SUPPLEMENTARY DATA

Supplementary Material

Title: Inositol Requiring Enzyme 1 Facilitates Diabetic Wound Healing through Modulating microRNAs

Authors: Jie-Mei Wang[#,1,2], Yining Qiu[1], Zeng-quan Yang[4], Li Li[1,5], Kezhong Zhang[#,1,3,4]
Institution information: [1]Center for Molecular Medicine and Genetics, [2]Department of Pharmaceutical Sciences, Eugene Applebaum College of Pharmacy & Health Sciences; [3]Department of Immunology and Microbiology, [4]Karmanos Cancer Institute, [5]Department of Internal Medicine, Wayne State University School of Medicine; Detroit, MI.

Running title: **IRE1α suppresses microRNAs in diabetic wound healing**

 # Corresponding to:
Jie-Mei Wang, PhD, Center for Molecular Medicine and Genetics, [2]Department of Pharmaceutical Sciences, Eugene Applebaum College of Pharmacy & Health Sciences, Wayne State University. Address: 259 Mack Ave, 3122 Applebaum Building, Detroit, MI 48202, USA. Tel: +1 313 577 1715; Fax: +1 313 577 5218; E-mail: jiemei.wang@wayne.edu.

Kezhong Zhang, PhD, Center for Molecular Medicine and Genetics; Wayne State University School of Medicine. Address: 540 E. Canfield Avenue, 3128 Scott Hall, Detroit, MI 48201, USA. Tel.: +1 313 577 2669; Fax: +1 313 577 5218; E-mail: kezhong.zhang@wayne.edu.

SUPPLEMENTARY DATA

The canonical unfolded protein response transcripts were not activated in diabetic BMPCs.
To test whether the canonical unfolded protein response (UPR) was activated in db/db type-2 diabetic BMPCs, the major transcripts in canonical UPR pathways including IRE1α, spliced XBP1 mRNA (XBP1s), Bip, ERO1, ERdj4, ATF4, ATF3, GADD34, CHOP, p58[ipk], Der1, in db/db BMPCs were detected using real-time PCRs. As shown in Supplemental Figure 1, our results suggested that none of these genes were significantly elevated (n=6 per group, p>0.05 vs. db/+). This evidence indicated that metabolic stress regulates ER function through non-canonical pathways in BMPCs.

Over-expression of Dicer improved BMPC tube formation
To determine the effect of Dicer on BMPC tube formation, we over-expressed Dicer in db/db BMPCs using adenovirus-mediated gene expression system. Western blot confirmed that Dicer protein expression in db/db BMPCs was significantly up-regulated after Ad-Dicer transfection (Supplemental Figure 2A). The tube formation assay was used to evaluate the effect of Dicer over-expression on BMPC function. Our results indicated that over-expression of Dicer improved BMPC tube formation (Supplemental Figure 2B, 2C), compared to their wildtype diabetic control

SUPPLEMENTARY DATA

Supplementary Figure 1. Unfolded protein response transcript expressions in db/db and db/+ BMPCs.

