STUDY ON MIR-210 PROMOTES LOCAL ANGIOGENESIS AFTER HYDRONEPHROSIS IN MICE BY REGULATING HIF-VEGF SIGNALING PATHWAY

Xiaojing Yang[1, 2, 3, #], Jun Zhao[4, 5, #], Yunhui Liao[6, *]
[1]Department of Pediatrics, The First Affiliated Hospital of Xiamen University, No. 55 Zhenhai Road, Xiamen 361003, China - [2]Pediatric Key Laboratory of Xiamen, No. 55 Zhenhai Road, Xiamen 361003, China - [3]Institute of Pediatrics, School of Medicine, Xiamen University, No. 55 Zhenhai Road, Xiamen 361003, China - [4]Department of Laboratory Medicine, The First Affiliated Hospital of Xiamen University, No. 55 Zhenhai Road, Xiamen 361003, China - [5]Xiamen Key Laboratory of Genetic Testing, No. 55 Zhenhai Road, Xiamen 361003, China - [6]Department of Clinical Laboratory, Xiamen Traditional Chinese Medicine Hospital, No. 1739 Xianyue Road, Xiamen 361009, China
[#]*These authors contributed equally to this work as co-first authors*

ABSTRACT

Objective*: To analyze the ability of micro-ribonucleic acid-210 (miR-210) to promote local angiogenesis by regulating the hypoxia-inducible factor-vascular endothelial growth factor (HIF-VEGF) signaling pathway after hydronephrosis in mice.*

Methods: *36 healthy male BALB/c mice were randomly placed into either the sham operation group or the hydronephrosis 2d, 7d, or 14d groups. Each group contained 9 mice. The histopathological changes in the kidneys were observed using a microscope. The levels of HIF-1α expression in mice from each group were determined by Western blotting. The levels of expression of miR-210, HIF-1α, and VEGF in mice from each group were determined by real-time fluorescence quantitative PCR. All mice were sacrificed following completion of the experiment.*

Results: *The mice in the control group exhibited normal kidney structure, even staining, and no infiltration of lymphocytes or plasma cells. The renal interstitia of mice in the hydronephrosis groups showed edema, infiltration of lymphocytes and plasma cells, dilation of the renal tubules, and swelling of the renal tubular epithelial cells. With the prolongation of hydronephrosis, the renal cortices became significantly thinner, and the volumes of hydronephrosis became significantly larger. The expression levels of miR-210, VEGF protein, and VEGF mRNA were significantly higher in the hydronephrosis 2d group than in the sham operation group. With the prolongation of hydronephrosis, the expression levels of miR-210, VEGF protein, and VEGF mRNA lessened significantly, while the expression levels of HIF-1αprotein and HIF-1αmRNA increased significantly ($p<0.05$).*

Conclusion: *miR-210 may promote local angiogenesis by regulating the HIF-VEGF signaling pathway following hydronephrosis in mice.*

Keywords: *miR-210, HIF-VEGF signaling pathway, hydronephrosis, local angiogenesis.*

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Introduction

Hydronephrosis is the expansion of the renal pelvis and calyces due to urinary tract obstruction and subsequent renal tissue atrophy. Stones, compression, deformities, tubal stenosis, benign prostatic hyperplasia, and neurogenic bladder can all cause hydronephrosis. When any of these factors remains untreated, urine presses on the renal cortex and gradually thins the kidney, leading to reduced renal function. This reduction increases the severity of hydronephrosis, causing the ischemia and hypoxia of the kidney tissue to worsen. The results of this process severely affect a patient's health and quality of life[(1)]. Therefore, methods must be established to reduce hydronephrosis and rescue the dying renal tubular epithelial cells in order to prevent a continued reduction of renal function.

Angiogenesis refers to the generation of new blood vessels, which depends on the stimulation of

cytokines, including vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF) [2]. Promoting the production of new blood vessels in an atrophic renal cortex may be important for functional recovery following hydronephrosis. Many protein signaling pathways are involved in the occurrence and development of fibrosis and renal function damage following hydronephrosis[3].

