Regenerative Medicine in Nephrology

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Abstract. *Currently, regenerative medicine is the most promising area, often considered as the future of medicine. Acute kidney injury (AKI) is a life-threatening condition characterized by high mortality. The increasing number of patients with AKI is a very significant problem for the public in societies around the world. Although AKI is often a reversible process, it progresses to chronic kidney disease (CKD). Therefore, it is very important to find methods preventing chronization. As there is no effective treatment at the moment, regenerative medicine is a very promising area that can offer other medical solutions. Stem cells are a potential way of treating kidney damage. A considerable number of studies have already been carried out to evaluate the effect of stem cells on AKI, but their influence on chronization has not been investigated well. This review presents the latest knowledge on nephrogenesis, various types of stem cells, transplantation methods and mechanisms in animal studies.*

Introduction

Acute kidney injury (AKI) is a dangerous condition characterized by a sudden decline of the renal function [1, 2]. The incidence of this disease is still increasing and has become more than 20 times higher over the past 20 years. Mortality is as well very high, especially in intensive care departments, where it reaches 60% [1, 3]. Due to this disease, about 2 million people die every year around the world [4]. Although there is certain pharmacological treatment in an AKI setting, including modern dialysis procedures, such as intermittent or long-term replacement therapy, these therapeutic approaches remain very limited in both their efficacy and spectrum. Therefore, a new therapeutic strategy is needed immediately [3].

Although AKI is a potentially reversible process, its episodes can lead to chronic kidney disease (CKD) and end-stage renal injury in the future [5, 6]. About 15–20% of AKI progress to late stage CKD [4, 6]. Although the understanding how AKI progresses to chronic is improving, there are no clinically effective therapeutic measures to stop the progression of this disease yet. The main goal is to prevent the chronization of AKI and to reduce the risk of death [5].

CKD, i.e., renal functional or structural changes, lasting for more than 3 months affect the health status [7]. This major global problem is receiving more and more attention because it is associated with high morbidity and mortality [5, 8]. All over the world, the disease is affecting younger people without chronic diseases such as diabetes or hypertension [8]. The number of patients with end-stage renal disease is rapidly increasing, which is a significant financial burden for the public [5, 8]. According to the data provided in 2012 by the European Kidney Association 2012 – European Association for Dialysis and Transplantation (ERA-EDTA – European Kidney Association – European Association for Dialysis and Transplantation), 716.7 inhabitants per million (million live births) in Europe were suffering from end-stage renal injury and were treated with renal replacement therapy [5].

Many treatments have been tried to slow down or stop the progression of CKD [9]. Despite preclinical studies, there is no effective and timely treatment. Therefore, it is very important to develop a new strategy for maintaining and improving the kidney function. One of the most promising ones is renal regenerative medicine, using cells that can effectively change various kidney structures [3]. Renal regeneration is described as the proliferation from residual living cells and necrotic cells into a new tubular epithelium [2].

Human Kidney Embryogenesis

In order to understand regenerative mechanisms better, a closer look at kidney development in embryos is needed. Nephrogenesis begins with the pronephros phase from fetal week 4. The phase of the pronephros is localized in the most cranial part. Further, nephrogenic mesenchyme differentiates into the pronephric duct and tubules. Degeneration lasts until week 5. The pronephric duct including intermediate mesoderm differentiates to mesonephric tubules and the mesonephric duct. This phase is called the mesonephros stage. The mesonephric duct develops into the genital system for males, but mesonephric tubules regress. On week 5, the metanephros phase begins, where the mesonephric duct undergoes the transformation into the ureteric bud and metanephric mesenchyme. Its cells derive from the intermediate mesoderm. It is the most cau-

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dal part. Renal calyces, the pelvis, colleting ducts and the ureter are formed from metanephric mesenchyme. Metanephric mesenchyme develops further collecting tubules and the glomerulus. The mentioned formation is called a nephron. Adult kidneys are formed in the metanephros. Human nephrogenesis develops from cranial to caudal direction until fetal week 34–35. The formation of the glomerulus occurs on week 36 [10–12].

