Effect of vitamin D on microRNAs in endothelial cells exposed to a diabetic-like environment

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BACKGROUND

Diabetes mellitus (DM) is associated with endothelial dysfunction including changes in barrier function and hemostasis, reduced vasodilator response, inflammatory activation, increased plasma levels of endothelial products and angiogenesis, all of which are associated with greater incidence and severity of cardiovascular diseases. Many of the pathogenic pathways known to arbitrate diabetic complications are associated with increased glucose blood levels and accumulation of cellular and extracellular advanced glycation end products (AGEs).

MicroRNAs (miRs) are non-coding RNAs of approximately 22 nucleotides that regulate gene expression in a sequence specific manner. MiRs have been shown to be important in various biological processes and serve as novel biomarkers, modulators and therapeutic targets for diseases such as cancer, heart disease, and diabetes.

AIM

Decipher miR expression changes following vitamin D treatment on human umbilical vein endothelial cells (HUVEC), in a diabetic-like environment (200 µg/ml AGE-HSA and 250 mg/dl glucose).

METHODS

HUVEC were treated for 24 h with 200 µg/ml human serum albumin (HSA) and 100 mg/dl glucose (control) or 200 µg/ml AGE-HSA and 250 mg/dl glucose (diabetic-like environment), and physiological calcitriol (vitamin D) concentrations (10^{-10} mol/L). MiR microarray analysis, validated by miR and mRNA real time PCR were performed to characterize the expression profiles of the treated HUVEC. Pathway analysis of the results was performed.

RESULTS

AGE - GLU vs. Control
Cluster analysis of differentially expressed miRs (p<0.05 and FC cutoff 1.5).

AGE - GLU + vit. D vs. AGE -GLU:
Cluster analysis of differentially expressed miRs (p<0.05 and FC cutoff 1.5).

Differentially expressed miRNAs lists

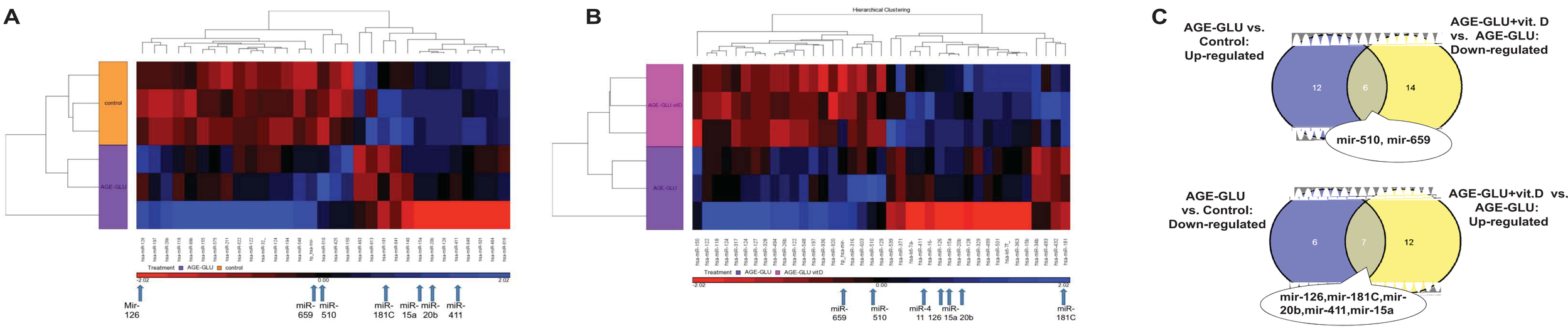

Fig. 1. Hierarchical clustering of miR expression in HUVEC. Seven focal miRs are indicated with arrows at the bottom of the clusters (A, B) and on the VENN diagram (C). Clusters present treatment with: (A) AGE-HSA and glucose 250 mg/dl (AGE-GLU), compared to control (HSA and 100 mg/dl glucose concentration); (B) AGE-HSA, glucose 250 mg/dl and 10^{-9} mol/l calcitriol (AGE-GLU+vit. D) compared to AGE-HSA and glucose 250 mg/dl (AGE-GLU); (C) Comparison between differentially expressed miR lists.