MicroRNA-210 (miR-210) exhibits hypoxia-inducing activity, which can regulate the expression of the ephrin-A3 gene, thereby affecting the functions of downstream genes[4]. Studies have found that miR-210 can induce angiogenesis. However, the underlying mechanism of action is still unclear[5]. In this study, BALB/c mice were used as study subjects, and the relevant mechanisms of miR-210 promoting local angiogenesis following hydronephrosis in mice were examined.

Materials and methods

Experimental animals

36 clean-grade healthy male BALB/c mice (Shanghai Rui Tai Mosi Biotechnology Co., Ltd., production license SCXK [Shanghai] 2018-0001) were randomly selected.

The mean±standard deviation (SD) body weight of the mice was 24±3 g. Under laboratory temperature and humidity conditions of 25±2 °C and 53±12%, respectively, with good ventilation, the mice were fed adaptively for 1 week, 12 hours each day and night.

Main instruments and reagents

In this study, we used the following: low-temperature high-speed centrifuge (Hebei Huicai Technology Co., Ltd., model: A1301022); -80°C ultra-low temperature refrigerator (Zhejiang Jiesheng Low Temperature Equipment Co., Ltd., model: DW-86L158); Real-time fluorescence quantitative PCR analyzer (Shenzhen Sanli Chemical Co., Ltd., model: TL988-IV); electronic balance (Shanghai Precision Instrument Co., Ltd., model: FA2004B); electron microscope (Shenzhen Haotel Electronic Technology Co., Ltd., model: HT-60S); and mouse anti-human HIF-1α antibody (Beijing Taize Jiaye Technology Development Co., Ltd.).

Grouping

The mice were randomly placed into either the sham operation group or the hydronephrosis 2d, 7d, or 14d groups, with 9 mice in each group.

Mouse model of hydronephrosis

Each mouse was fasted for 10 hours prior to surgery. After successful anesthesia on the operating table, preparation was conducted on the abdomen. The abdomen was immersed in iodine for 10 minutes. Alcohol was then used to deiodize, sterile tissue was used to cut approximately 1cm to the left of the abdominal line, and the abdominal cavity was opened longitudinally. Subsequently, the left kidney pedicle of the mouse was fully exposed, the ureter was located down the kidney pedicle, and the middle and upper ureter was doubly ligated with sterile silk thread. The ureter was cut, the abdomen was sutured layer by layer, and the abdominal cavity was closed. The abdominal cavities of mice in the experimental groups were opened 2 days, 7 days, and 14 days after this operation, and a swollen and full pelvis was regarded as successful modeling.

Observation indicators

The renal capsule was separated, and the left kidney was retrieved and washed clean. A section of the renal cortex was removed and placed in liquid nitrogen, and the rest of the renal cortex was fixed in 4% formaldehyde solution for future use.

The kidney tissues were removed and paraffin cuts were made. The blocks were dehydrated with alcohol, dewaxed with xylene, and cut into slices 4 μm thick. They were stained with hematoxylin for 2 min, rinsed with distilled water, and stained with eosin for 2 min. After alcohol dehydration and xylene dehydration, they were sealed with neutral gum. Four fields were randomly selected under the microscope to observe the pathological changes in each renal tissue.The levels of HIF-1α expression in mice from each group were determined by Western blotting. One hundred mg of the previously preserved renal cortex was weighed using an electronic balance, placed into a test tube, ground into a slurry using a homogenizer, and placed on ice for 15 min. Subsequently, 0.2 mL of chloroform was added, and the samples were shaken vigorously up and down for 15 min before resting on the block for 10 minutes.

Samples were centrifuged at 4°C before 0.5 mL of supernatant was removed and ice-cold isopropanol was added. The samples were placed in the refrigerator to stand and then centrifuged again. The supernatant was removed, the precipitate was dried, and 50 μL of diethypyocarbonate treatment water was added. Finally, the samples were fully dissolved by shaking. The expression levels of miR-210, HIF-1α, and VEGF in mice from each group were

determined by real-time fluorescence quantitative PCR. All mice were sacrificed after completion of the experiment.

Statistical methods

SPSS 22.0 software was used for statistical data analysis. Comparisons of measurement data were performed using independent sample t-tests. Comparisons of the same indicators at different time points were performed using repeated measurement analysis of variance.

Comparisons of differences between groups at each time point were conducted using independent sample t-tests. The time difference of each group was compared with an LSD t-test, and p-values of <0.05 were considered statistically significant.

