Original article

Nephroprotective effects of subcapsular transplantation of metanephric mesenchymal cells on gentamicin-induced acute tubular necrosis in rats

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Background: **The subcapsular transplantation of metanephric mesenchymal cells (MMCs) may be a new therapeutic approach for the treatment of acute tubular necrosis (ATN). To investigate this hypothesis and provide evidence for its possible use in the clinic, we evaluated the nephroprotective effects of transplanting MMCs into the renal subcaspsule of rats with ATN induced by gentamicin.**

Methods: **MMCs were expanded in culture. After gentamicin-induced ATN was established, fluorescently-labeled cells were transplanted and traced in kidney tissues by fluorescence microscopy. Serum creatinine (Cr), urea nitrogen (BUN), and N-acetyl-b-D-glucosaminidase (NAG) levels were determined at different time points. Kidney pathology was studied by hematoxylin-eosin staining. Apoptosis was examined by the TUNEL assay.**

Results: **In the MMCs-treated group, the mortality rate decreased; BUN, Cr, and NAG levels peaked at 8 days, and were significantly lower than those in the other groups at 11 and 14 days. RIMM-18 cells locally recruited through precise tropism to sites of injury had the ability to migrate into the tubuli from the renal subcapsule. Damage to the cell-treated kidneys was reduced. The pathologic lesion scores of tubular damage reached the highest values at 8 days in the treated kidneys and 11 days in the untreated ones. The apoptotic index showed that the peaks of apoptosis occurred at earlier stages of the injury process in cell-treated than in untreated kidney and thereafter declined in a time-dependent manner.**

Conclusion: **The subcapsular transplantation of MMCs could ameliorate renal function and repair kidney injury.**

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Key words: **acute tubular necrosis; metanephric mesenchymal cells; subcapsular transplantation**

Introduction

Acute renal failure is a serious medical complication characterized by the sudden loss of the ability of the kidneys to excrete wastes, concentrate urine, conserve electrolytes, and maintain fluid balance.[1] Currently, routine treatment options such as dialysis and other supportive measures can not completely replace renal function. The mortality associated with this syndrome is still as high as 50%-80% and has not changed significantly over the last 50 years.[2,3] Renal biopsies have demonstrated that acute tubular necrosis (ATN) is the cause of 70%-80% of acute renal failure cases. Recovery of renal function after ATN is dependent on having sufficient surviving epithelial cells to proliferate, differentiate into mature tubular cells, and restore the injured tubular structure and function. Nevertheless, endogenous surviving cells are usually not sufficient to regenerate even parts of such a complicated structure after injury.[4,5] Therefore, the discovery and exogenous administration of renal stem cells or progenitor cells may provide a more effective treatment.[6,7]

The existence of stem cells in the adult kidney is currently controversial.[8] Most renal studies have focused on the role of bone marrow stem cells (BMSCs) in basal cell repair in various renal injury models, which have shown that BMSCs were able to migrate to damaged kidney tissue, cross lineage boundaries, and

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differentiate into renal tubular epithelial cells, improving renal function.[8-10] However, these findings have also proved to be controversial.[11-13] Moreover, it has been reported that a small percentage of bone marrow cells may consist of mesenchymal stem cells (estimated at about 0.001% to 0.01%).[14] Previous studies have estimated that there are very few mesenchymal stem cells, which have been reported to protect renal function, in freshly-isolated whole bone marrow cells.[11,15,16] In addition, the precise cell population in bone marrow responsible for these putative therapeutic effects is uncertain. Therefore, BMSCs do not currently appear to be ideal to treat renal disease.

A second possible solution to the problem of stem cell availability is the transplantation of renal progenitor cells.[17] Oliver et al[18,19] demonstrated that metanephric mesenchyme contains embryonic renal stem cells. A single metanephric mesenchymal cell can generate all the epithelial cells of the nephron (except the collecting duct), indicating that these cells are renal epithelial stem cells, which are pluripotent and have ability to differentiate into nephron epithelia in a suitable environment.

