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# The Changes of MicroRNA Expression in the Corpus Cavernosum of a Rat Model With Cavernous Nerve Injury

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## ABSTRACT

**Background:** MicroRNAs (miRs) were found to be dysregulated in erectile dysfunction (ED) related to aging, type 2 diabetes mellitus, and vasculogenic abnormalities. However, miR expression in ED after radical prostatectomy (RP) is not known.

**Aim:** To detect abnormal miR expression in post-RP ED and analyze target genes and pathways.

**Methods:** 16 Sprague Dawley rats were divided into bilateral cavernous nerve crush (BCNC) and control groups. 4 weeks after surgery, erectile function and histological change in the corpus cavernosum were evaluated. Total RNA from 3 rats from each group was isolated and processed to analyze the miR expression profiling by RNA sequencing. The top 10 up-regulated miR profiles were chosen directly and further validated in another 5 rats per each group by quantitative real-time polymerase chain (PCR) reaction. The target genes were predicted by online databases, including: TargetScan, mirwalk, miRanda, miRDB, and DIANA. The enrichment analysis of gene ontology-term analysis and Kyoto Encyclopedia of Genes and Genomes were performed by DAVID database.

**Outcomes:** Intra-cavernosal pressure, mean arterial pressure, smooth muscle content, and miR expression were measured.

**Results:** Compared to the control group, the BCNC group had decreased intra-cavernosal/mean arterial pressure ratio and smooth muscle marker ($\alpha$-smooth muscle actin). The sequence results showed that 124 miR expression dysregulated in the BCNC group, in which 122 miR expression were up-regulated. Of the 122 miRs, 21 miR expressions were increased above 2-fold. Among the top 10 up-regulated miRs, 4 miRs (miR-101a, miR-138, miR-338, and miR-142) levels were finally validated for over-expression by quantitative (PCR) reaction. The gene ontology analysis results showed that these 4 miRs could regulate the processes of cell apoptosis, fibrosis, endothelium, and smooth muscle cells function. The Kyoto Encyclopedia of Genes and Genomes pathway analysis showed the target genes were involved in 7 pathways related to ED.

**Clinical Translation:** Our findings provide novel insights into post-RP ED that may stimulate further studies to develop miR targeted therapy or damage detection for ED.

**Strengths & Limitations:** To our knowledge, this is the first study to identify the miR profiling and function in the BCNC rat model. The rat model might not represent the human condition and the miR was only detected at 1 period. Besides that, there is a high probability of false positives for RNA sequence results.

**Conclusion:** 4 dysregulated miRs were found in the BCNC rat model, which may be related to post-RP ED by regulating apoptosis, fibrosis, endothelial, and smooth muscle cells. **Liu C, Cao Y, Ko TC, et al. The Changes of MicroRNA Expression in the Corpus Cavernosum of a Rat Model With Cavernous Nerve Injury. J Sex Med 2018;15:958–965.**

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**Key Words:** microRNA; erectile dysfunction; radical prostatectomy; sequence

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## INTRODUCTION

Prostate cancer is the most common solid-organ cancer in the United States. According to the American Cancer Society, approximately 161,360 new cases and 26,730 prostate cancer—associated mortalities occurred in the United States in 2017.[1] Among all of new cases, approximately 95% were clinically localized prostate cancer and radical prostatectomy (RP) is a commonly used procedure for these patients.[2,3] However, RP is associated with 2 major quality-of-life issues: urinary incontinence and erectile dysfunction (ED).[4] With technique improvements, especially the wide use of robotic-assisted RP, incontinence rates have dropped to an acceptable level.[5,6] However, the same cannot be said for erectile function. Therefore, the risk of ED after RP impacts the decision-making process among physicians, patients, and their partners. Penile rehabilitation after RP with phosphodiesterase type 5 inhibitors, intracavernosal injection, vacuum erection device, or a combination of these has not proven to be effective to improve recovery of spontaneous erection based on current understanding of the pathophysiology of post-RP ED.[7] Therefore, identifying additional mechanisms of post-RP ED is important for finding new therapeutic options.