Types of Stem Cells

Several researchers have suggested the use of kidney precursors isolated from adult kidneys. They have an important role in restoring damaged kidney structures in various pathological conditions. However, the adult human kidney contains very few kidney precursor cells, which, in addition, have limited proliferation and differentiation. Therefore, the clinical use of these is unavailable for patients [2].

Embryonic stem cells (ESC) have the ability to regenerate and differentiate into many types of cells. This is very important in regenerative medicine. A lot of research has already been done to evaluate the benefits of stem cells in treating AKI. ESC could differentiate into kidney cell lines in vitro. Research has shown that renal progenitors derived from ESC can integrate into proximal tubules with normal morphology and development of mouse newborn kidneys. However, the use of embryonic cells in clinical practice is limited by ethical and legal aspects [2].

Since it has been found that kidneys have stem cells, the idea that the physiological kidney regeneration mechanism is based on increased proliferation and differentiation of mesenchymal stem cells has arisen. These cells also contribute to the regeneration of many other injured tissues. This has led to the search for new therapies to achieve a better result [9].

Mesenchymal stem cells (MSC) are multipotent mesodermal non-embryonic stem cells that are capable of self-renewal and differentiation into new cell lines (e.g., blood, nerve cells) [13–16]. MSCs can be isolated from a number of tissues, such as the adipose tissue [17], bone marrow [18], muscle [19], cord blood [20], synovium [21], umbilical cord [22], dental pulp [23] and amniotic fluid [24].

A new type of stem cells are called induced pluripotent stem cells (iPSCs) [2, 3]. Using selected transcription factors, the reprogramming of somatic cells is achieved. This leads to forming of embryonic stem cell-like cells. The similarity of these cells with ESC appears in self-renewal, plasticity and differentiation. The value of iPSCs is already known in regenerative medicine [25]. iPSC development into one or another nephron progenitors (NP) can be achieved by inducing special growth factors in suitable concentrations. These factors can be activin, retinoic acid (RA), bone morphogenetic protein (Bmp), fibroblast growth factor (FGF) and canonical Wnt agonist (CHIR) [26, 27]. iPSC can differentiate into NP as well as into a late primitive streak, a posterior mesoderm, a posterior intermediate mesoderm or metanephric mesenchyme. In these processes, it is noted that NP rebuilds self-organized nephron-like 3D structures in vitro. These include a glomerulus with podocyte cells, proximal tubules, loops of Henle and distal tubules [26-29]. As mentioned, Wilms tumor protein 1 (Wt1), paired box 2 (Pax2), Sal-like 1 (Sall1), homeodomain transcriptional regulator (Six2), Odd-skipped related 1 (Osr1), glomerulus markers, including Wt1/nephrin+, Claudin1, differentiated renal tubules markers Pax2 and Sall, proximal tubules markers cadherin6+, Aquaporin 1 (AQP1), Lotus tetragonolobus lectin (LTL), and distal tubules marker E-cadherin+ express intermediate mesoderm and/or metanephric mesenchyme markers. Thus, iPSCs transformation into NPs repeats the formation of the human embryonic kidney [26, 28, 29]. The investigation of in vivo NPs differentiation capability is not yet performed; however, the evidence of efficient vascularization of the glomerulus upon transplantation is noticed [26].

Stem Cells in Kidney Injury

The efficacy of MSC in the treatment of AKI has already been proven. However, controversial opinions that MSC can help prevent the development of AKI to CKD are still present [30]. Recently, positive effects of iPSC in various CKD animal models have been observed. A decrease in creatinine, urea plasma concentrations, proteinuria, canal dilatation, tubular tuberculosis, kidney fibrosis, glomerulosclerosis, macrophage and inflammatory cytokines infiltration and improvement of GFR has been noticed [13, 30]. However, in other studies, improvement in the renal function after treatment with stem cells has not been observed but histological changes have been significantly reduced [9, 31]. MSC could be used as a prevention tool of AKI development to CKD with the exception in stable CKD [31]. The ability of MSC to remain alive after one or several months in vivo has not been proven in these studies [30, 31].

