Serum miRNA-155 as a potential biomarker of degenerative disc disease in patients with low back pain

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INTRODUCTION

Extracellular miRNAs are stable molecules that can be readily detected in patient serum and provide an insight into current disease states (Figure 1). Degenerative disc disease (DDD) is a common cause of low back pain in clinical practice, however it poses a diagnostic challenge. Several miRNAs have been shown to be dysregulated in DDD and in particular, local tissue levels of miRNA-155 are downregulated in patients. However, studies looking at serum levels of miR-155 as a potential biomarker for DDD are lacking.

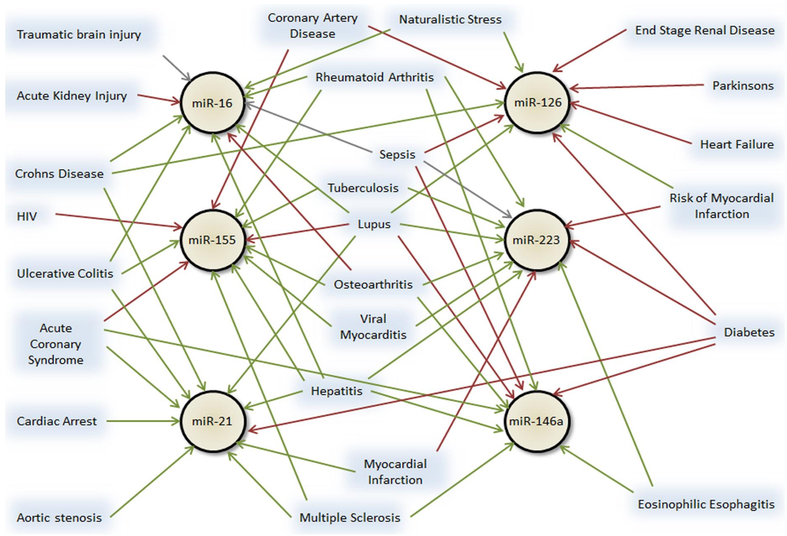

Figure 1. The role miR-155 has been identified in several chronic diseases, however no studies have studied serum miR-155 in DDD. *Reproduced from Haider BA et al. (2014) A Critical Evaluation of microRNA Biomarkers in Non-Neoplastic Disease. PLoS ONE 9(2). © CC by 4.0*

MATERIALS & METHODS

The study was approved by the Thomas Jefferson University IRB. Patients greater than 18 years undergoing single-level lumbar fusion and young, healthy controls were enrolled. Those with history of previous surgery, active infection, or any chronic systemic illnesses were excluded. Venous blood and serum were isolated preoperatively. miRNA was isolated using miRNeasy Serum/Plasma Advanced Kit (Qiagen, Germany). Initial screening for differential gene expression of miRNAs was performed using TaqMan™ Advanced miRNA Human Serum/Plasma 96-well plates (Applied Biosystems, USA) on a small subset of patient samples. Real-time qRT-PCR analysis was completed for confirmation of expression of miR-155-5p. Bioinformatics analysis was performed using Ingenuity Pathway Analysis (Qiagen, Germany) to provide a robust network analysis of gene targets for miR-155-5p and other miRNAs.