Results

Histopathological changes of the kidneys from each group

Kidneys from mice in the control group exhibited normal structure, even staining, and no infiltration of lymphocytes or plasma cells. The renal interstitia of mice in the hydronephrosis groups showed edema, infiltration of lymphocytes and plasma cells, dilation of the renal tubules, and swelling of the renal tubular epithelial cells. With the prolongation of hydronephrosis, the renal cortices became significantly thinner and the volumes of hydronephrosis became significantly larger (see Figure 1).

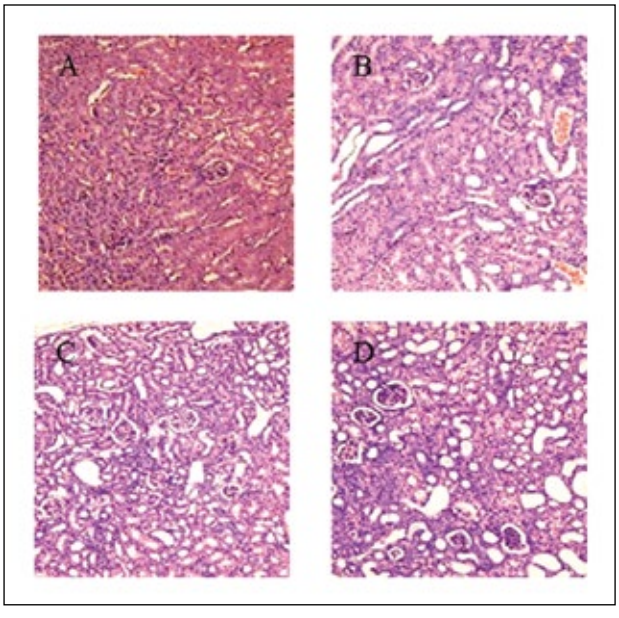

Figure 1: Histopathological changes of kidneys from mice in each group.
A: sham operation group; B: hydronephrosis 2d group; C: hydronephrosis 7d group; D: hydronephrosis 14d group.

Comparison of miR-210 expression levels of mice in each group

The levels of miR-210 expression in the hydronephrosis 2d group were significantly higher than those in the sham operation group. With prolonged hydronephrosis, the levels of miR-210 expression significantly decreased (p<0.05) (see Table 1).

Groups	Cases	miR-210 (mean±SD)
Sham operation group	9	1.00±0.02
Hydronephrosis 2d group	9	4.76±0.27[a]
Hydronephrosis 7d group	9	1.29±0.13[ab]
Hydronephrosis 14d group	9	0.21±0.04[abc]
F		1593.32
P		<0.001

Table 1: Comparison of miR-210 expression levels of mice in each group ($\bar{x}\pm s$).
Note: [a]p<0.05 compared with sham operation group; [b]p<0.05 compared with hydronephrosis 2d group; [c]p<0.05 compared with hydronephrosis 7d group.

Comparison of mouse HIF-1α and VEGF protein expression levels

The expression of VEGF protein was significantly higher in the hydronephrosis 2d group than in the sham operation group. With prolonged hydronephrosis, the levels of VEGF protein expression significantly decreased, and the levels of HIF-1α protein expression significantly increased (p<0.05) (see Figure 2).

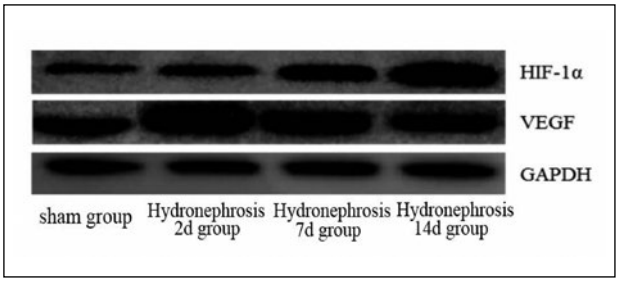

Figure 2: Levels of HIF-1α and VEGF protein expression in mice from each group.

Comparisons of the expression levels of HIF-1α and VEGF mRNA of mice in each group

The expression of VEGF mRNA was significantly higher in the hydronephrosis 2d group than in the sham operation group. With increased hydronephrosis time, the VEGF mRNA expression decreased significantly, while HIF-1αmRNA expression increased significantly (p<0.05) (see Table 2).

