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Molecular crosstalk between microRNA-34a and SIRT1 in hyperglycaemia-induced impaired angiogenesis: Effects of metformin

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Background: Angiogenesis plays an essential role in the maintenance and repair of tissues. An imbalance of angiogenesis critically contributes to the pathogenesis of diabetes-associated cardiovascular disease (CVD). MicroRNAs (miR), small non-coding RNAs, have been identified as important transcriptional and post-transcriptional regulators of gene expression and also regulators of angiogenesis. Deregulated miR expression and its translational output (protein expression) have been critically implicated in the pathogenesis of diabetes-mediated impaired angiogenesis (1). Clinical data suggests that metformin, in addition to its hypoglycaemic action, may also have a direct protective action on the vasculature (2)**.** miR-34a has been reported to target SIRT1 and to be critically involved in angiogenesis. In-silico analysis and cell culture studies indicate that SIRT1 is a potential target for endothelial cell specific miRs (3). In the current study, we investigated the molecular crosstalk between miR-34a and SIRT1 in metformin-treated endothelial cells.

Methods: Mouse microvascular endothelial cells (MMECs) were maintained in culture under either normoglycaemic (NG, 11 mM glucose) or hyperglycaemic (HG, 40 mM) conditions. miR-34a expression, SIRT1 and downstream signalling molecules were analysed by real-time PCR and western blotting. Statistical analysis (GraphPad Prism 5.0) of the data was performed by one-way analysis of variance (ANOVA). Post-hoc comparisons between the groups were performed by Tukey Multiple Comparisons Test. Results are presented as Mean±SEM with $P < 0.05$ used to indicate statistical significance. Results were normalized to controls (NG).

Results: Real-time PCR analysis reveals that exposure of MMECs to HG resulted in a significant ($p<0.05$) increase in miR-34a expression (1.80±0.03 fold increase) and this induction paralleled and correlated with altered expression of SIRT1 (2.41±0.2 fold decrease), ratio of phospho/total eNOS (1.40±0.15 fold decrease), as well as altered markers of angiogenesis (VEGF, Ang-1, Ang-2, TSP-1) when compared to MMECs exposed to NG. Inhibition of miR-34a (anti-miR) significantly ($p<0.05$) increased SIRT1 (2.46±0.2 fold increase) expression, attenuated changes in downstream signalling and impaired angiogenesis in HG-exposed MMECs. Conversely, treatment with metformin (50 μM) also inhibited miR-34a expression and attenuated HG-induced impaired angiogenesis in MMECs.

Conclusion: miR-34a, via the regulation of SIRT1 expression, has an anti-angiogenic action in microvascular endothelial cells that is attenuated by metformin. miR-34a may represent a new therapeutic target for the prevention/treatment of diabetic vascular disease.

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