Studies on the repair mechanisms after kidney injury have shown the occurrence of proximal tubular cell dedifferentiation, re-entry into the cell cycle, and epithelial-to-mesenchymal conversion.[20-22] The dedifferentiated cells have the ability to migrate to the injured regions, replenish necrotic or dead cells, and repair injured tissues.[23] This repair process has similarities to embryonic kidney development. Evidence has shown that a major contributor to the development and progression of ATN is the loss of functioning tubular epithelial cells by various processes of cell deletion or death. Recovery is dependent on the ability of the remaining tubular cells to dedifferentiate, proliferate, reline the damaged areas along the nephron, and redifferentiate. Hence, we speculated that metanephric mesenchymal cells (MMCs) implanted beneath the renal capsule or in tunnels fashioned into the renal parenchyma might become incorporated into the tubular system of the host and thereby increase host renal function. Renal stem/progenitor cells have been found in the embryonic metanephron (ED13.5 in rats[24]), whose immunogenicity is lower than their adult counterparts. Transplantation between inbred strains allows cells to differentiate and develop without using immunosuppressive drugs,[25] giving it immunodominance. For this reason, we used an immortalized cell line of metanephric mesenchymal cells to evaluate the protective effects of transplanting them into the subrenal capsule in a model of gentamicin-induced nephrotoxicity ATN rats.

Methods

Cell culture

Embryonic metanephric mesenchymal cells (RIMM-18 cells) were presented by Levashova et al and cultured.[26] The cells were maintained in Dulbecco's modified Eagle's media (DMEM)/F12 (1:1) (Gibco/BRL, USA) supplemented with 5% fetal bovine serum (HyClone, USA), basic fibroblast growth factor (1 g/mL), and 17β-estradiol (100 nmol/L) (Sigma Chemical Co., USA). Cultures were incubated in a humidified incubator at 37°C in 5% CO_2 in air. For passage, the cells were dissociated with 0.25% trypsin/0.02% EDTA (1:1) for 3-5 minutes at 37°C and rinsed with serum-containing medium to remove residual trypsin/EDTA. The medium was changed regularly every 2-3 days and washed with D-hank's solution (Sigma Chemical Co., USA). The cells were collected by centrifugation at 1200 rpm for 5 minutes and seeded into fresh flasks at a 1:3 split. Cultured cells were used for the *in vivo* transplantation.

Rat model of ARF

Female Sprague Dawley rats weighing 180 to 210 g were obtained from the Second Xiang Ya Hospital animal laboratory (Hunan, China SYXK (xiang) 2004-0013). The rats were reared in metabolic cages under ordinary conditions (24±1°C, 12-hour light and 12-hour dark) and allowed free access to food and water. ATN was induced in 8- to 10-week-old rats by subcutaneous injection of gentamicin (TianWu, Tianjin, China) at 200 mg/kg daily for 4 days. The dose was based on preliminary experiments. The rats were randomly divided into five different groups (n=24 for each group): group 1, normal control group; group 2, ATN group (gentamicin injection only); group 3, ATN plus sham-operated group: gentamicin injection and injected saline (0.2 mL) to the right subrenal capsule 6 hours after the first dose of gentamicin; group 4, ATN plus medium group: gentamicin injection and injected cultural medium alone (0.2 mL, without donor cell administration) to the right subrenal capsule; group 5, ATN plus cellular transplantation group: gentamicin injection and injected cells (1×10^7 of cells in 0.2 mL of fresh serum-free medium) to the right subrenal capsule. The rats in all groups were killed at 5, 8, 11, or 14 days after the first injection of gentamicin (The day of grafting or the first injection was day 1). Six rats were needed at each time point for each group. Urine was collected daily and urine samples were used to determine the levels of urinary protein, by the biuret test (urine protein quantitative kit, Jancheng Bio. Co., Nanjing, China). Blood was drawn from the abdominal aorta and heart under anesthesia, and serum creatinine (Cr), urea nitrogen (BUN), and

N-acetyl-b-D-glucosaminidase (NAG) levels were determined at the different time points to estimate renal function or tubular and cellular integrity.

Cell labeled and transplantation of RIMM-18 cells underneath the renal capsule

Prior to *vivo* injection, cells were harvested with 0.25% trypsin/0.02% EDTA and suspended as single cells at a concentration of 1×10^7 cells/mL with fresh serum-free medium. The cells were then incubated with 4, 6-diaminidino-2 phenylindole (DAPI) for 40 minutes at 37°C and 5% CO_2, and a sample of the cells was examined under a fluorescent microscope or injected within 1 hour. The rats were anesthetized with 10% chloral hydrate (0.35 mL/100 mg body weight, intraperitoneally), laid on their left side, and a posterior subcostal flank incision was made at their right side. The kidney was slipped out of the body cavity by applying pressure on the other side of the organ using a forefinger and thumb. Immediately before implantation, the cell suspension was drawn up into a syringe, and three injections of cells (0.2 mL totally) were slowly made underneath the capsule. After grafting, the kidney was placed back into the proper position and the edges of the body wall and skin were aligned and closed with suture.[27] The animal was kept at 37°C with a heating blanket. After injection, the rats were returned to their cages.