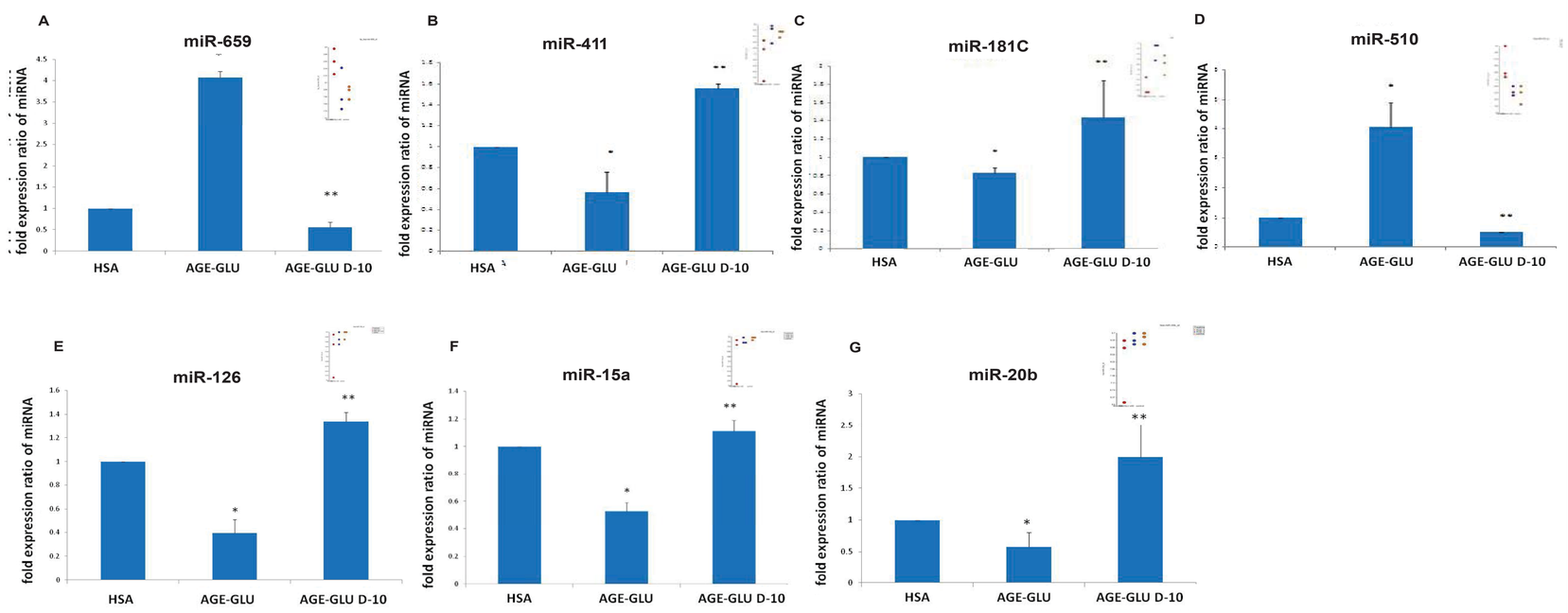

Fig 2. Validation of miR expression results by real time PCR. The miR set included (A) miR-659, (B) miR-411,(C) miR-181C, (D) miR-510, (E) miR-126, (F) miR-15a, and (G) miR-20b. The expression patterns determined by real-time PCR confirmed the microarray analysis. MiR-659 and miR-510 were significantly elevated following stimulation by the diabetic environment, while the addition of calcitriol (10^{-10} mol/l) significantly reduced the expression. MiR-411,miR-181C, miR-15a and miR-20b were significantly decreased following stimulation by diabetic environment, while the addition of calcitriol (10^{-10} mol/l) significantly up-regulated the expression. Insets show miR microarray expression results. *P<0.05 compared to control group-HSA. ** P<0.05 compared with AGE-Glucose.

Table 1: Putative target genes for the selected miRs (TargetScan analysis)

	miR-181c	miR-20b	miR-15a
KLF6	KLF6		
KLF15	KLF15		
KLF3		KLF3	
KLF9	KLF9		
KLF10	KLF10	+	
KLF12		KLF12	
IL8		IL8	
TXNIP		TXNIP	+

Fig 3. Predicted miR-target protein interactions including direct (physical) and indirect (functional) associations. Real-time PCR showed that TXNIP, IL8 and KLF6 mRNA expression were significantly up-regulated in HUVEC exposed to a diabetic-like environment. The addition of calcitriol significantly down-regulated IL8, KLF6, KLF9 and KLF10 mRNA expression.

CONCLUSIONS

1. The miRs identified in this study might provide new information about gene regulation in a diabetic-like environment after addition of calcitriol. Several of the miRs such as miR-510, miR-126, miR-15a and miR-20b are already known to respond to a diabetic environment.
2. Three miRs ,miR-181C, miR-15a and miR-20b, which were up-regulated after the addition of vitamin D, were further analyzed as a basis to explore the function of their putative targets.
3. This study highlights gene targets from the Kruppel-like family (KLF6, KLF9 and KLF10), which are transcription factors that play key regulatory roles in cellular growth, differentiation, proliferation, apoptosis and angiogenesis.
4. TXNIP, IL8 and genes from the Kruppel-like family, were found to correspond to molecular and biological processes such as nucleic acid binding transcription factor activity, chemokine receptor binding and positive regulation of RNA metabolic processes, which are part of the biological and molecular mechanisms in DM.
5. These findings might in the future contribute to the understanding of the role of vitamin D in treating patients with diabetes.

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246-MP
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