BMPCs from db/+ and db/db mice were cultured for 7 days as described in Methods section. Total RNA from BMPCs was isolated by RNeasy Mini Kit (Qiagen). For mRNA expression analysis, qRT-PCR was performed using primers synthesized by IDT Technologies. The primer sequences are shown in the Supplemental Table 1. Amplification and detection of specific RNA products were performed with the ABI PRISM 7500 Sequence Detection System, using GAPDH as an internal control. n=4 per group. No statistical significance was found in the detected genes between db/db and db/+ BMPCs.
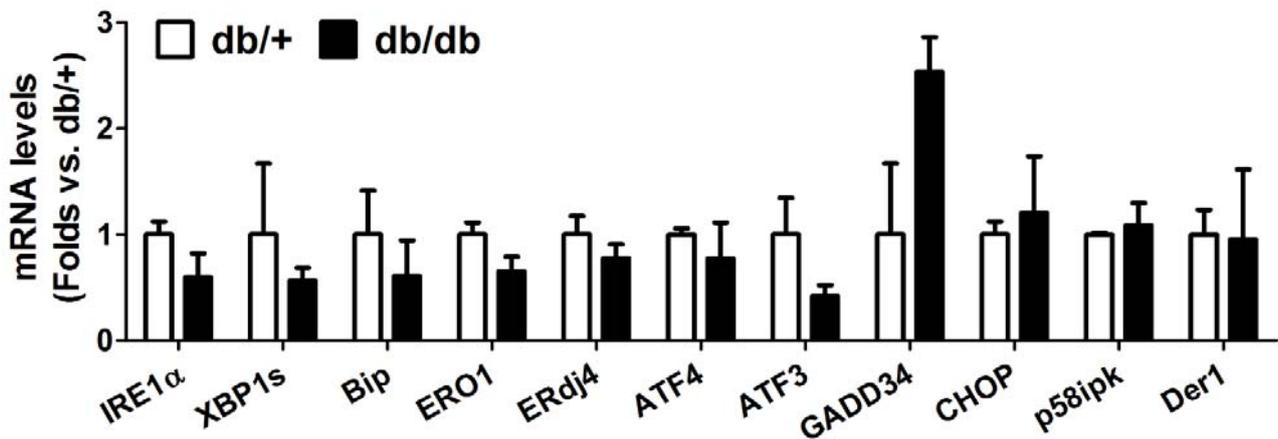
SUPPLEMENTARY DATA

Supplementary Figure 2. Dicer over-expression improved diabetic BMPC tube formation.
BMPCs from db/+ and db/db mice were cultured for 7 days as described in Methods section. Adenovirus expressing human Dicer1 and eGFP were purchased from VectorBiolabs, Inc. For transfection of cells with adenovirus, cells were seeded in six-well plates. After 24 hours, cells were transfected with Ad-Dicer1 (50 MOI) Ad-GFP (50 MOI) for 48 hours. After the transfection, cells were harvested for tube formation on Matrigel and cell protein lysate was collected for Western Blot analysis. **(A).** Western Blot analysis of Dicer protein expression in db/db BMPCs transfected with Ad-Dicer1 or Ad-GFP using db/+ BMPCs transfected with Ad-GFP as normal controls. n=4 per group, *p<0.05 vs. db/+ - GFP, # p<0.05 vs. db/db-GFP. **(B).** Accumulated tube formation in db/db BMPCs transfected with Ad-Dicer1 or Ad-GFP using db/+ BMPCs transfected with Ad-GFP as normal controls. n=4 per group, *p<0.05 vs. db/+ - GFP, # p<0.05 vs. db/db-GFP. **(C).** Representative Pictures showing tube network formed by db/db BMPCs transfected with Ad-Dicer1 or Ad-GFP using db/+ BMPCs transfected with Ad-GFP as normal controls.
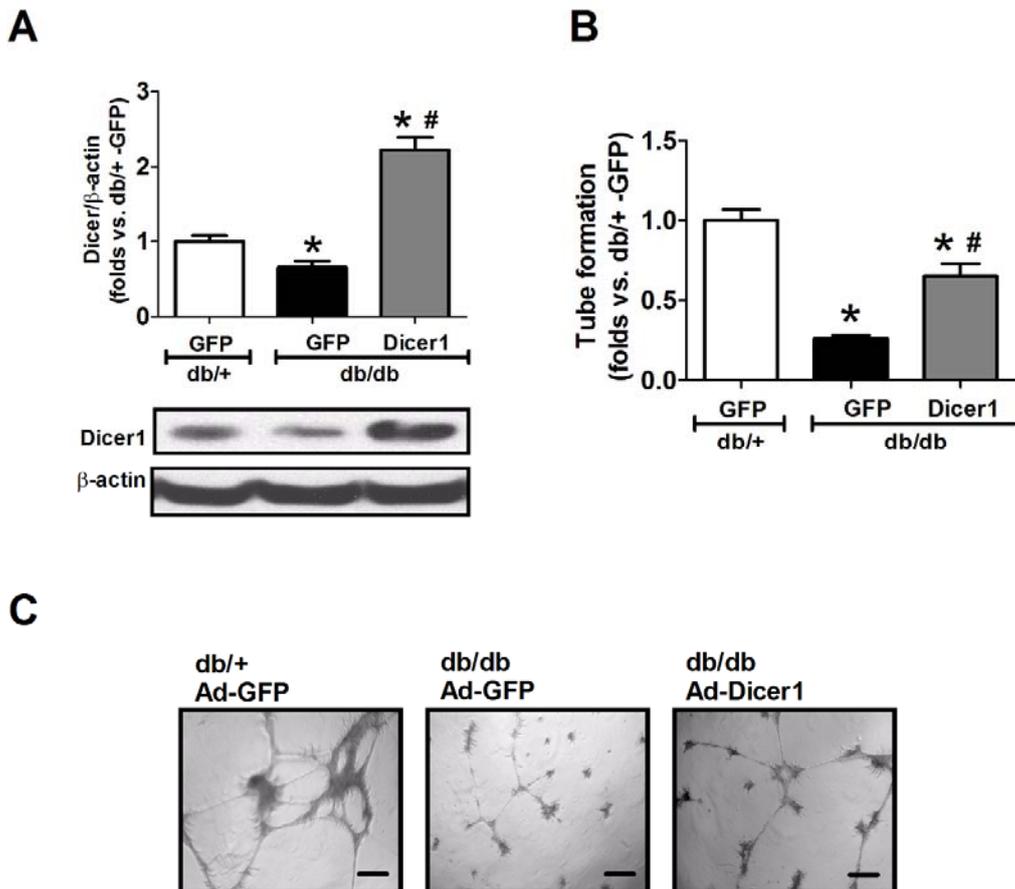
SUPPLEMENTARY DATA

