Summary

White adipose tissue plays an important role in maintaining energy balance and thus homeostasis of the body. Excessive accumulation of fat by adipocytes impairs their proper lipid storage and secretory functions. Enhanced production of proinflammatory cytokines leads to the development of chronic inflammation in adipose tissue, which is the main cause of metabolic diseases. In order to understand biological process of adipocytes it is necessary to identify factors regulating both adipocytes differentiation and inflammatory response. Such properties possess MCPIP1 protein. As an RNase, MCPIP1 decreases level of transcripts coding for proinflammatory cytokines and negatively regulates NF-κB and AP-1 activity. In addition, MCPIP1 plays an important role in the regulation of adipogenesis *in vitro* by degrading transcript encoding C/EBPβ.

This work characterized the MCPIP1 protein as a ribonuclease, whose anti-inflammatory and adipogenesis-regulating properties have been demonstrated in a model of adipogenesis *in vitro* using the mouse 3T3-L1 cell line, a population of human stem cells derived from subcutaneous adipose tissue (sASC), and biopsy material obtained from murine and human adipose tissue.

To determine link between MCPIP1 and obesity development, we analyzed changes in MCPIP1 levels depending on the amount of fat mass. We found reduced level of Mcpip1 in adipose tissue isolated from mice subjected to a 12-week high fat diet in comparison to animals fed a control diet. Using biopsy material of subcutaneous (SAT) and visceral fat (VAT) obtained from obese (BMI $\geq$ 39 kg/m^2) and lean patients (BMI $\leq$ 28 kg/m^2), we observed decreased amount of MCPIP1 (both at the mRNA and protein level) along with an increase in body mass index (BMI) in SAT. In addition, MCPIP1 negatively correlated with the amount of transcripts coding for IL-6 and MCP-1, GLUT4 and the macrophage marker, CD68 in SAT. Elevated levels of proinflammatory cytokines observed in adipose tissue of obese subjects might be explained by a reduced MCPIP1 level in this tissue.

Our studies of adipogenesis *in vitro* with the use of sASC showed that the MCPIP1 mRNA level was significantly decreased in the course of adipogenesis, and it was inversely correlated with the expression of transcripts encoding key adipogenic factors, C/EBPβ and PPARγ. In contrast, MCPIP1 at the protein level underwent dynamic changes during the adipogenesis of sASC, which may indicate its posttranslational modifications. Increased level of mutated MCPIP1, with the point mutation (D141N) in the PIN domain, depriving it of

RNase activity, did not affect the adipogenesis of 3T3-L1 preadipocytes, in contrast to wild-type form, which reduced levels of C/EBPα and PPARγ and in consequence disturbed the differentiation process into mature adipocytes.

To further understand the mechanisms of adipocyte-specific processes regulated by MCPIP1, we performed global transcriptome and proteome analysis in 3T3-L1 adipocytes with exogenous $_{WT}$MCPIP1 or $_{D141N}$MCPIP1 and cells with an empty vector, used as a control in the study, on day 2 and 4 of adipogenic differentiation, respectively. Our RNA-Seq analysis followed by confirmatory qRT-PCR revealed that elevated $_{WT}$MCPIP1 levels in 3T3-L1 adipocytes upregulated transcripts encoding proteins involved in signal transmission and cellular remodeling and downregulated transcripts of factors involved in metabolism. These data are consistent with our proteomic analysis, which showed that adipocytes with exogenous $_{WT}$MCPIP1 exhibited upregulation of proteins involved in cellular organization and movement and decreased levels of proteins involved in lipid and carbohydrate metabolism.

Next, we examined impact of $_{WT}$MCPIP1 or $_{D141N}$MCPIP1 protein on insulin-stimulated glucose uptake in 3T3-L1 adipocytes. We observed that exogenous $_{WT}$MCPIP1 in 3T3-L1 adipocytes resulted in a decreased level of glucose transporter, Glut4, both at the mRNA and protein levels. Moreover, 2-deoxyglucose uptake was significantly reduced in $_{WT}$MCPIP1 adipocytes as compared to mutant and control cells. In addition, elevated level of $_{WT}$MCPIP1 inhibited activation of the insulin signaling pathway in 3T3-L1 adipocytes, manifested by a decrease in insulin receptor and Akt kinase phosphorylation levels. These results indicate that MCPIP1 may inhibit adipogenesis by reducing Glut4 level and attenuating glucose uptake in response to insulin stimulation. We draw this conclusion because the differentiation impairment of 3T3-L1 adipocytes with exogenous $_{WT}$MCPIP1 protein has been rescued by the exogenic expression of transcript coding for Glut4 in these cells.

The data obtained so far indicate that MCPIP1 acts also as a suppressor of miRNA activity and biogenesis counteracting Dicer. In our study we analyzed the miRNA profile in 3T3-L1 adipocytes with exogenous $_{WT}$MCPIP1 and control cells, using Next-generation sequencing. Our results indicated that increased level of $_{WT}$MCPIP1 resulted in modulated levels of 58 miRNAs on day 2 of adipocyte differentiation, of which one third of the identified molecules have not been correlated with the adipogenesis process yet. Using the qRT-PCR method, we confirmed decreased level for 5 selected miRNA (miR-9-3p, miR-32-5p, miR-152-3p, miR-26b-5p, miR-30e-5p). Moreover, in reporter assays, we proved that two

of the selected transcripts, *Mapk8* and *Tiam1* are direct targets for miR-32-5p and miR-9-3p. In addition, activation of MAP kinases pathway (Jnk and p38), proposed as being modulated by downregulated miRNAs, was strongly activated in 3T3-L1 adipocytes with exogenous wild-type MCPIP1. We conclude that the increased level of $_{WT}$MCPIP1 decreases the expression of miRNAs important for the regulation of adipogenesis, which may help to explain the negative role of MCPIP1 in preadipocyte differentiation.

The integrative transcriptome and proteome analyses presented here show that in addition to the well-established anti-inflammatory role of MCPIP1, this protein is also an important regulator of adipogenesis and adipocytes metabolism. However, the mechanisms underlying the strong reduction in MCPIP1 in adipose tissue of obese patients remain unclear. Our data partially clarify the importance of MCPIP1 level in adipose tissue and show that its lack or reduced level, as in the case of people suffering from obesity, results in the presence of chronic inflammation, which negatively affects the homeostasis of the whole body. Additionally, further studies are interesting in terms of searching for MCPIP1 modulators preventing from its degradation in fat tissue of obese subjects.