Functional and molecular analysis of aberrant expression of microRNA-133a in endothelial cells during cardiovascular disease

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INTRODUCTION: MicroRNA (miRNA) molecules are a class of small non-coding RNA molecules (~22 nucleotides), which target the 3' untranslated region (3' UTR) of mRNA and either induce mRNA degradation or suppress protein translation. Emerging evidence indicates miRNA molecules play a key role in the regulation of cardiovascular pathophysiology. miR-133a is mainly expressed in cardiomyocytes and skeletal muscle cells. In endothelial cells miR-133a is expressed at very low levels in physiological conditions however, increased expression of this microRNA in the endothelium has been strongly associated to cardiovascular disease. Although aberrant expression of miR-133a has been linked to endothelial dysfunction, the molecular and cellular mechanisms deregulated in endothelial cells by high expression of miR-133a remain largely unknown.

METHODS: Here, we have evaluated the consequences of aberrant expression of miR-133a in endothelial cells by transfecting primary Human Umbilical Vein Endothelial cells (HUVEC) with a double-stranded, miRNA-like mimic of miR-133a. This strategy simulates the situation observed in pathological conditions. A scramble miRNA-like mimic was used as negative control. The effect of "miR-133a mimic" in endothelial cell migration and tubular morphogenesis has been determined by performing wound-healing migration and matrigel assays. Changes in the expression of angiogenic genes caused by overexpression of miR-133a have been investigated by qPCR.

RESULTS: We show here that ectopic expression of miR-133a in endothelial cells robustly attenuates endothelial cell migration and VEGF-induced angiogenesis. As a first step to elucidate the molecular mechanisms underlying this inhibitory effect of miR-133a, we have screened gene arrays to identify changes in the expression of genes involved in cell motility and angiogenic signalling, and validated potential changes in gene expression by qPCR. Our results show that transfection of "miR-133a mimic" into primary endothelial cells strongly downregulates the expression of genes implicated in cell motility (such as *PLAUR* and *MSN*) and angiogenic signalling (such as *CD44* and *ID1*).

CONCLUSION: These data indicate that enhanced expression of miR-133a in endothelial cells impairs pro-angiogenic cellular processes by altering the expression of specific, target genes. Our results suggest that blockage of miR-133a function in endothelial cells might have important therapeutic applications to treat patients suffering from cardiovascular pathologies that occur with excessive angiogenesis.