Penile erection is defined as a neurovascular event, where both neuronal and vascular components are essential in the physiological pathway.[8] The prostatectomy damages the cavernous nerve. This injury induces Wallerian degeneration of the nerves, thus leading to the denervation of the corpora cavernosa, then it leads to penile hypoxia and fibrosis and finally results in venous leakage.[9] The bilateral cavernous nerve crush (BCNC) model is one of the most commonly used models to study post-RP ED. In this model, the axonotmesis leads to decrease of erectile function and loss of smooth muscle cells after BCNC. Besides that, the study showed that the injury-induced damage to corporal smooth muscle cells was irreversible.[10]

MicroRNAs (miRs) are small, non-coding, conserved RNA molecules that are about 22 nucleotides in length.[11] It has been well demonstrated that miRs are critical for the physiological and pathological processes, including proliferation, differentiation, apoptosis, and metabolism.[12,13] miRs were found dysregulated in ED related to aging, type 2 diabetes, and vasculogenic abnormalities.[14–17] This study was conducted to identify abnormal miR expression in post-RP ED corpora cavernosa tissues and analyze the target genes and pathways regulated by abnormal miRs.

## METHODS

### Animal Model

8-week-old male Sprague Dawley rats were obtained from Envigo RMS Inc, Indianapolis, IN. Care and treatment were approved by the institutional animal care and use committee at our institution (University of Texas McGovern Medical School at Houston). Prior to the study, the rats were allowed time to become accustomed to the new environment, as required by our institutional animal care and use committee. Rats were divided into BCNC and control groups (N = 8 for each group). In the BCNC group, a rat model of BCNC-induced ED was established according to our previously described methods.[18] Briefly, the rats were anesthetized with isoflurane. After the surgical area was disinfected, a mid-line supra-pubic incision was made. The bladder and prostate were exposed. The major pelvic ganglion (MPG) and cavernous nerves were identified on both sides of the prostate. The bilateral cavernous nerves, 5 mm distal to the MPG, were crushed using an ultra-fine hemostat with full tip closure for 30 seconds, removed for 30 seconds and then re-applied for another 30 seconds. The incision was closed with 3—0 Vicryl sutures (Ethicon, San Angelo, TX, USA). In the control group, the MPG and cavernous nerves were exposed, but no further surgical manipulation was performed before closing the incision.

### Erectile Function Evaluation

Erectile function was evaluated using the ratio of intracavernous pressure (ICP) to mean arterial pressure (MAP) 4 weeks after surgery. Briefly, rats were anesthetized with pentobarbital sodium (30 mg/kg) via an intra-peritoneal injection. The left carotid artery was exposed and cannulated with a polyethylene-50 tube filled with heparinized saline (250 IU/mL) to monitor MAP. For the ICP measurement, a 25-gauge needle connected to a polyethylene-50 tube was inserted into the left corpus cavernosum. The cavernous nerve was identified and isolated with a mid-line laparotomy. Stimulations were performed at 16 Hz with duration of 5 milliseconds at 5 V for 60 seconds with 5 minutes between subsequent stimulations.

### Immunohistochemistry

The penile tissues were fixed in 4% paraformaldehyde overnight and embedded in paraffin. Paraffin sections (5-$\mu$m thick) were then incubated with primary antibody, mouse anti-$\alpha$ smooth muscle actin (SMA), 1:500 (Santa Cruz Biotechnology, Dallas, TX, USA), at 4°C overnight, followed by DyLight 488 conjugated secondary antibody (1:500) (Vector Laboratories, Burlingame, CA, USA) for 1 hour at room temperature. The mean fluorescence intensity was quantified by Image Pro Plus (Media Cybernetics, Rockville, MD, USA).

### DNA Transcript Sequence

The penile tissue samples from 3 rats of both groups were collected for RNA isolation after removal of urethral and adventitial tissues. Total RNA was extracted using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration and quality of the extracted RNA were measured with a NanoDrop 200 (Madison, WI, USA) at 260nm and 280nm. The integrity of total RNA was analyzed by Agilent 2100 Bioanalyzer using Agilent RNA 6000 Nano Kit (Agilent). Total DNA transcript for each rat was individually processed for RNA