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microRNA-17-5p and microRNA-20a-5p downregulation by atorvastatin in Hepg2 cells: a new mechanism involved in the lipid-lowering therapy

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Background: Hypercholesterolemia increases the risk of coronary artery disease and its pharmacological treatment has demonstrated, besides reducing cholesterol levels, a decrease in the incidence and mortality from coronary events. The treatment of hypercholesterolemia is mainly driven by using statins. However, the response to pharmacological therapy shows high inter-individual variability, resulting in a variable effect in both lipid lowering and risk reduction. Thus, a better understanding of lipid-lowering mechanism and response variability at molecular level is required. Previously, we demonstrated a deregulation of microRNA (miR) profile in HepG2 cells after atorvastatin treatment, including the downregulation of miR-17-5p and miR-20a-5p which potentially targets the LDL receptor gene (LDLR), suggesting that it might be involved in allowing the LDLR overexpression on the surface of hepatic cells to subsequently capture circulating LDL and to reach the expected lipid-lowering effect.

Purpose: To determine the role of miR-17-5p and miR-20a-5p on the regulation of LDLR gene expression in HepG2 cells.

Methods: Cells HepG2 were treated with atorvastatin 10µM for 24 hours. RNA extraction and enrichment of smallRNAs were performed. The gene expression of miR-17-5p, miR-20a-5p, miR-24-3p, miR-93-5p, miR-106a-5p and LDLR were evaluated. To evaluate the effect of miR-17-5p and miR-20a-5p on LDLR gene expression, both miRs were overexpressed or repressed by transfection of mimics or inhibitors respectively into HepG2 cells for 24, 48 and 72 Hours. The gene expression of LDLR was quantified by real time PCR using RPL27 gene as reference gene. The protein expression of LDLR and beta actin were evaluated using western blot and quantified using the ImageJ software.

Results: Our data showed that atorvastatin significantly repressed the expression of miR-17-5p (P<0.0001) and miR-20a-5p (P=0.0456) in HepG2 cells. In silico studies showed that miR-17-5p interact with the 3’-UTR region of the LDLR. Consistently, when miR-17-5p or miR-20a-5p were overexpressed by using mimics, we observed that gene and protein expression of LDLR decreased significantly (P<0.0001 and P<0.05 respectively). Consistently, when miR-17-5p or miR-20a-5p were repressed by the use inhibitors, we observed that the gene and protein expression of LDLR increases significantly (P < 0.005).

Conclusions: In conclusion, we demonstrate that atorvastatin induces a significant down-regulation of the miR-17-5p and miR-20a-5p in HepG2 cells. The overexpression or repression regulate the gene and protein expression of LDLR.