Histological examination

After dissection, kidney tissues were fixed in 10% neutral-buffered formalin for 24 hours, transferred to a graded series of ethanol (100%, 90%, 70%), treated with xylene, embedded in paraffin at 56°C, and finally cut into 3 μm thick sections with a microtome (RM 2135, LEICA, Japan). The sections were deparaffined with xylene and rehydrated in the graded ethanol series to phosphate buffer saline. For histological analysis, the sections were stained with hematoxylin and eosin and observed using a light microscope (Nikon eclipse E400, Tokyo, Japan). Later, they were graded by Miller's pathologic lesion scoring standard of renal tubular injury.[28] Twelve high-power fields were chosen randomly to be scored for each section. The total score for each kidney examined was divided by 8 to give a final score of 0-3.

The number of neutrophils in the kidney was expressed as 400 kidney cells in three high-power fields (original magnification × 400) per slide.[29] The mean value from three high-power fields was used for statistical analysis.

Detection of apoptotic cells

DNA fragmentation in apoptotic cells was examined using the terminal deoxynucleotidyl transferase (TdT)-mediated nick-end labeling (TUNEL) method (MK1020, Boster, Wuhan, China).

Distribution of DAPI-labeled cells

Frozen sections of the samples were used to detect the distribution of DAPI-labeled cells in cell-treated kidneys using a fluorescence microscope (Nikon eclipse E400, Tokyo, Japan). In a dark chamber, fresh tissues from the grafted kidney specimens were embedded in Tissue-Tek OCT (Sakura Finetek, USA) compound and cut into 5 μm thick frozen sections using a cryostat (Thermo, SN:cs3298L0311, UK). The frozen sections were immediately examined under a fluorescence microscope. A 490-nm laser line generated by an argon laser was used to detect DAPI fluorescence.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Significance at the 95% level was established using one-way ANOVA followed by Dunn's post-hoc test. The presence of significant differences between the groups was examined by ANOVA LCD using SPSS 11.5 software (Jandel, San Rafael, USA), which was provided by the School of Public Health, Central South University, China.

Results

Evaluation of function and mortality

We first determined whether the engraftment of RIMM-18 cells improved functional deficits in the rat model of gentamicin-induced nephrotoxicity. Renal function, as measured by serum BUN, Cr, and NAG levels, was noticeably impaired in the observation period. Subcutaneous injection of 200 mg/kg of gentamicin daily for 4 days induced significant increases in these serum parameters compared with normal controls ($P<0.05$). No rats died in the control group. Compared to group 2 (30.6%), group 3 (35.0%) and group 4 (33.3%), the mortality significantly decreased in the ATN plus cellular transplantation group (16.7%) ($P<0.05$). The engraftment of RIMM-18 cells significantly ameliorated renal function as compared with groups 2-4 (Fig. 1) and it decreased mortality.

Histological examination

We investigated whether the renal function improvement by RIMM-18 cells treatment was associated with preservation of tubular structure. Pathologic examination using light microscopy revealed different extents of tubular lesions post injection. No pathological changes were observed in renal tissues from the control group.

