Received: 3 July 2019 | Revised: 4 October 2019 | Accepted: 8 October 2019

DOI: 10.1096/fj.201901599RR

RESEARCH ARTICLE

THE FASEB JOURNAL

# MiR-9-5p protects from kidney fibrosis by metabolic reprogramming

**Marta Fierro-Fernández[1] | Verónica Miguel[1] | Laura Márquez-Expósito[2] | Cristina Nuevo-Tapioles[1] | J. Ignacio Herrero[1] | Eva Blanco-Ruiz[1] | Jessica Tituaña[1] | Carolina Castillo[3] | Pablo Cannata[2] | María Monsalve[4] | Marta Ruiz-Ortega[2] | Ricardo Ramos[5] | Santiago Lamas[1]**

[1]Department of Cell Biology and Immunology, Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid, Spain

[2]Instituto de Investigación Sanitaria, Fundación Jiménez Díaz (UAM), Madrid, Spain

[3]Hospital Universitario "Príncipe de Asturias", Madrid, Spain

[4]Instituto de Investigaciones Biomédicas "Alberto Sols", (CSIC-UAM), Madrid, Spain

[5]Servicio de Genómica, Fundación Parque Científico de Madrid, Madrid, Spain

**Correspondence**
Santiago Lamas, Department of Cell Biology and Immunology, Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid, Spain
Email: slamas@cbm.csic.es

**Funding information**
Ministerio de Economía y Competitividad (MINECO), Grant/Award Number: SAF2015-66107-R, SAF 2012-31388, SAF2015-63904-R, BES-2013-065986 and BES-2014-068929; MINECO |

**Abstract**

MicroRNAs (miRNAs) regulate gene expression posttranscriptionally and control biological processes (BPs), including fibrogenesis. Kidney fibrosis remains a clinical challenge and miRNAs may represent a valid therapeutic avenue. We show that miR-9-5p protected from renal fibrosis in the mouse model of unilateral ureteral obstruction (UUO). This was reflected in reduced expression of pro-fibrotic markers, decreased number of infiltrating monocytes/macrophages, and diminished tubular epithelial cell injury and transforming growth factor-beta 1 (TGF-β1)-dependent de-differentiation in human kidney proximal tubular (HKC-8) cells. RNA-sequencing (RNA-Seq) studies in the UUO model revealed that treatment with miR-9-5p prevented the downregulation of genes related to key metabolic pathways, including mitochondrial function, oxidative phosphorylation (OXPHOS), fatty acid oxidation (FAO), and glycolysis. Studies in human tubular epithelial cells demonstrated that miR-9-5p impeded TGF-β1-induced bioenergetics derangement. The expression of the FAO-related axis peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α)-peroxisome proliferator-activated receptor alpha (PPARα) was reduced by UUO, although preserved by the administration of miR-9-5p. We found that in mice null for the mitochondrial master regulator PGC-1α, miR-9-5p was unable to promote a protective effect in the UUO model. We propose that miR-9-5p elicits a protective response to chronic kidney injury and renal fibrosis by inducing

**Abbreviations:** 2,4-DNP, 2-4 dinitrophenol; 2-DG, 2-deoxyglucose; ADGRE1, adhesion G protein-coupled receptor E1; AKI, acute kidney injury; BPs, biological processes; BSA, bovine serum albumin; CKD, chronic kidney disease; COL1α1, collagen 1α1; CPT1A, carnitine palmitoyl-transferase 1a; CTGF, connective tissue growth factor; DAVID, database for annotation, visualization, and integrated discovery; ECAR, extracellular acidification rate; ENA, European nucleotide archive; ESRD, end-stage renal disease; FAO, fatty acid oxidation; FBS, fetal bovine serum; FMO, flow minus one; FN1, fibronectin; FPKM, fragments per kilobase of exon model per million reads mapped; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H&E, Hematoxylin and EOSIN; IHC, immunohistochemistry; IPA, ingenuity pathway analysis; ITS, insulin-transferrin-selenium; KEGG, Kyoto encyclopedia of genes and genomes; miRNA, microRNA; NOX4, NADPH-oxidase 4; OCR, oxygen consumption rate; OSR, oligomycin-sensitive respiration; OXPHOS, oxidative phosphorylation; PAS, periodic acid-Schiff; PCNA, proliferating cell nuclear antigen; PDGFR β, platelet-derived growth factor receptor beta; PGC-1α, proliferator-activated receptor gamma coactivator 1 alpha; PPARα, peroxisome proliferator-activated receptor alpha; RNA-Seq, RNA-sequencing; TCA, tricarboxylic acid; TGFBR2, transforming growth factor-beta receptor II; TGF-β1, transforming growth factor-beta 1; UUO, unilateral ureteral obstruction; α-SMA, alpha-smooth muscle actin.

Verónica Miguel and Laura Márquez-Expósito contributed equally as second authors.