Changes of MicroRNA-377 in Diabetic Nephropathy

Thesis

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Mervat Hussein

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List of Abbreviations

Abb.	Full term
ACE	*Angiotensin-Converting Enzyme*
ARP	*Angiotensin Receptor Blocker*
CDNA	*Complementary DNA*
CKD	*Chronic Kidney Disease*
COL	*Collagen*
C_T	*Cycle Threshold*
DGCR8	*Digeorge Syndrome Critical Region 8*
DM	*Diabetes Mellitus*
DN	*Diabetic Nephropathy*
E111A	*Alternatively Spliced Fibronectin Type 111 Repeat A*
E111B	*Alternatively Spliced Fibronectin Type 111 Repeat B*
ESRD	*End Stage Renal Disease*
FN	*Fibronectin*
GFR	*Glomerular Filtration Rate*
GLP-1	*Glucagon Like Peptide 1*
LDL	*Low Density Lipoprotein*
MAPK	*Mitogen Activated Protein Kinase*
miRISC	*RISC with Incorporated miRNA*
miRNA	*Mature Form of microRNA*
miRNP	*miRNA Ribonucleo Protein*
NF-KB	*Nuclear Factor Kappa B*
NIDDM	*Non-Insulin Dependent Diabetes Mellitus*
NOX4	*NADPH Oxidase Subunite 4*

List of Abbreviations (Cont...)

Abb.	Full term

NTP.....*Nucleotide Triphasphate*

P38 MAPK.....*P38 Mitogen Activated Protein Kinase*

PAK.....*p21 Activated Kinase*

Pri-miRNA.....*Primary microRNA*

qRT- PCR .....*Quantitative Reverse- Transcription Polymerase Chain Reaction*

RISC .....*RNA Induced Silencing Complex*

rpm.....*Round per Minute*

RT .....*Reverse Trascription*

Rt-PCR.....*Real Time Polymerase Chain Reaction*

SGLT2 .....*Sodium-Glucose Cotransporter 2*

SMA.....*Smooth Muscle Actin*

SOD .....*Super Oxide Dismutase*

STZ.....*Streptozotocin*

TGFB .....*Transforming Growth Factor B*

TIMP.....*Tissue Inhibitor of Metallo-Proteinase*

UAE .....*Urinary Albumin Excretion*

VEGF.....*Vascular Endothelial Growth Factor*

YWHAZ.....*Tyrosine 3- Monooxygenase /Tryptophan 5-Monooxygenase Protein Zeta*

ABSTRACT

Background: as one of the most important long term complications of diabetes, Diabetic nephropathy is the major cause of end stage renal disease and high mortality.

Purpose: to identify the pattern of microRNA-377 changes specific for diabetic nephropathy in diabetic patients and in patients with chronic kidney disease of different etiology.

Patients and Methods: the study was conducted from 2016 to 2018, included 50 patients for analysis of MicroRNA-377 and its control gene U18 at El Demrdash Hospital Ain shams university and Quessena Hospital El Monfia. The miRNA-U18 was analyzed for normalization of correction ratio.

Results: the results of our research found that the highest median IQR of miR-377 was significantly present in DN stage 1&2 and the lowest median IQR was significantly present in CKD stage 1&2, and there was significant difference between group 1 (DN stage 1&2) versus group 2 (DN stage 3&4), group 3 (Diabetics without nephropathy) and all stages of CKD.

Conclusion: in diabetic nephropathy stage 1&2, serum miR-377 was highly significant increased more than diabetics without nephropathy, diabetic nephropathy stage 3&4 and all satges of CKD.

Keywords: *MicroRNA - Diabetic Nephropathy - Chronic Kidney Disease*

INTRODUCTION

As one of the most important long term complications of diabetes, Diabetic nephropathy is the major cause of end stage renal disease and high mortality ***(Dronavalli et al., 2008).***

The major pathological features of diabetic nephropathy are charactized by hypertrophy and expansion in the glomerular mesangium and tubular compartments along with podocyte dysfunction and accumulation of extracellular matrix proteins.