Supplementary Table 1. Primer sequences for real-time PCRs.

Gene name	Forward primer	Reverse primer
IRE1α	5'-TGTGGTCAAGATGGACTGGC-3'	5'-TCGGAGGAGGTCTCTCACAG-3'
XBP1s	5'-AGTCCGCAGCAGGTGCA-3'	5'-GTCAGAGTCCATGGGAAGATG-3'
Bip	5'-CATGGTTCTCACTAAAATGAAAGG-3'	5'-GCTGGTACAGTAACAACTG-3'
ERO1	5'-AGCTGGTCCCAGTGACAGAAA-3'	5'-ACAGCCCTGCATTACAGAGGA-3'
ERdj4	5'-CCCCAGTGTCAAACTGTACCAG-3'	5'-AGCGTTTCCAATTTTCCATAAATT-3'
ATF4	5'-ATGGCCGGCTATGGATGAT-3'	5'-CGAAGTCAAACTCTTTCAGATCCATT-3'
ATF3	5'-AATCGGCTAACCCGCGCTCC-3'	5'-GGGACAATGGCGGTCGCACT-3'
GADD34	5'-AGCCGCGTGGACGATGTTGG-3'	5'-AGCCAGCGGGTTCATGTCGC-3'
CHOP	5'-CTGCCTTTCACCTTGGAGAC-3'	5'-CGTTTCCTGGGGATGAGATA-3'
p58ipk	5'-TCCTGGTGGACCTGCAGTACG-3'	5'-CTGCGAGTAATTTCTTCCCC-3'
Der1	5'-CATCACGCGCTACTGGTTTG-3'	5'-CTTGCCGATCAAGGGGACAG-3'
GAPDH	5'- CCAGGAAGACGCTTGAAAAG-3'	5'-TCTGAGCCTCCTCCAATTC-3'