genes was found at the end of the treatment with LZ for 1 h. Gene expression, compared to untreated controls, decreases at 2 h after the 1 h exposure and also after 24 h of continuous cell exposure. Although LZ modifies the gene expression level of a few dozen genes, the gene expression fold change is rather high, suggesting a significant functional impact. Moreover, some genes (CKMT2, TMEM150C, DLG2) recur differentially expressed at all times of analysis respect to the controls, pointing out to their likely master role in the effects of LZ. To determine the significant pathways of differentially expressed genes Ingenuity□ Pathway Analysis (IPA□) was applied for the analysis, integration and interpretation of data derived from the RNA-seq experiment. This preliminary evaluation uncovers LZ effects on cell circuitries involving signalling and signal transduction pathways. In particular, the alterations of the gene expression pattern observed are consistent with a down-regulation of NF-□B, and the consequent suppression of pro-inflammatory cytokines. These findings, besides looking into the pathways related to the immune-modulatory mechanism of action of LZ, also serve to get more and new information on the genomic activities of this natural and ubiquitary peptide.

Acknowledgements

We thank the Integrative Food and Nutrition Center (EPFL) for financial support.

Cocchietto et al. (2008). Diabetes and Metabolism. 34, 597-4.

Gallo et al. (2014). Exp Biol Med. 239, 337-46.

Jollés. (1996). Lysozymes: Model Enzymes in Biochemistry and Biology. Birkhäuser Verlag, Basel.

Yeboah et al. (2003). WO 2003/0332969.

Zorzin et al. (2006). J Drug Del Sci Tech. 16, 413-20.