miRNA-218TARGETS LIPIN-1AND GLUCOSE TRANSPORTER TYPE 4GENES IN 3T3-L1CELLS TREATED WITH LOPINAVIR/RITONAVIR

Elena Bresciani[1], Cecilia Saletti[1], Nicola Squillace[2], Laura Rizzi[1], Laura Molteni[1], Ramona Meanti[1], Robert J. Omeljaniuk[3], Giuseppe Biagini[4], Andrea Gori[5], Vittorio Locatelli[1], Antonio Torsello[1]

[1]School of Medicine and Surgery, University of Milan-Bicocca, Monza - Italy, [2]Division of Infectious Diseases, Department of Internal Medicine, San Gerardo Hospital, Monza - Italy, [3]Department of Biology, Lakehead University, Thunder Bay, P7B 5E1ON - Canada, [4]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena - Italy, [5]Infectious Diseases Unit, Department of Internal Medicine, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, University of Milan, Milan - Italy

Introduction: Metabolic complications represent a common and serious problem associated with HIV infection and combined Antiretroviral Therapy (cART). Alterations in body fat distribution are associated with significantly increased risks of (i) metabolic derangements, (ii) cardiovascular pathologies, and (iii) insulin resistance. A case control study showed that in subcutaneous adipose tissue from HIV-infected patients on cART presenting lipodystrophy (LS), the levels of miRNA-218were upregulated and those of lipin-1, a putative target gene of miRNA-218, were downregulated compared with HIV-negative subjects. Lipin-1is one of the most important factors linked to development of LS. Lipin-1, by controlling PPARγ2, regulates the expression of specific genes, such as that of Glucose Transporter Type 4(GLUT-4), required for maturation and maintenance of adipocytes. The aim of this research is to determine whether lopinavir/ritonavir (LPV/RTV) can modulate lipogenesis in adipocytes affecting miRNA-218and lipin-1mRNA expression, and to investigate the functional link between miRNA-218and GLUT-4mRNA expression.

Material and methods: Differentiated 3T3-L1cells were treated with various combinations of LPV/RTV, followed by measurements of cell viability, lipid accumulation, lipin-1and GLUT-4mRNA and miRNA-218levels. Transfection of anti-miR-218or a miRNA-218mimic were used to investigate the role of miRNA-218in lipogenesis.

Results: LPV/RTV treatment did not affect the viability of differentiated 3T3-L1cells, but caused (i) a significant decrease of lipid accumulation, (ii) an overexpression of miRNA-218, and (iii) a reduction of lipin-1and GLUT-4mRNA levels. The anti-miR-218transfection of 3T3-L1cells significantly ameliorated the adipogenic dysfunction and restored mRNA levels of lipin-1and GLUT-4consequent to LPV/RTV treatment. By contrast, 3T3-L1cells transfected with a specific miRNA-218mimic showed (i) an overexpression of miRNA-218, (ii) a reduced cellular lipid fraction, and (iii) decreased levels of mRNA for lipin-1and GLUT-4.

Discussion and conclusions: 3T3-L1cells, treated with LPV/RTV, show altered lipid content due to increased miRNA-218levels, which affects lipin-1mRNA. Moreover, increased miRNA-218levels were inversely correlated with changes in GLUT-4expression, which suggests a role for miRNA-218in mediating the insulin resistance consequent to cART.