Endothelin regulation by miR-218: A target in scarring
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The adult human dermis scars; scarless repair occurs in the oral cavity. Alpha-smooth muscle (α-SMA)-expressing myofibroblasts are responsible for scarring. TGFβ1 causes myofibroblast differentiation in dermal but not gingival fibroblasts (N = 3, p<0.05). Gingival fibroblasts express less focal adhesion kinase, display less focal adhesion kinase phosphorylation and do not induce endothelin-1 (ET-1) in response to TGFβ1 (N = 3, p<0.05). The induction of ET-1 in response to TGFβ1 in dermal fibroblasts does not occur in the absence of FAK or in the presence of the FAK/src inhibitor PP2 (N = 3, p<0.001). Expression profiling revealed that, compared to gingival fibroblasts, dermal fibroblasts overexpress a variety of miRNAs including miR-218. Addition of miR-218 to gingival fibroblasts results in enhanced focal adhesion kinase expression and phosphorylation, cell spreading, endothelin-1 production and in the ability of TGFβ1 to induce myofibroblast formation (N=3, p<0.01). Knockdown of miR-218 abolishes the ability of TGFβ1 to induce myofibroblast formation in dermal fibroblasts. These results strongly suggest that the presence or absence of ET-1 is responsible for myofibroblast formation in fibroblasts. Targeting ET-1 might be a viable approach to prevent scarring.


miRNA-1 Regulates endothelin-1 in diabetes
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MicroRNAs-1 (miR-1) plays important roles in several biological processes. ET-1 is upregulated in chronic diabetic complications. In this study, we investigated the role of miR-1, an ET-1 targeting miRNA, in the endothelial cells (ECs) and in the organs of diabetic animals. PCR array was used to identify alteration of miR expressions in the ECs exposed to glucose. miR-1 expression was validated by TaqMan Real-Time PCR assay. Human retinal ECs (HRECs) exposed to various glucose levels with or without miR-1 mimic transfection as well as tissues from streptozotocin-induced diabetic animals after 2 months of follow-up, were examined for ET-1 mRNA and protein levels, fibronectin (FN) mRNA and miR-1 expression. Array analyses showed glucose-induced alterations of 125 miRNAs (out of 381) in ECs exposed to 25 mM glucose (HG) compared to 5 mM glucose. Fifty-one miRNAs were upregulated and 74 were downregulated. HG decreased miR-1 expression and increased ET-1 mRNA and protein levels. miR-1 mimic transfection prevented HG-induced ET-1 upregulation. Furthermore, glucose induced upregulation of FN, which is mediated in part by ET-1, was also prevented by such transfection. Diabetic animals showed decreased miR-1 expression in the retina, heart, and kidneys. In parallel, ET-1 mRNA expressions were increased in these tissues of diabetic animals compared to controls. Furthermore these tissues showed upregulation of FN. These studies indicate a novel glucose-induced molecular mechanism of tissue damage, in which miR-1 regulates ET-1 expressions in diabetes. Identifying such mechanisms may lead to potential RNA based treatment for diabetic complications. Supported by Canadian Diabetes Assn. and Heart and Stroke Foundation of Canada.

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Endothelin-1 mediates downstream profibrotic effects by transforming growth factor-beta 1 in systemic sclerosis skin fibroblasts
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Systemic sclerosis (SSc) is an autoimmune connective tissue disorder characterized by excess collagen deposition and vascular changes that affect multiple organs. Although transforming growth factorβ1 (TGF-β1) and endothelin-1 (ET-1) are known to be potent fibrotic factors in SSc, the relationship between them is not fully understood. The aim of our study was to examine the effects of TGF-β1 on the fibrogenic phenotype of SSc skin fibroblasts through ET-1 production. Human skin fibroblasts obtained from SSc patients were incubated with TGF-β1 in the presence of SIS3 (an inhibitor of Smad3 phosphorylation). In addition, the effects of ETRA, ETRB and dual ETRA/ETRB antagonist were explored. Expression of ET-1, CTGF and type I collagen was evaluated by using ELISA and real time RT-PCR. ETRA and ETRB expressions were assessed by immunohistochemistry. We found that TGF-β1 increased ET-1 mRNA and protein expression and this increase in ET-1 was suppressed by SIS3. Upregulation of COL1A1 and