MicroRNAs- biomarkers of diagnosis and prognosis in diabetic cerebrovascular complications

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microRNAs (miRNAs) are endogenously produced short noncoding 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.

miRNAs have a high potential to modulate cellular and biochemical functions, leading to the initiation or progression of various diseases, including diabetic nephropathy.


MicroRNAs

- miRNAs are transcribed in the nucleus by RNA polymerase II into primary transcripts (pri-miRNAs)
- within the nucleus, the ribonuclease III Drosha cuts pri-miRNAs to precursor miRNAs (pre-miRNAs), which are ~70 nucleotide stem-loop structures
- these pre-miRNAs are transferred to the cytoplasm by a protein called exportin-5
- in the cytoplasm, pre-miRNAs are further processed by a second ribonuclease, Dicer, to mature miRNAs

MicroRNAs

- Mature miRNA duplexes are then incorporated into the RNA-induced silencing complex (RISC), which also contains the Argonaute family of proteins within this complex, miRNAs interact with the 3'-untranslated region of the target mRNA species and induce translational repression or degradation.
MicroRNA biogenesis and repression of gene expression

MicroRNAs in diabetic cerebrovascular complications

- miRNA profiles in in vitro and in vivo studies as well as in human peripheral blood are quite representative of the miRNA expression in human atherosclerotic plaque

- miRNAs are stably expressed and in circulation even after several months from the onset of stroke

- significant higher expression in patients with asymptomatic carotid artery stenosis progression
MicroRNAs in diabetic cerebrovascular complications

miR-146a

- miR-146a is a cytokine-responsive miRNA that regulates distinct components of NF-κB signaling and confers atheroprotective properties in the vessel wall

- miR-146a overexpression inhibited cytokine responsiveness in ECs, suggesting that it may participate in a negative feedback mechanism to limit EC inflammatory signaling

- miR-146a expression is also increased in human and mouse atherosclerotic plaques
MicroRNAs in diabetic cerebrovascular complications

miR-126

- is among the most abundantly expressed miRNAs in ECs and has been implicated in regulating both inflammation and angiogenesis in a flow-dependent manner.
- In the context of atherosclerosis, it has been demonstrated that miR-126 is the most abundant miRNA expressed in EC-derived apoptotic bodies.

MicroRNAs in diabetic cerebrovascular complications

miR-126

- consistent with this pathway, intravenous delivery of endothelial apoptotic bodies mobilized progenitor cells in the circulation and enhanced their incorporation into aortic plaques, an effect that suppressed atherosclerotic progression in a miR-126–dependent manner implicated in maintaining an atheroprotective VSMC contractile phenotype

MicroRNAs in diabetic cerebrovascular complications

**miRNA-126**

- neuroprotective effects of ischemic preconditioning supported by the upregulated pro-survival miRNAs in MCA infarcts - reduction in infarction size
  

- decreases vascular remodelling
  

- is a differentially regulated miRNA which appears to be involved in proliferative or vascular inflammatory functions – decreases vascular inflammation
  

- plays essential roles in endothelial cells in maintenance of vascular integrity and angiogenesis
  
MicroRNAs in diabetic cerebrovascular complications

miRNA-21

- critical regulator in vascular neointimal lesion formation; neutralization of miR-21 reduced neointimal lesion formation in response to mechanical balloon injury
  

- correlates with carotid IMT
  

- found to be differentially regulated under ischemic conditions - increases vascular remodelling
  

- increases proliferative and vascular inflammatory functions
  
MicroRNAs in diabetic cerebrovascular complications

miRNA-21

- progression of atherosclerotic plaque in experimental animal models by regulating either endothelial cell function or vascular smooth muscle cell proliferation

- significantly up-regulated in human atherosclerotic plaque - stabilizes the atherosclerotic plaque
  [Raitoharju E, et al. *Atherosclerosis* 2011]

- up-regulated in asymptomatic atherosclerotic plaque - enhanced plaque stability
MicroRNAs in diabetic cerebrovascular complications

miRNA-21

- post-stroke angiogenesis
- dynamics of brain tissue
- protects neurons from ischemic death by targeting water channel modulators, aquaporins, and the Fas ligand gene
- vascular remodelling in young stroke patients


MicroRNAs in diabetic cerebrovascular complications

miRNA-21

- could negatively regulate LPS-induced lipid accumulation and inflammatory responses in macrophages by the TLR4-NF-κB pathway
- protective in bacterial infection-induced vascular remodelling
- reversely abrogates bacterial infection-induced pathological processes of atherosclerosis, indicating a promising therapeutic prospect for the prevention and treatment of atherosclerosis by miR-21 overexpression

MicroRNAs in diabetic cerebrovascular complications

miRNA-21

- miR-21 and CRP levels were significantly higher, whereas nitric oxide and endothelial nitric oxide synthase levels were significantly lower in patients with increased carotid IMT.
- In normoalbuminuric patients, the increased miR-21 expression in patients with increased carotid IMT lead to the conclusion that this miRNA might be involved in the early stages of atherosclerotic process in hypertensive patients.

- Stroke patients and atherosclerosis subjects had significantly higher miR-21.


