Regenerative medicine in AKI

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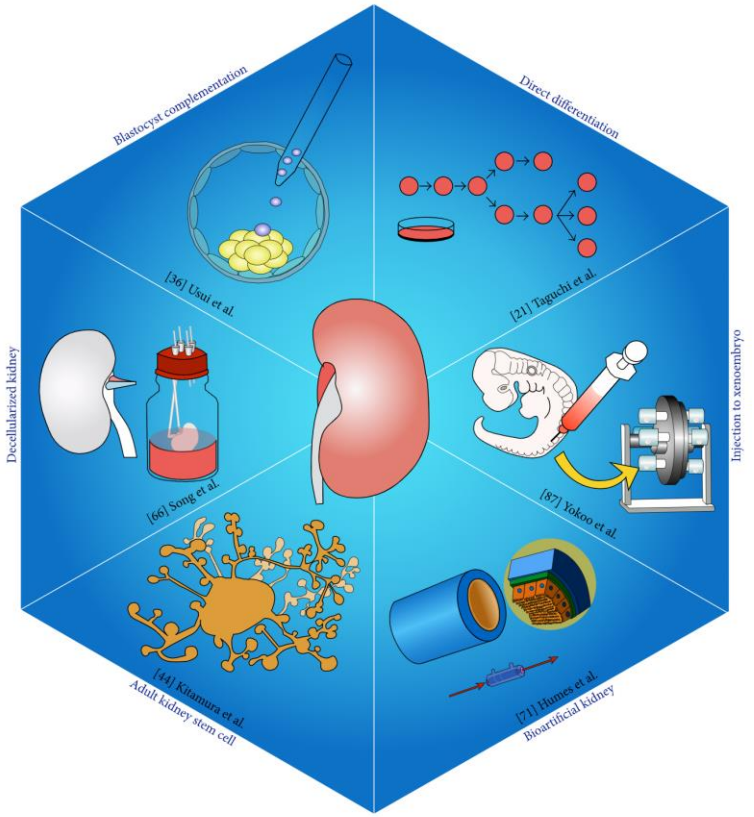
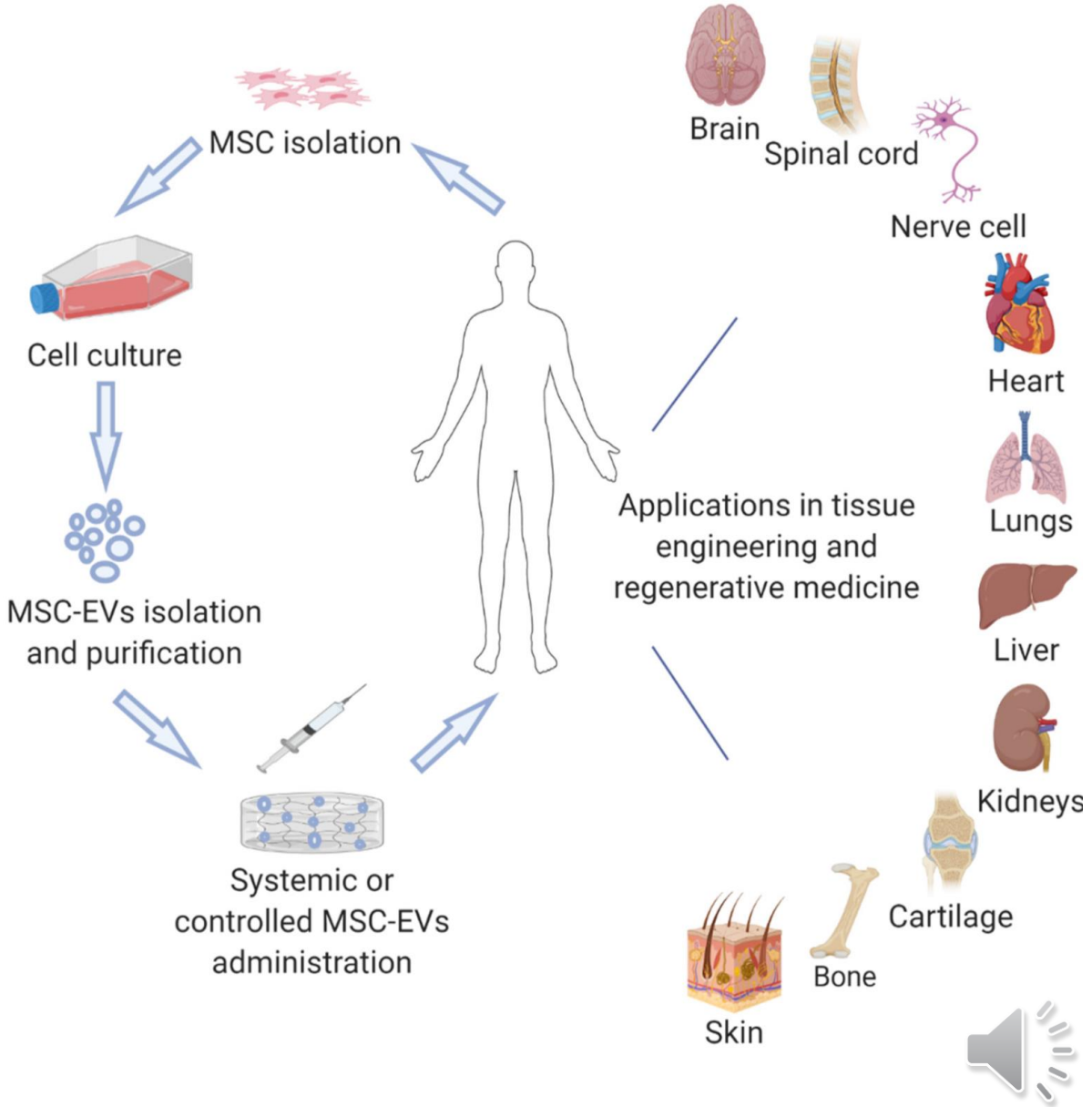
- **The term “regenerative medicine” is** used to describe any **biomedical approach to the replacement or regeneration of human tissues or organs for therapeutic purposes.**
- pluripotent stem cells (PSCs) from human somatic cells,
- the isolation of tissue-specific stem cells, and
- the reprogramming of non stem cells to a stem cell state .