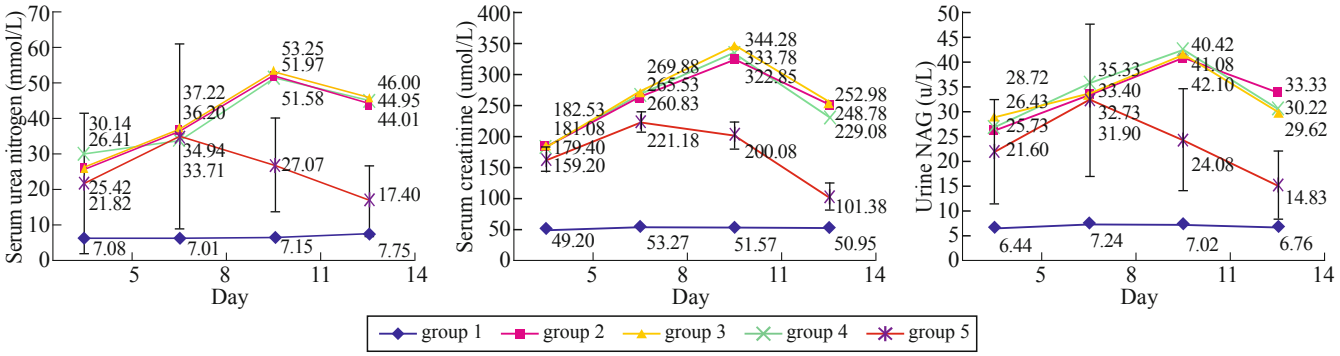

Fig. 1. Changes in serum urea nitrogen (BUN), serum creatinine (Cr), and N-acetyl-b-D-glucosaminidase (NAG) levels in the five groups at different time points. In group 5, BUN, Cr, and NAG levels peaked at 8 days; at 11 and 14 days, their values were significantly lower than those in groups 2-4 (P<0.05), even though we only injected cells into the unilateral capsule. There were no significant changes among groups 2-4. The data indicate that administration of RIMM-18 cells at the time of the first gentamicin injection significantly ameliorates subsequent renal function impairment.