MicroRNAs in diabetic cerebrovascular complications

miRNA-124

- neuroprotective effects of ischemic preconditioning supported by the upregulated pro-survival miRNAs in MCA infarcts- reduction in infarction size

- brain-specific miRNA-biomarker of cerebral ischemia induced by middle cerebral artery occlusion in rats

- increases as early as 6 hours following reperfusion
MicroRNAs in diabetic cerebrovascular complications

miRNA-125

- significantly overexpressed in symptomatic vs. asymptomatic plaques
- miR-125a expression was significantly inversely correlated with the circulating level of low-density lipoprotein cholesterol in the symptomatic group
- a potential regulatory role in the evolution of the plaque towards growth, instability and rupture

Personal research

- atherosclerosis and microangiopathy within the brain parallel diabetic nephropathy (DN) in the course of Type 2 diabetes mellitus (DM)
- the aim of our study was to evaluate the time frame of vascular remodelling in the course of Type 2 diabetes mellitus (DM) in two vascular territories, the kidney and the brain, both affected by diabetic vasculopathic complications
- the pattern of vascular involvement was evaluated in relation to miRNAs profile
Methods

- 76 patients with Type 2 DM and 11 healthy subjects
- urine albumin: creatinine ratio (UACR)
- urinary nephrin, podocalyxin, and synaptopodin as biomarkers of podocyte injury
- urinary N-acetyl-β-D-glucosaminidase (uNAG), urinary kidney injury molecule-1 (uKIM-1) as biomarkers of proximal tubule dysfunction;
- Cystatin C; eGFR(sCreat-cystatin C)
- miRNAs were quantified in blood and urine by a real-time PCR System
- the neurosonology methods included intima-media thickness (IMT) in the common carotid arteries, the resistance index (RI) in the internal carotid arteries and the middle cerebral arteries; the cerebrovascular reactivity was assessed through the breath-holding index (BHI)
Methods

MicroRNAs assessment

- Total RNA was isolated from the blood and urine samples using the Blood and the Urine microRNA Purification Kit, NORGEN BIOTEK CORPORATION, 3430 Schmon Parkway, Thorold, ON, Canada Cat. 29000. Total RNA was stored at −80 °C. The quantity and quality of total RNA was verified, and reverse transcription was performed using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems [ABI], Foster City, CA). cDNA were amplified using the TaqMan MicroRNA Assay for specific miRNAs (miRNA21, miRNA124, miRNA192, miRNA125a, miRNA126, miRNA 146a) and TaqMan® Universal PCR Master Mix. The PCR reaction was performed on a 7900HT real-time PCR System. Blood and urine samples were run in triplicate, and relative expression level of specific gene were calculated using the SDS software v.2.4., and U6 small nuclear RNA as the endogenous control. Fold-change of expression was calculated using the comparative Ct method (Δct).
Methods

Statistical analysis

- clinical and biological data are presented as medians and IQR, as for variables with skewed distribution.
- differences between groups were analysed with the **Mann-Whitney U test** for comparison of 2 groups and the **Kruskal-Wallis test** for comparison of 4 groups.
- **simple linear regression analysis** was carried out to evaluate the significance of the relation between continuous variables for all groups together (pooled data).
- only significant variables yielded by univariate regression analysis were introduced in the models for **multivariable regression analysis** (Cox & Snell R square).
- the P values for all hypothesis tests were two-sided, and statistical significance was set at P<0.05.
- All analyses were conducted with **Stata 15.1** (Statacorp, Texas, USA).
Discussion

- Urinary miRNA 21 and 124 correlated directly with nephrin, podocalyxin, synaptopodin, UACR, uNAG, uKIM1, and indirectly with eGFR, thus showing negative effects upon the podocytes, the proximal tubule, and renal function.

- Urinary miRNA 125a, 126, 146a and 192 correlated indirectly with nephrin, podocalyxin, synaptopodin, UACR, uNAG, uKIM1, and directly with eGFR, suggesting renoprotective effects of these miRNAs.
Discussion

- Plasma miRNA124, miRNA125a, miRNA126, miRNA146a correlated indirectly with IMT, RI, and directly with BHI, suggesting neuroprotective effects.
- Plasma miRNA 21 and miRNA192 correlated directly with IMT and RI, and indirectly with BHI, pointing to negative effects of these miRNAs to cerebral vessels remodelling.
in Type 2 DM patients, miRNAs could impact both the cerebral vessels and the kidney

most likely, vascular remodelling related to miRNAs profiles variability may occur initially in the brain vasculature, even in the normoalbuminuria stage

thus, involvement of the cerebral vessels appears to be dissociated from glomerular injury and PT dysfunction in early DN
Conclusion

- it may be assumed that in the course of Type 2 DM there are distinct time frames of vascular remodelling, highly dependent on miRNAs expression at a certain point in the course of Type 2 DM

- this phenomenon may be explained by the variability of miRNAs expression within the two organs

- this observation draws the attention to the clinical implication of an early approach to cerebral vessels impairment in asymptomatic normoalbuminuric patients with Type 2 DM, often perceived as a group of patients at low risk of developing cerebrovascular complications
THE TIME FRAME OF VASCULAR REMODELLING IS DISSOCIATED WITHIN THE BRAIN AND THE KIDNEY AND MAY BE EXPLAINED BY THE VARIABILITY OF miRNAs EXPRESSION IN TYPE 2 DIABETES MELLITUS PATIENTS

Concluding remarks

- The use of angiomiR mimics and angiomiR inhibitors are two therapeutic approaches to promoting and inhibiting angiogenesis.
- The use of these strategies depends on the requirements of upregulating or downregulating target angiomiRs to inhibit or enhance the angiogenic process according to the pathological angiogenic status of relevant diseases.
- In ischemic diseases with insufficient angiogenesis such as myocardial infarction, ischemic stroke, and peripheral artery diseases.
Concluding remarks

- there are several potential challenges or limitations for application of miR and angiomiR therapeutics in ischaemic stroke
- lack of understanding the function of individual angiomiRs in stroke pathogenesis
- a less than effective delivery system to achieve neural cell specific delivery *in vivo*, limitations pertaining to miR target specificity, and the unique structure of the blood-brain barrier