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Research Progress of Urogenic Stem Cells and Exosomes in Chronic Kidney Disease

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Abstract: Chronic kidney disease (CKD) is a chronic metabolic disease with renal function injury or glomerular filtration rate <60% caused by a variety of factors. Due to the powerful compensatory function of the kidney, the onset of the disease is hidden, and if it cannot be detected and intervened in time, it can eventually progress to end-stage renal disease. At this time, alternative drugs such as dialysis and kidney transplantation can be used to maintain life. Urine-derivedstemceslls (USUs) are stem cells obtained from human blood with very high expansion function and multi-differentiation potential, and have the characteristics of unlimited source, non-invasive and self-derived culture. Many scientific studies have shown that USCs also has very important physiological and pathological effects in the fields of cell inflammatory response, cell apoptosis and cellular oxidative stress. Therefore, the search for potential biomarkers of chronic kidney disease has become an urgent problem. In this paper, the origin, isolation and culture of urogenic stem cells, their application in chronic kidney disease and the research progress of exosomes were reviewed.

1. Introduction

Chronic kidney disease (CKD) is mainly caused by various causes of renal structural and functional damage (albuminuria, glomerular and tubular related lesions, imaging abnormalities, etc.) for more than 3 months, or glomerular filtration rate <60%. The common causes of chronic kidney disease are diabetes, hypertension and so on. Some patients with chronic kidney disease develop from childhood CKD, and the causes of chronic kidney disease in children include hereditary kidney disease (such as eye-ear-kidney syndrome and polycystic kidney disease), primary glomerular disease (such as IgA nephropathy, primary nephrotic syndrome and glomerular sclerosis), and secondary glomerular disease (such as diabetic nephropathy and Henoch purpura nephritis, etc.)[1,2].Chronic kidney disease caused by these reasons will develop renal tissue fibrosis and eventually evolve into end-stage renal disease with the development of the disease [3].Among them, 70% of children with chronic kidney disease will develop end-stage renal disease by the age of 20, and the fatality rate is 30 times higher than that without end-stage renal disease [4].The prevalence of CKD in the United States increased from 10.0% in 1999 to 13.1% in 2005[5].An epidemiological survey in 2012 showed that the prevalence rate of adult CKD in China

was as high as 10.8%[6].In addition, the number of kidney patients in China is also growing very fast, reaching 100 million per year [7].At present, the diagnosis of chronic kidney disease is mainly through kidney puncture biopsy, but this method has a certain degree of trauma, most patients are difficult to accept, so the early screening and repeated detection of the disease is limited. In addition, once a chronic kidney disease patient develops end-stage renal disease, only dialysis therapy (peritoneal and hemodialysis) and kidney transplantation can be performed. Dialysis is expensive, has many complications, and affects patients' quality of life. At the same time, due to the lack of transplant donors, postoperative rejection, and the stimulating effect of drugs on patients and other factors, the development of kidney transplantation has increased a lot of difficulty. If chronic kidney disease is not diagnosed and intervened in time, the symptoms will be further aggravated, the condition will be delayed, and the health of the patient will be affected. Therefore, a non-invasive, highly specific and sensitive biomarker is urgently needed to facilitate the early diagnosis of chronic kidney disease.

USCs can be extracted from human urine, which is simple and harmless to the human body, and has become the focus of many scholars. The causes of kidney disease are complex, with high morbidity and mortality. At present, the treatment of kidney disease usually adopts alternative therapy, such as dialysis or transplantation. These therapies can only temporarily prolong the survival of patients, but cannot fundamentally solve the problem of kidney disease. However, in recent years, USCs has provided a new option for the treatment of kidney disease[8].

2. Origin, isolation and culture of USCS

In 2008, Zhang et al. [9] extracted progenitor cells from healthy human urine for the first time and found that they had the characteristics of mesenchymal stemcells and were called urine-derivedstemcells (USCs). Compared with mesenchymal stem cells, these cells have the advantages of being simple, non-invasive and easy to isolate and culture. In a study conducted by Bharadwaj et al.[10], it was found that USCs extracted from urine of female patients contained Y chromosome and could express kidney specific genes CD146, CD13 and PAX2. It was found that this female patient had received kidney transplantation from a male donor, indicating that USCs originated from the kidney.