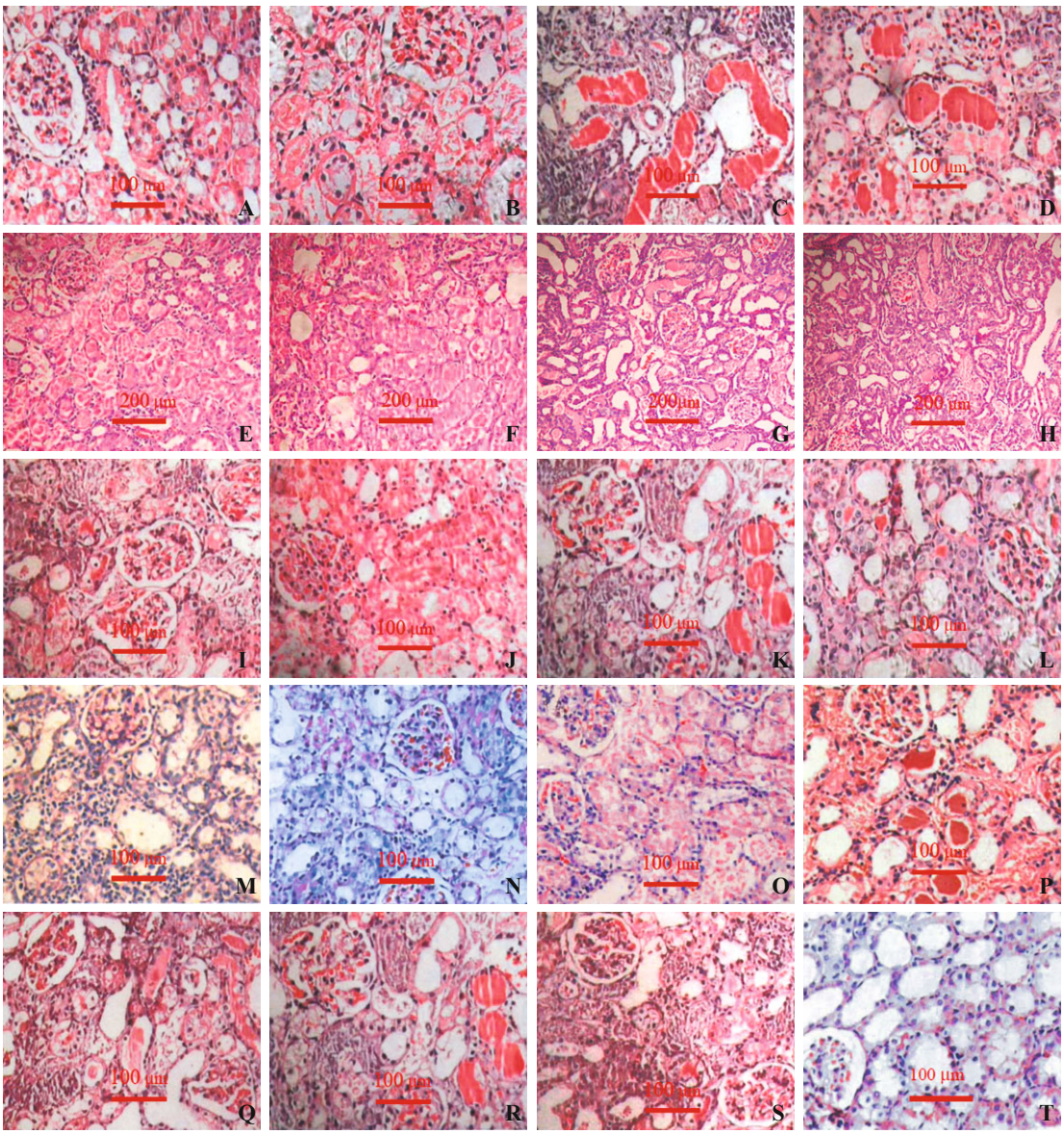

Fig. 2. A, **B**, **C**, and **D** represent 5, 8, 11 and 14 days respectively for the right kidney of group 2. **E**, **F**, **G** and **H** represent 5, 8, 11 and 14 days respectively for the right kidney of group 3. **I**, **J**, **K** and **L** represent 5, 8, 11 and 14 days respectively for the right kidney of group 4. **M**, **O**, **Q** and **S** represent 5, 8, 11 and 14 days respectively for the left kidney of group 5. **N**, **P**, **R** and **T** represent 5, 8, 11 and 14 days respectively for the right kidney of the same group. Photos of **E**, **F**, **G** and **H**, original magnification × 200 and the others × 400.

Taking the right kidneys in groups 2-4 and the hibateral kidneys in group 5 (Fig. 2), we found at 5 days after the gentamicin injection, examination of the proximal tubuli revealed loss of the brush border, cytoplasmic swelling, vacuolization, and a large number of inflammatory cells. In the right kidneys in groups 2-4 (Fig. 2A-L) and the left kidneys in group 5 (Fig. 2M, O, Q, S), the pathologic changes increased with time up to 14 days, with extensive loss of epithelial cells and protein casts. In contrast, the pathologic changes were milder in the right kidneys (Fig. 2N, P, R, T) of group 5. The pathologic lesion scores of tubular damage peaked at 8 days in the right kidneys and 11 days in the left ones (1.854±0.471 and 2.160±0.358, respectively) in group 5. Kidney comparison showed significant differences at 11 days (1.310±0.575 and 2.079±0.298) and 14 days (1.135±0.344 and 1.891±0.419). Similar to their effects on renal dysfunction, RIMM-18 cells exerted effect on renal pathologic changes. Injection of RIMM-18 cells ameliorated tubular damage to some extent. No apparent structural glomerular alterations were observed.

The number of infiltrating neutrophils in the right kidneys in groups 1-5 is shown in Fig. 3. Infiltrating neutrophils were significantly lower in the rats after gentamicin injection and cellular transplantation. There were no significant changes in groups 2-4.

Tracing experiment *in vivo*: migration of cells after injection into the renal subcapsule

If RIMM-18 cells participate in renal tubular repair, they or their progeny should be capable of migrating toward and incorporating into the tubular epithelium of the kidney. To test this hypothesis and examine the fate of transplanted RIMM-18 cells, the cells were labeled

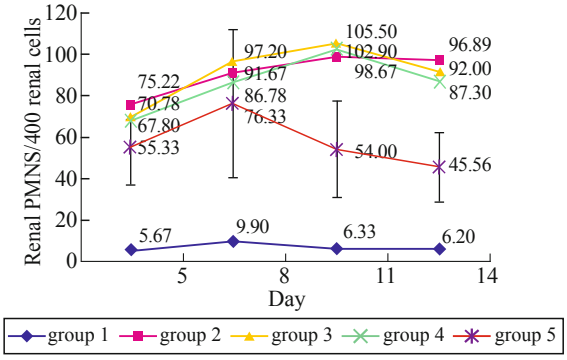

Fig. 3. The number of neutrophils infiltrating into the kidney in the five groups at different time points. Renal tissue samples were collected from the right kidney in each group. The number of neutrophils was observed after hematoxylin staining. Values were determined by counting neutrophils in three high-power fields (original magnification × 400) per section. The number of renal cells was also counted in each field, and the number of cells was expressed as per 400 renal cells. Infiltrating neutrophils in group 5 was lower than in groups 2, 3 and 4, and peaked at day 8.