Several mechanisms including hyperglycemia, advanced glycation end products, protein kinase c, oxidative stress inflammation and poly (ADP ribose) polymerase activation, are believed to contribute to the pathogenesis of diabetic nephropathy ***(Sun et al., 2013).***

Several typical cell signaling pathways have been proven to be involved in diabetic nephropathy. for example transforming growth factor (TGFB) is a well-known pathway leading to accumulation of extracellular matrix in diabetic nephropathy ***(Chen et al., 2014; Reeves et al., 2000; Ziyadeh and Sharma, 2003).***

Phosphoinositide3 kinase protein kinase B (PI3KAKt) pathway is considered to result in glomerular hypertrophy and extracellular matrix accumulation ***(Dey et al., 2012; Habib et al., 2012).***

Nuclear factor Kappa light chain enhancer of activated B cells (NFk_{β}) is a key in the inflammatory pathway, It recruits a variety of inflammatory cytokines involved in diabetic nephropathy ***(Ka et al., 2012; Xie et al., 2013).***

However the molecular pathogenesis hidden behind is still not fully understood. MicroRNAs (miRNAs) are endogenously produced short non coding RNAs of about 21-25 nucleotides that have been shown to play important roles in modulating gene expression thus affecting almost every key cellular function ***(Bhatt et al., 2011).***

It is estimated that about 60% of the human protein-coding genes can be targeted by miRNAs thus research on miRNA has attracted a high level of interest so that miRNAs are found to regulate signaling pathways involved in the pathogenesis of diabetic nephropathy.

Mature miRNA recognizes target mRNAs through sequence complementarity, resulting in either degradation of the target mRNA (perfect complementarity to 3′UTR) or more frequently inhibition of translation (imperfect complementarity to 3′UTR).

Previous work in diabetic renal disease (performed in cell culture, animal models or formalin fixed human biopsy) has linked a number of microRNA to the development of nephropathy.

MicroRNA-21 targeted phosphatase and tensin homolog (PTEN) to induce the over activation of Akt (protein kinase) signaling pathway, followed by renal fibrosis and hypertrophy ***(Dey et al., 2011).***

MicroRNA -451 over expression inhibits glomerular mesangial cells proliferation through down regulation of p38-MAPK signaling via YWHAZ (tyrosine 3 monooxygenase / tryptophan 5monooxygenase activation protein, zeta) down regulation ***(Zhang et al., 2012).***

MicroRNA-93 targeted vascular endothelial growth factor (VEGF-A), so inhibitors increase (VEGF-A) secretion ***(Long et al., 2010).***

MicroRNA-192 targeted zing finger E-box binding homeobox1/2 (ZEB1/2) to activate transforming growth factor B (TGF- B) signaling pathway leading to increase COLLa2 and fibrosis ***(Kato et al., 2011).***

MicroRNA-29 family repressed the expression of targeted COL1 and COL4 may exert protective effect by inhibiting TGF-B /SMAD3 signaling pathway ***(Wang et al., 2012).***

MicroRNA-25 was significantly reduced accompanied by increase in NOX4 regulating NADPH oxidase activity level ***(Fu et al., 2010).***

Nevertheless there have been no comprehensive studies examining microRNA changes in human bio fluids in diabetic nephropathy in relation to the level of urinary albumin and clinical outcome.

Up regulated microRNA-377 led to reduced expression of P21-activated kinase and superoxide dismutase which enhanced fibronectin production a key matrix protein accumulated in excess in diabetic nephropathy ***(Wang et al., 2008).***

Micro RNA-377 targets and suppresses translation of important mesangial protein, include SoD_1, SoD_2, and PAK_1, this leads to enhanced susceptibility to oxidant stress and accumulation of the extracellular matrix protein, fibronectin, hence, micro RNA-377 is positioned to have a critical role in the mesangial cell response to the diabetic milieu.

Aim of the Work

To identify the pattern of microRNA-377 changes specific for diabetic nephropathy in diabetic patients and in patients with chronic kidney disease of different etiology.

Chapter 1

MICRO RNA

Mi RNAs are naturally expressed small non-coding RNA 20–22-nucleotides) that regulate gene expression through posttranscriptional mechanisms in general, miRNAs lower the expression of target genes by imperfect base pairing to the 3`-un translated regions of target mRNAs leading to translation inhibition. More than 2.000 human mature miRNAs have now been identified and at least 60% of all human protein- coding genes are known to be regulated by miRNAs ***(Bartel, 2009).***

Therefore, miRNAs have tremendous potential to modulate the expression of numerous genes and thereby to change cellular and biochemical function, potentially affecting the development or progression of various diseases, since their discovery 20 years ago, miRNA have been implicated in numerous biological processes as well as in the pathology of several human diseases.

Nomenclature of Micro RNAs:

Names are assigned to experimentally confirmed miRNAS before publication. ***(Griffiths, et al., 2006)***

The prefix "miR" is followed by a dash and a number the latter often indicating the order of naming e. g miR- 124 was named and likely discovered prior to miR- 456. A capitalized "miR".