Compared with other adult stem cells, USCs has a strong multidirectional differentiation ability in the treatment of chronic kidney diseases, and can rapidly and efficiently differentiate into glomerular epithelial cells and renal tubule skin cells [10].Studies have shown that USCs can successfully differentiate into urothelial cells, urinary smooth muscle cells, nerve cells, as well as fat cells, bone cells, and chondrocytes [11].Finally, urine-derived stem cells have a unique appearance structure that allows them to remain stable and not be destroyed during differentiation, and can be cultured and proliferated more efficiently. Experiments have shown that USCs can express active markers on the cell surface, such as the surface markers of mesenchymal stem cells such as CD73, CD44 and CD54, and the surface markers of embryonic stem cells such as SSEA4 and TRA-1-81 [12].Studies have shown that USCs can produce a variety of trophic growth factors through paracrine effects to promote cell repair and inhibit fibrosis and podocyte apoptosis[13].Animal experiments have proved that USCs plays an important role and value in the cardiovascular system, nervous system, bone repair system, urogenital system, kidney system, etc., and can improve the function of multiple organs [14].

At present, the culture method of USCs is as follows: (1) Urine obtained from normal human body is loaded into a sterile centrifuge tube, and the bottom precipitation is collected after centrifugation;(2) Add 10ml phosphate buffer to clean, continue to centrifuge and collect liquid precipitation;(3) The precipitation was placed into DMEM/F-12 cell culture medium for

re-suspension, and the medium was transferred into a 12-well plate for static culture in a cell culture bottle at 37℃ and 5%CO2.1 ~ 3d, replace the medium once a day, and then replace it to observe whether the medium is polluted. If there is pollution, it needs to be replaced once. After about 3 days of culture, some cells were cloned, and 80%-90% fusion was achieved in 9-12 days, at which time the cells had strong reproductive ability and could be passed through [15].

3. Application of USCs and exosomes in CKD

Diabetic nephropathy is one of the most common complications of diabetes and an important type of chronic kidney disease, and poor treatment will lead to end-stage renal disease[16].Jiang et al. [17] constructed a rat model of type 1 diabetes mellitus and found that exosomes secreted by urine-derived stem cells could inhibit caspase-3 overexpression in diabetic rats, reduce urine volume and urinary microalbumin excretion, inhibit the apoptosis of renal tubular epithelial cells, increase the proliferation of glomerular endothelial cells and promote the regeneration of vascular endothelial cells by injecting them intravenously. In addition, exosomes were found to inhibit podocyte apoptosis in a rat model of podocyte injury under high glucose culture. Other studies have found that the course of chronic kidney disease or renal fibrosis is related to the reduction of miR-29c and miR-181a [18]. Exosomes also contain potential therapeutic factors such as transforming growth factor β (TGF-β1), bone morphogenetic protein-7 and angiopoietin, which promote cell proliferation and angiogenesis to prevent kidney damage from diabetes. Zhao and Zhang et al. [19,20] demonstrated that in the constructed rat model of chronic kidney injury, USCs was injected into renal parenchyma, and the results showed that USCs could significantly improve glomerular filtration rate, improve renal histopathological changes, inhibit monocyte infiltration, and reduce blood creatinine level. In the diabetic rat model studied by Dong et al. [21], after the intervention of urine-derived stem cells, the number of apoptotic cells in kidney tissue, renal fibrosis and podocytes damage were significantly reduced, and various indicators were significantly better than the positive control group, such as 24h urinary protein content, urinary creatinine and urea nitrogen. Studies have shown [22, 23] that the development of diabetic nephropathy is related to vascular endothelial growth factor A, because it can lead to abnormal formation of blood vessels. Therefore, Duan et al. [24] selected miRNA-16-5p, which can regulate vascular endothelial growth factor A, expressed it highly in urine-derived stem cells, and then injected it into diabetic rats, and found that mirNA-16-5P can reduce the podocyte damage induced by high sugar, and reduce the increased urine volume and urinary microalbumin due to kidney damage. It has obvious effect on the treatment of diabetic nephropathy. Other studies have pointed out that exosomes may also be an important marker to evaluate the diagnostic effectiveness of diabetic nephropathy [25]. Based on the above results, exosomes play an important role in the diagnosis and treatment of diabetic nephropathy.