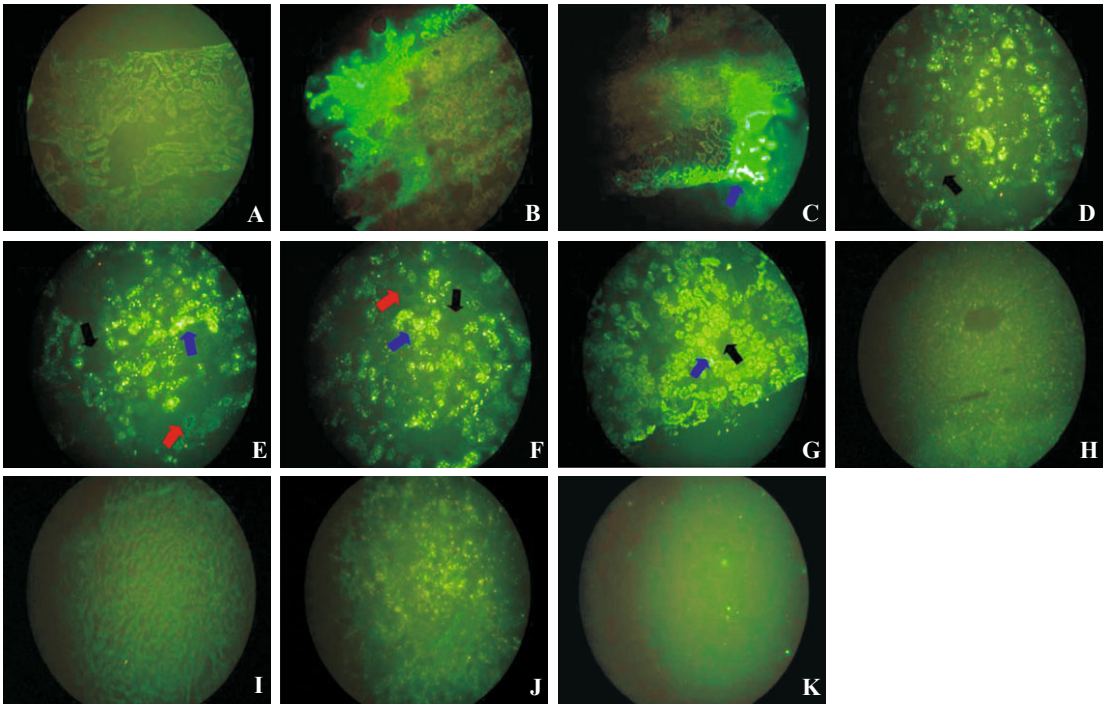

Fig. 4. Fluorescence distribution in rat kidney, heart, liver, spleen and urine. DAPI-labeled cells were injected into the renal capsule on the day of first gentamicin administration. Frozen sections were processed for DAPI detection. Untreated kidneys were negative (**A**). At 2 days, a few scattered DAPI-labeled cells with some cell aggregates were observed near the capsule (blue arrows, **C**). At 5 days, some positive cells were observed in the cortex (blue arrows, **D**). At 8, 11, and 14 days, the cortex tubuli contained numerous positive cells (**E**, **F**, **G**, respectively). **B** indicates positive cells mainly in the capsule. Glomerulus (black arrows, **A**, **D**, **E**, **F**, **G**), interstitial arteriole (red arrows, **E**, **F**), liver (**H**), heart (**I**), spleen (**J**), and urine (**K**) were negative.

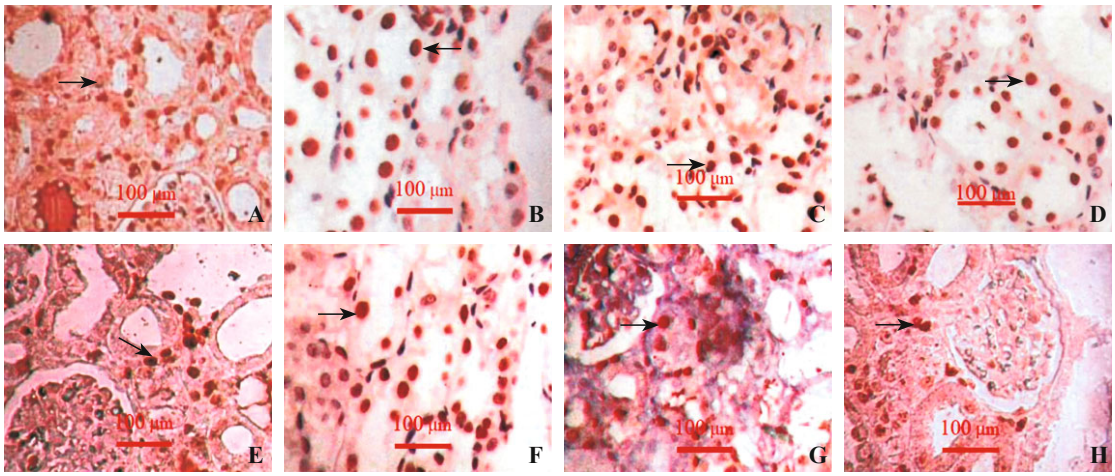

Fig. 5. A, **C**, **E** and **G** represent 5, 8, 11 and 14 days respectively for the left kidney of group 5. **B**, **D**, **F** and **H** represent 5, 8, 11 and 14 days respectively for the right kidney of the same group. *In situ* detection of apoptosis in the kidney. TUNEL-positive nuclei were stained condensed brown (black arrows). At 5 days, there were many TUNEL-positive cells distributed near the proximal tubule (**A**, **B**). At subsequent time points, the number of TUNEL-positive cells gradually decreased (**C**, **D**, **E**, **F**, **G**, **H**) in both kidneys. TUNEL-positive cells decreased more clearly in the right kidney of group 5 (P<0.05) (original magnification × 400).

