Mir-431 as a Potential Master Regulator in Angiotensin Ii-induced Vascular Injury

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Objective: Vascular injury is an early manifestation and a cause of end-organ damage in hypertension. microRNAs (miRNAs) play an important role in cardiovascular disease, but their implication in vascular injury is remains unclear. We aim to use RNA sequencing (seq) and a systems biology approach to identify master regulators that mediate global gene expression changes in the course of vascular injury.
Methods and Results: Ten week-old male C57BL/6 mice were infused or not with angiotensin (Ang) II (1 μg/kg/min, SC) for 14 days. Blood pressure (BP) was measured by telemetry. Total RNA was extracted from the mesenteric vasculature for total RNA and small RNA-seq. Differentially expressed (DE) miRNAs (23 up and 12 down) and mRNAs (550 up and 256 down) were identified (1.5-fold, q<0.05). Molecular networks were constructed to integrate predicted interactions between DE miRNAs and inversely expressed DE mRNAs and between DE transcription factors (TF) and DE genes. Gene enrichment analysis revealed DE mRNAs involved in extracellular matrix (ECM) and developmental processes regulated by DE miRNAs (q<1.5E-11). Seventeen upregulated miRNAs are located in the miRNA cluster of the Dlk1-Dio3 region that is highly conserved in humans, 9 of which had expression levels correlated with BP (P<0.05). Among those 9, miR-431 that ranked first as DE miRNA (q<0.0005) and is 100% conserved in humans, and a conserved putative DE target, a BP-correlated (P<0.05) TF ETS homologous factor (*Ehf*), which regulates numerous ECM genes including collagen type I α1 (*Col1a1*), were selected for functional studies. Transfection of a miR-431 mimic in human aortic smooth muscle cells (HASMCs) decreased *Ehf* (0.1±0.1-fold, P<0.001) and increased *Ehf*-suppressing target *Col1a1* (1.7±0.5-fold, P<0.001) mRNA levels. Transfection of a miR-431 inhibitor caused reciprocal effects (P<0.05). *Ehf* siRNA knockdown increased *Col1a1* (1.2±0.1-fold, P<0.001) mRNA levels.
Conclusions: Ang II infusion altered expression of miRNAs in the Dlk1-Dio3 cluster and genes involved in ECM and developmental processes. miR-431 targets TF *Ehf*, which leads to increased *Col1a1* in HASMCs. miR-431 may act as a master regulator for vascular injury and could be a potential therapeutic target.

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