RESULTS

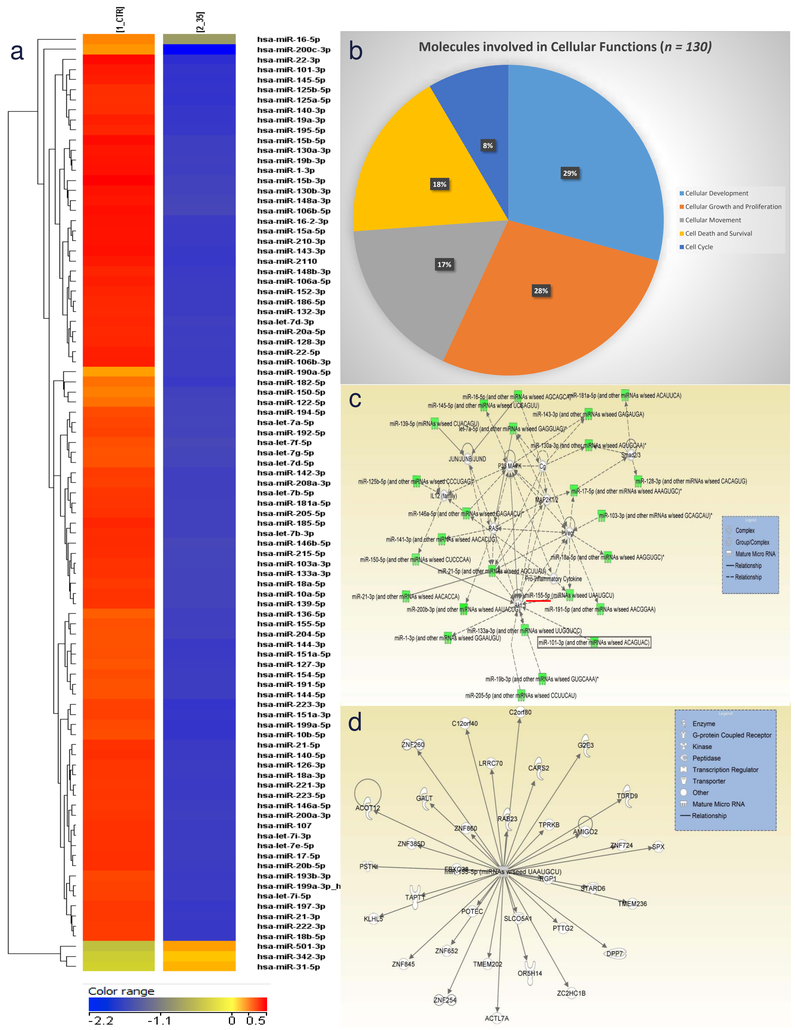

Figure 2. Bioinformatics Analysis for screening assay. a) Heatmap showing differential gene expression for control (left) and patients with DDD (right). b) Proportion of gene targets involved in cellular functions. c) miRNA association with common pathways d) Most common downstream gene targets of miR-155-5p

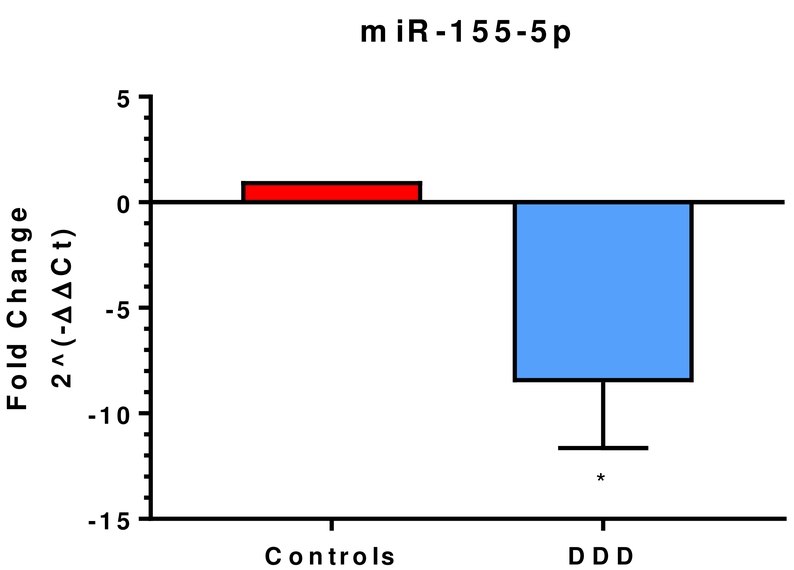

Figure 3. Validation of expression level of miR-155-5p. Quantitative RT-PCR results showed a significant eight-fold downregulation of miR-155-5p in patients with DDD (n = 63) compared to healthy controls (n = 15) (Mean -8.523, 95% CI [-5.461, -11.585], p < 0.001)

RESULTS

78 patients were included (15 controls, 63 patients with DDD, Table 1). Analyzing the results from the screening assay for serum-detected miRNAs (Figure 2), there was significant downregulation of the majority of miRNAs in patients with DDD. Network analysis showed involvement of miR-155-5p in mostly cellular development and cellular growth and proliferation. It also showed significant interactions with cell cycle and apoptosis pathways (P38/MAPK, Akt) as well as with pro-inflammatory cytokines.

		Healthy Controls *n = 15*	DDD group *n = 63*
Age		29.5 ± 10.2	57.5 ± 13.6
Gender			
	F	6 (37.5%)	33 (52.4%)
	M	10 (62.5%)	30 (47.6%)
Race			
	Caucasian	10 (62.5%)	53 (84.1%)
	Black	1 (6.3%)	7 (11.1%)
	Hispanic	0	2 (3.2%)
	Asian	5 (31.2%)	1 (1.6%)
Pfirrmann Grade†			
	3	-	7 (11.1%)
	4	-	34 (54.0%)
	5	-	22 (34.9%)
Back Pain*		0	6.43 ± 2.94
Leg Pain*		0	6.13 ± 3.05

Table 1. Patient Demographics. †*Graded on preoperative T2 sagittal MRI.* **Preoperative numeric pain rating scale*

DISCUSSION

Many studies have found dysregulation of local levels of miRNAs in degenerative disc tissue. In particular, miR-155 is noted to be downregulated, which increases Fas-mediated apoptosis in nucleus pulposus cells, contributing to intervertebral disc degeneration. However, to date, no study has identified dysregulation of miRNA in the serum.

This is the first study to detect downregulation of miR-155-5p in the serum in patients with degenerative disc disease, correlating with *in situ* levels. Further studies are needed to identify other miRNAs involved in the regulation of intervertebral disc degeneration to create a robust gene profile that can be used as a biomarker for patients with DDD.

ACKNOWLEDGEMENTS

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* Denotes equal contributors to this work