with fluorescent dye DAPI *in vitro*, then microinjected into the subcapsular area of the right kidney. At 2, 8, 11, and 14 days, both kidneys were harvested and tissue sections were analyzed for the presence of labeled cells. As shown in Fig. 4, DAPI-positive cells were detectable mainly in the proximal tubuli of the right kidney. The DAPI-positive cells were mainly present in the subcapsular region at 2 days (Fig. 4C), then were found in the subcapsule and parenchymal regions of the host kidneys at 5 days (Fig. 4D). At 8, 11, and 14 days, there was widespread distribution of DAPI-labeled cells, mostly located in the cortical tubuli (Fig. 4E, F, G). Nevertheless, no DAPI-positive cells were found in the glomeruli and interstitial vessels (Fig. 4E, F). Our findings suggested that RIMM-18 cells locally recruited through precise tropism to the sites of injury have the ability to migrate into the tubuli from the renal subcapsule. This provided evidence that mesenchymal cells actively participated in the reconstitution of the differentiated epithelial lining. There were no cells positive for DAPI in urine and extrarenal organs (liver, heart, and spleen) (Fig.4 H, I, J, K). When cells were transplanted into normal kidneys, DAPI-positive cells were limited in the graft sites (the renal subcapsule) and only a few of them were integrated into epithelial structures (Fig. 4B). In addition, no tumor growth was observed in any tissue throughout the experiment.

Renal apoptosis

To examine whether apoptosis is an important reason for this severe disease and whether RIMM-18 cells have the ability to reduce the number of apoptotic cells, we examined apoptosis in both kidneys at each indicated time using TUNEL staining of fragmented DNA. We consistently observed significant levels of apoptosis and necrosis in gentamicin-induced ATN. We emphasized that the increased levels of apoptosis occurred mainly in the proximal tubular epithelium after injury. In the cell-treated group, the apoptotic index showed that the peaks of apoptosis occurred at earlier stages of the injury process in cell-treated than untreated kidneys (5 days post injury, 32.17±11.36 and 25.67±11.52, respectively) (Fig. 5B, A), and thereafter declined in a time-dependent manner. Apotosis was decreased more quickly in the cell-treated kidney than in the untreated one (11 days: 10±4.43 and 19.33±7.15; 14 days: 6.67±2.73 and 13.67±6.53, P<0.05) (Fig. 5F, E, H, G). We also observed similar differences between the treated kidney and the untreated kidney in groups 2-4 (data not shown).

Discussion

RIMM-18 cells were isolated from embryonic metanephrogenic mesenchyme. These pluripotent renal progenitor cells can differentiate into multiple lineages and have the ability to proliferate, including self-renewal and clonal propagation. In contrast to the diverse stem cell sources used for cellular transplantation, our study showed that mesenchymal stem cells have several potential advantages for tissue repair. Firstly, RIMM-18 cells have an unlimited capacity to propagate clonally *in vitro*, while the remaining undifferentiated. Secondly, the cells come from the embryonic metanephron and can differentiate into organ-specific cell types, including various epithelial cells in response to injury, and ameliorate damaged renal function. Under our

culture conditions, the properties of metanephrogenic mesenchymal cells agreed with the report of Levashova and co-workers.[26] RIMM-18 cells can maintain an undifferentiated, stable phenotype over many generations (at least 20 passages). These characteristics might make them suitable targets for *ex vivo* genetic manipulation and an option to promote kidney healing. The aim of cell culture was for subsequent *in vivo* implantation study. The first issue we encountered was to effectively distinguish engrafted and host cells.