Groups	Cases	HIF-1α mRNA	VEGF mRNA
Sham group	9	1.01±0.01	1.00±0.01
Hydronephrosis 2d group	9	2.13±0.09[a]	2.22±0.13[a]
Hydronephrosis 7d group	9	3.07±0.14[ab]	0.77±0.10[ab]
Hydronephrosis 14d group	9	4.21±0.18[abc]	0.21±0.06[abc]
F		1108.68	845.25
P		<0.001	<0.001

Table 2: Comparison of the expression levels of HIF-1α and VEGF mRNA of mice in each group ($\bar{x}\pm s$).
Note: [a]p<0.05 compared with sham operation group; [b]p<0.05 compared with hydronephrosis 2d group; [c]p<0.05 compared with hydronephrosis 7d group.

Discussion

Hydronephrosis can result in renal urinary dysfunction due to kidney malformations, stenosis, stones, ureteral tumors, or other causes. When the urinary tract is partially or completely obstructed, the urine produced by the kidneys cannot be discharged normally into the bladder. Instead, it accumulates in the renal pelvis, which increases intrarenal pressure, causing the renal pelvis and calyces to gradually expand, thereby resulting in atrophy and destruction of the renal parenchyma[6]. The different causes of hydronephrosis can categorize it as either congenital hydronephrosis or acquired hydronephrosis, the latter of which often has no obvious clinical manifestations. If it is not resolved in a timely manner, there is a high probability of expansion of the renal pelvis and calyces and irreversible atrophy of renal parenchyma. It can even cause renal failure, which severely impacts a patient's physiology and psychology[7]. Therefore, an obstruction must be resolved as quickly as possible in order to restore the patient's renal function before damage occurs.

Recent studies have found that local microvascular injuries in the kidney can cause disturbances in microcirculation, thus resulting in glomerular interstitial fibrosis, which ultimately leads to renal failure[8]. Injury of local microvessels in the kidney may be an important factor affecting decreased renal function after hydronephrosis. Angiogenesis refers to the process of capillary endothelial cell proliferation and migration and the remodeling and formation of blood vessels[9]. Neovascular endothelial cells primarily originate from existing microvascular endothelial cells[10]. Under normal circumstances, endothelial cells and vascular smooth muscle cells are in a relatively stable state without mitosis. However, under the influence of tissue ischemia, hypoxia, inflammatory edema, or other factors, endothelial cells migrate and divide, thus resulting in the formation of new blood vessels[11]. Several studies have found that miRNAs can regulate relevant signaling pathways, participating in the change to renal function after hydronephrosis and promoting local angiogenesis. miRNAs, a class of small RNA molecules, exist widely in eukaryotes and can regulate the expression of various proteins and influence their biological effects[12]. miR-210 is a microRNA with hypoxia-inducing activity. This study found that under hypoxic conditions, miR-210 can be upregulated through the protein kinase B-p53 (Akt-p53) signaling pathway, thereby inducing the production of HIF and promoting angiogenesis[13]. Primarily, the present study aimed to explore the mechanism through which miR-210 promotes local angiogenesis following hydronephrosis in mice.

According to related reports, transforming growth factor, HIF, and VEGF are all angiogenic factors. Among them, HIF is a dimeric transcription factor produced under hypoxic conditions and has hypoxia-inducing activity[14]. When hypoxia occurs, HIF-1α can specifically sense hypoxia sites and improve tissue hypoxia through various signaling pathways and transcriptional regulation of target genes. As one of the target genes of HIF-1α, VEGF is currently considered the most important angiogenic factor. Studies have found that when the body is in a state of ischemia and hypoxia, VEGF can play an angiogenic role through ligand receptor binding, thereby compensating and alleviating tissue hypoxia, especially in tumor tissues[15]. The higher the malignancy of the tumor, the more serious the tumor spread, and the higher the VEGF level. The results of the present study found that miR-210 and VEGF expression increased after hydronephrosis in mice and subsequently decreased, while HIF-1α expression gradually increased.

In conclusion, miR-210 may promote local angiogenesis through regulation of the HIF-VEGF signaling pathway following hydronephrosis in mice.

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Corresponding Author:
Yunhui Liao
Email: w249lx@163.com
(China)