

Alterations in microRNA Expression in a Murine Model of Diet-Induced Vasculogenic Erectile Dysfunction

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DOI: 10.1111/jsm.12793

ABSTRACT

Introduction. MicroRNAs (miRs) are noncoding, endogenous RNA molecules that regulate gene expression and play roles in response to vascular injury.

Aim. The aim of this study was to identify miRs expressed in corporal tissue (CT) and to determine whether miRs demonstrate differential expression in a mouse model of diet-induced erectile dysfunction (ED).

Methods. RNA was isolated from the CT from control mice and mice with diet-induced ED. A quantifiable miR profiling technique (NanoString) was used to determine the expression of over 600 miRs.

Main Outcome Measures. Differential expression analysis was performed using a negative binomial regression model for count-based data. Mean expression levels, fold change, and false discovery-corrected *P* values were determined. Candidate miRs were validated via quantitative polymerase chain reaction (Q-PCR).

Results. In control mice, NanoString analysis revealed that 181 miRs were expressed above background levels and 5 miRs were expressed at high levels. Diet-induced ED resulted in the up-regulation of 6 miRs and the down-regulation of 65 miRs in the CT compared with mice on control diet. Focusing on the upregulated miRs, we chose five for Q-PCR validation. Of these five, two (miR-151-5p and miR-1937c) demonstrated significance via Q-PCR, whereas the other three (miR-720, miR-1937a, miR-205) trended in the correct direction.

Conclusions. MiRs may play a significant role in mRNA regulation in CT and specific miRs may be involved in diet-induced vasculogenic ED. Future studies are aimed at determining the mRNA targets of these miRs. **Barbery CE, Celigoj FA, Turner SD, Smith RP, Kavoussi PK, Annex BH, and Lysiak JJ. Alterations in microRNA expression in a murine model of diet-induced vasculogenic erectile dysfunction. J Sex Med 2015;12:621–630.**

Key Words. Erectile Dysfunction; microRNA; NanoString; Diet-Induced ED

Introduction

Our understanding of the role and function of microRNA (miR) has grown exponentially in the past years. It is now estimated that miRs regulate the expression in over 60% of mRNA transcripts in the human genome [1]. The central dogma of protein expression and gene regulation has been overhauled by these small, noncoding

RNAs. miRs begin as long precursor molecules that undergo essential processing catalyzed by enzymes such as Drosha and Dicer before reaching their mature forms [2]. Once in the cytoplasm, they associate with the RNA-induced silencing complex that facilitates their interaction with target mRNA molecules. By binding to the 3'-untranslated region of target mRNAs, miRs can inhibit their translation through either causing

degradation of the mRNA, repressing translation of the mRNA, or deadenylating the mRNA [2].

While some studies have shown that miRs can cause disease, they appear to have a greater role as a modifier of disease severity and thus miRs can serve as therapeutic agents [3]. A somewhat unique property of miRs lies in their ability to regulate the expression of several functionally related genes, thus possibly affecting an entire biologic pathway [4]. Additionally, some miRs have been shown to be stable in plasma when bound to specific carrier molecules, such as Argonaut or high-density lipoprotein, and have been suggested to be biomarkers for certain disease states [5].

The role of miRs in the context of benign urological disease, specifically erectile dysfunction (ED), remains limited [6]. Current estimates place the number of men with ED in the United States to be greater than 30 million [7]. When considering the entire population of men with ED, a sizeable fraction will have diet-induced ED. Obesity, hyperglycemia, hyperinsulinemia (i.e., insulin resistance), and type 2 diabetes mellitus (DM) have all been linked to a high-fat diet (HFD) and all are contributing factors to ED [8]. The overwhelming majority of preclinical models of DM-induced ED use approaches where hyperglycemia is present, but hyperglycemia is often not associated with obesity and is not associated with insulin resistance. For example, when hyperglycemia is induced by streptozotocin injection [9] or using the Akita genetic model [10], ED will be observed; however, obesity is not observed. Thus, most preclinical studies of DM-induced ED fail to incorporate some of the most essential aspects of the human condition of ED in adult onset DM, which includes not only hyperglycemia but obesity and insulin resistance.

Studies from our group have shown that feeding C57BL/6 mice with an HFD where 45% of its daily calories come from fat caused obesity, hyperglycemia, and insulin resistance. By examining the corpus cavernosum in these mice, we found that a number of findings were consistent with ED including: (i) abnormalities in corporal endothelium-dependent and endothelium-independent vasoreactivity; (ii) a decrease in the ratio of the smooth muscle to collagen content; (iii) a reduction in NADPH diaphorase staining (measure of bioavailable NO); and (iv) increases in apoptosis, as measured by TUNEL staining [11,12]. When these results were compared with

information in other modes of ED (type 1 DM [13] and hypercholesterolemia [14]), it appeared that some of the mechanisms for the HFD-induced vascular ED were different from findings in these other models of ED [13,14].

Aims

By employing a quantifiable miR profiling technique termed nCounter® miR Expression Assay (NanoString Technology, Inc., Seattle, WA, USA), the current study aims to identify miRs that are expressed in the corporal tissue (CT) as well as miRs that are differentially expressed in the CT of mice with diet-induced ED compared with control normal mice. Through the identification of differentially expressed miRs and their respective targets, we will gain a better understanding of the pathophysiologic mechanisms underlying diet-induced ED as well as develop new therapeutic targets.

Methods

Murine Model of Diet-Induced Vasculogenic ED

Animal studies were approved by the Institutional Animal Care and Use Committee and conformed to the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health. Mice were fed freely and maintained on a 12-hour dark–light cycle. Our laboratory has previously reported that mice fed with a diet consisting of 60% fat will develop ED compared with mice on normal chow diet [12,15]. C57BL/6J mice were put onto a 60% HFD (Jackson Laboratories, Bar Harbor, ME, USA) at 6 weeks of age and remained on the HFD for 22 weeks. Age-matched control mice were placed on normal chow diet.

Validations of the Murine Model System

At 28 weeks of age, the mice were fasted for ~16 hours and placed on Sani-Chip bedding. They were weighed and injected intraperitoneally with D-glucose (1 g/kg body weight; Sigma, St. Louis, MO, USA); for example, for a 25-g mouse, 0.1 mL was injected and for a 40-g mouse, 0.16 mL was injected. Blood samples were then taken at various time points (0–120 minutes) from the tail vein. Glucose measures were made using a glucometer (One Touch Profile meter; LifeScan, Milpitas, CA, USA).

Endothelium-dependent relaxation was assessed using acetylcholine (Ach) on corporal

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