DAPI was used to examine the behavior of labeled cells on nephrotoxicity in ATN rats. We injected labeled cells directly beneath the right renal capsule and found that after a 2-week chase period, without use of any inductor, DAPI-positive cells were mainly distributed under the renal capsule at 2 days. At 5 days, we observed that DAPI-positive cells gradually migrated into the renal cortex. Furthermore, the number of DAPI-positive cells in the kidney increased with time. At 8 days, DAPI-labeled cells were numerous in the tubular epithelium, persisting for up to 14 days after transplantation. We also found that RIMM-18 cells entered the tubular epithelial lining during the repair phase of gentamicin-induced ATN. We first reported this phenomenon and proposed that RIMM-18 cells are a kind of renal stem cells with high plasticity. It is important to note that fluorescent-positive cells can only be found in the cell-treated renal tubuli, but no positive cells can be detected in the glomerulus of the cell-treated kidney, contralateral kidney, urine, and other extrarenal organs. These observations are important in at least three respects. First, subrenal capsular injection avoids many of the side-effects of systematic drug administration. This technique should be used as an accurate delivery route for renal therapy and it provides a new and feasible approach for using stem cells. Second, we present evidence that DAPI-labeled RIMM-18 cells have the ability to migrate to and reside in injured renal tubules. Third, our findings also suggested that the cells can survive under hypoxic and inflammatory conditions to some extent. However, further investigation should be conducted to verify whether RIMM-18 cells have the potential to differentiate into tubular epithelial cells.

The tracer experiment *in vivo* demonstrated that the implanted RIMM-18 cells can migrate to the site of injury. Do these cells have the capacity to further repair or replenish damaged or aging tubular cells and then function as mature tubular cells? We found some indirect evidence to verify our hypothesis in this study. In our experiments, BUN, Cr, and NAG levels in the cell-treated group decreased more significantly than in the other groups (excluding the normal group), with significant distinctions at 11 days and 14 days. Morphological analysis of kidney repair after injury supported the above findings. Moreover we found that the mortality of the disease also decreased (the cell-treated group: 16.7%; the untreated group: 30.6%-35%). It was noteworthy that in the cell-treated rats, there were unparalleled improvements in pathology, demonstrating that transplanted RIMM-18 cells played an important role in the damaged kidney. These findings indicate that when RIMM-18 cells reach the lesion site, they contribute to healing of tissue integrity and also to the functional recovery of the injured kidney.

Since Gobe and Axelsen[30] first defined the presence and role of apoptosis in a renal disease termed hydronephrosis in 1987, apoptosis has been given more and more attention in the pathogenesis of established nephropathies including ATN. Apoptosis or programmed cell death is necessary for normal renal organogenesis and tissue homeostasis and plays an important role in renal physiology and pathology. Cellular death or survival depends upon a balance between death and survival signals. If survival signals are deficient or decreased in the injured kidney, cell death or senescence may be accelerated.[31] Schumer et al[32] found that renal tubular cellular death in ATN was partially induced by apoptosis. Using an *in situ* TUNEL assay, we found that apoptosis also occurred in the gentamicin-induced acute renal failure model. At 5 days, the apoptotic index peaked in the proximal tubuli, confirming a role for apoptosis at the early stages of gentamicin-induced ATN. Further analysis found that the apoptosis index in the cell-treated kidney significantly decreased at 8, 11, and 14 days. These data indicate that RIMM-18 cells have ability to decrease apoptosis in the damaged kidney. Between 5 days and 14 days after injury, the percentage of cells undergoing apoptosis fell in each group. One possibility is that internal anti-apoptotic mechanisms in the organism are initiated at the time and gradually predominate over apoptotic mechanisms. Another one is that the intrinsic renal repair process begins to play an important role. The rapid decrease in the apoptotic index in the cell-treated kidney in this study confirmed that transplanting cells regulate this process.

Although we did not observe any tumor growth in any tissue throughout the experiment, a potential risk of MMCs therapy may be paradoxically due to the ability of MMCs to promote tumor growth and metastasis. MMCs proliferation *in vitro* may acquire cytogenic abnormalities after long-term passage in culture and subsequent differentiation into tumor cells after *in vivo* administration. Our observation time is as short as 14 days. Follow-up studies are needed to determine the safety and long-term efficacy of MMCs.

In conclusion, the present study demonstrates the feasibility of transplanting allograft MMCs subcapsularly in adult rat hosts as well as the first long-term survival (greater than 10 days) and migration of metanephron

mesenchymal cells without immunosuppression. The injured rats in the study showed improved renal function change after transplantation of MMCs. We suggest that subcapsular transplantation of RIMM-18 cells into the damaged kidney may provide a new therapeutic approach for kidney disease in the future. The results of the present study will provide new insights into the mechanism of tubular regeneration after injury.

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Competing interest: No benefits in any form have been received or will be received from any commercial party related directly or indirectly to the subject of this article.
Contributors: Liu L proposed the study and wrote the first draft. Chen D analyzed the data. All authors contributed to the design and interpretation of the study and to further drafts. Yi ZW is the guarantor.

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