As iPSCs regenerate, their usage can contribute to drug screening or cell therapies preventing diseases as well as in modeling kidney development [2, 3, 27]. The issue of iPSC-derived NP is discussed in three articles [2, 3, 32]. Research of NP in AKI models, including the impact of fibrosis, showed that iPSC had no protective effect on the renal function and renal histology [32]. Another research drew a conclusion that iPSC had positive results on the renal function as well as decreasing histological changes. Consequently, differences in opinion appear [33]. However, the iPSC kidney progenitor in preventing CKD after AKI has not been analyzed yet. Therefore, the iPSC differentiation into all kid-

ney structures and improvement of their functionality in AKI or CKD models with animals should be analyzed. Accordingly, iPSC-derived NP have not been sufficiently studied and the risk of tumors persists. However, as these cells are more differentiated than iPSC for treatment, their tumorogenic potential is lower [2, 3].

Mechanism of Stem Cells

It is still hard to understand how MSC improves the renal function and minimizes the damage. Although MSC has the ability to differentiate, most scientists tend to believe that their protective and regenerative properties are mainly due to the mechanism of paracrine action [13, 14, 30, 34]. The most probable mechanism is based on the protective properties of the blood vessels that prevent development of renal glomerulosclerosis, help to regenerate tubules, protect against apoptosis and promote epithelization processes [9, 35].

There are also divergent views on the functioning of iPSC. Opinions about the functioning properties of iPSC differ. While some authors state that kidney structures can be formed using transplanted iPSC-derived NP, other authors deny it as NPs do not differentiate to the tubules of the kidney in vivo. It should be mentioned that as iPSCs express early stage markers of differentiation, they are not approved as NPs [2, 3]. On the other hand, it has been noticed that the method of differentiation is not always suitable [32]. However, it is believed that better results can be achieved by using different iPSC differentiation protocol. It can serve to prove as well that apart from paracrine effects, iPSC can differentiate into kidney strains in vivo. Therefore, using another iPSC, it is expected to obtain better results and to demonstrate that iPSC has not only paracrine effects but also the ability to differentiate into renal tissues.

Route of Stem Cells Transplantation

Stem cell transplantation can be performed in different ways; however, the most appropriate method for the placement of stem cells has not yet been determined. In the past, one of the most popular methods was intravenous infusion of MSC. In this route, only few stem cells reach the kidneys, and most of the transplanted stem cells are deposited in the lungs, the spleen, and the liver [36, 37]. Therefore, in order to increase the number of cells that reach the target organ, it is necessary to infuse more stem cells. This is dangerous because it can increase the risk of side effects, especially that of pulmonary embolism. This is due to the small capillary shaft, the large capillary network and the strong mesenchymal cell adhesion properties [36]. It is believed that it is more efficient to allow stem cells to enter the artery, as more stem cells enter the kidney and, thus, prevent arterial thrombosis, infections or other adverse reactions [36, 38].

Fatma et al. did not show a significant difference between intravenous, intra-arterial mesenchymal stem cell allocation and subcapsular transplantation. Allocating stem cells in all three ways reduces tubular necrosis and infiltration of inflammatory cells, but benefits of the renal function have been observed with subcapsular transplantation [39].

Controversy on iPSC-derived transplantation is still present. While some state that transplantation of iPSC had positive effects on the renal function and fibrosis in animal studies [2], others claim that improvement in the renal function appears due to subcapsular transplantation of iPSC-derived NP excluding intraparenchymal transplantation of these cells [32].

Conclusion

Investigations of the number of stem cells type usage in AKI settings reveal promising benefits for treating both AKI and AKI to CKD conversions. Further research on animals is essential for regenerative medicine to be adapted to clinical practice. This will help reduce the incidence of chronic kidney disease-related complications, deaths, disability and economic burden around the world. Stem cell therapy will definitely determine the breakthrough of medicine in the future and will eventually change the use of various medications.

Conflict of Interests

This manuscript have no conflicts of interest.

Authors' Contributions

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

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