4. Protective mechanisms of USCs and exosomes against CKD

In animal models of chronic kidney injury, USCs has played a very good role in protecting the structure and function of the kidney, and the exosomes secreted by urine stem cells play a key role. Exosomes are one of the main types of extracellular vesicles, containing DNA, mRNA, miRNA, other non-coding RNA, proteins and other substances, ranging in diameter from 40 to 200nm, and are cystic vesicles formed by the fusion of multiple vesicles and cell membranes. Exosomes are formed in the form of exocytosis, mainly undergo three transformations, and are released into the cell through plasma membrane fusion and used for corresponding target cells [26].Exosomes secreted by urinary stem cells are cystic vesicles with lipid bilayer structure secreted by various epithelial cells of the kidney and reproductive system. The phospholipid bilayer structure of exosomes can safely transport active substances to corresponding target cells for intercellular information exchange, and play a key role in cell homeostasis, cell proliferation and inflammatory

response. In terms of kidney diseases, bioactive ingredients carried by exosomes can be taken up and absorbed by renal tubular epithelial cells, thereby preventing renal tubular injury and cell apoptosis, and achieving the purpose of alleviating local inflammation [27].Currently, exosomes are effective in the treatment of nervous system injury, bone repair, urinary system diseases, cardiovascular diseases, kidney diseases, etc., providing great help for the early diagnosis and prognosis of diseases[28,29]. Detection of exosomes expression in body fluids has become a new hot spot in the medical field.

Mirnas are mostly derived from small RNA molecules in non-coding regions and consist of about 20 to 27 nucleotides. miRNA is highly stable and conserved in gene location, and these advantages are related to their functions. The structure of miRNA regulatory genes is very complex, and the expression of one gene can be regulated by the combination of several mirnas, while the expression of multiple genes can also be regulated by one miRNA. Some studies have found that miRNA plays an important regulatory role in the occurrence and development of diabetic nephropathy and renal fibrosis[30,31].miRNA can inhibit the expression of messenger ribonucleic acid (mRNA), so as to interfere with cell proliferation, apoptosis, transformation, etc., as a supplementary detection method for chronic kidney disease. Studies have shown that more than 10 kinds of miRNA are abnormal in the urine of patients with type 2 diabetic nephropathy, and miRNA is found to be significantly correlated with urinary albumin excretion, platelet reaction-1, and transforming growth factor-β signaling pathways. Therefore, miRNA will become the target and breakthrough point of new diagnosis and treatment of diabetic nephropathy.

Studies have shown that exosomes are an important medium for information exchange between cells, and the paracrine mechanism of exosomes plays an important role in the repair of CKD [32].In addition, exosomes can increase the levels of anti-inflammatory factors such as transforming growth factor-β and interleukin-10, and decrease the levels of pro-inflammatory factors such as tumor necrosis factor-A and interferon Y [33].Therefore, exosomes secreted by urinary stem cells in the treatment of chronic kidney diseases have become a hot topic in the medical field.

5. Summary and outlook

In summary, most researchers have demonstrated that exosomal mirnas play an important role in the diagnosis and treatment of kidney diseases, and their high specificity and sensitivity may make some mirnas become clinical diagnostic markers for chronic kidney diseases. At the molecular level, specific markers such as proteins, nucleic acids and lipids carried by exosomes can reflect the pathological information of chronic kidney disease, thus providing great help for the specific diagnosis of the disease. In terms of detection, exosomes have the advantages of non-invasive access, stable content of bioactive substances, repeatable detection, etc., which can be used for repeated monitoring of kidney diseases. The dynamic changes of active substances in exosomes play an important role in the prognosis of kidney disease and can detect the development and outcome of the disease in real time. Although exosomes have many advantages in the treatment of kidney diseases, there is a lack of a large number of clinical studies to prove the feasibility and safety of their use in humans, and a large number of clinical trials are needed to continuously explore them, explore how to isolate and purify exosomes, and explore the molecular mechanism of action of exosome mirnas in chronic kidney disease. Finding exosomes with high specificity for chronic kidney disease is an important issue in current clinical research. In addition, there is still a lack of a large number of evidence-based studies to further clarify the clinical application of miRNA in kidney disease.

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