Utilization of CRISPR/Cas9 to Knock Down Inflammatory and Angiogenic MicroRNAs in Endothelial Cells

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Pathological angiogenesis and fibrosis are the hallmark features of several cardiovascular and inflammatory diseases, including diabetic retinopathy, cardiomyopathy, atherosclerosis, and cancer. Emerging evidence indicates that an effective therapy may require targeting multiple genes that become aberrantly expressed in diseased tissues/organs. MicroRNAs are short nucleotide sequences that bind to the untranslated region of targeted genes and regulate their expression. Particularly, miR-155 is closely associated with the inflammation of numerous tissues including blood vessels, while miR-21 has been characterized as a pro-angiogenic and fibrotic molecule. They each play significant roles in post-transcriptional regulation of angiogenic, fibrotic, and inflammatory genes which become aberrantly expressed in diseased tissues. Thus, targeting miR-155 and/or miR-21 can effectively curtail the inflammatory, fibrotic, and/or aberrant angiogenic responses that initiate and exacerbate the disease process. CRISPR/Cas9 is a novel technology designed to edit parts of the genome by altering sections of the genomic DNA. Conceivably, editing miR-155 and miR-21 sequences using CRISPR/Cas 9 will provide a better control of the expression of angiogenic and inflammatory genes. For this purpose, RNA-guided Cas9 vectors specific to miR-155 and miR-21 were designed and their ability to edit the corresponding genomic DNA sequences was further tested in cultured endothelial cells. The data showed that both miR-155 and miR-21 were effectively targeted with the RNA-guided Cas9 and inactivated in endothelial cells. This study demonstrates the feasibility of CRISPR/Cas9 to edit microRNA sequences and provides the basis for the potential therapeutic utility of this approach.