- Immunomodulation or the administration of biologicals.

Figure 1

Schematic representation of the main strategies for kidney regeneration.





- All of these options can be envisaged for any organ;
- however, the barriers to success increase as histological and functional complexity increases.
- Hence, the replacement of skin or the bioengineering of simple epithelial structures, such as bladder wall, have seen substantial progress.

- The kidney has been a far greater challenge.

However, the last decade has seen significant changes in what we can achieve with this organ.

The kidney is not a static organ, rather it is repairing and remodeling itself throughout life.

- Acute injury can rapidly trigger extensive cellular proliferation.

- This endogenous repair potential gives the kidney a capacity to repopulate and repair damaged structures, **even though nephron formation has ceased shortly before birth**

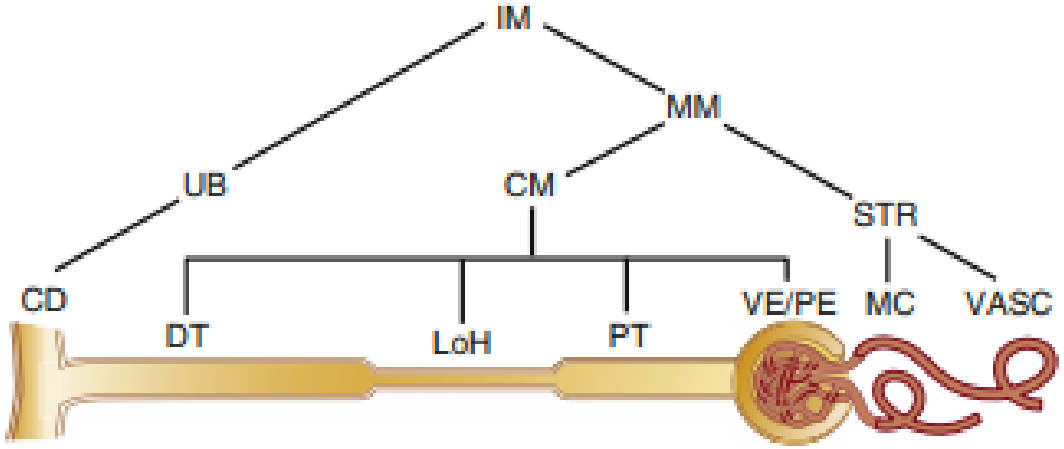


Figure 2 | Cellular origins of the kidney cell types required for repair or regeneration. The kidney arises from the intermediate mesoderm (IM), which forms both the ureteric bud (UB) and the metanephric mesenchyme (MM). The UB gives rise to all of the epithelium of the collecting ducts (CDs). The MM gives rise to the stroma (STR) and the cap mesenchyme (CM). All epithelial cells along the nephron other than the collecting duct arise from the CM. This includes the parietal epithelium (PE) and visceral epithelium ([VE], podocytes) of the glomeruli, the proximal tubules (PTs), loops of Henle (LoHs), and distal tubules (DTs). The STR gives rise to the mesangial cells (MCs), pericytes, and interstitium. It is also thought that some of the vascular endothelium (VASC) arises from this compartment.

Kidney International (2016) **90,** 289–299

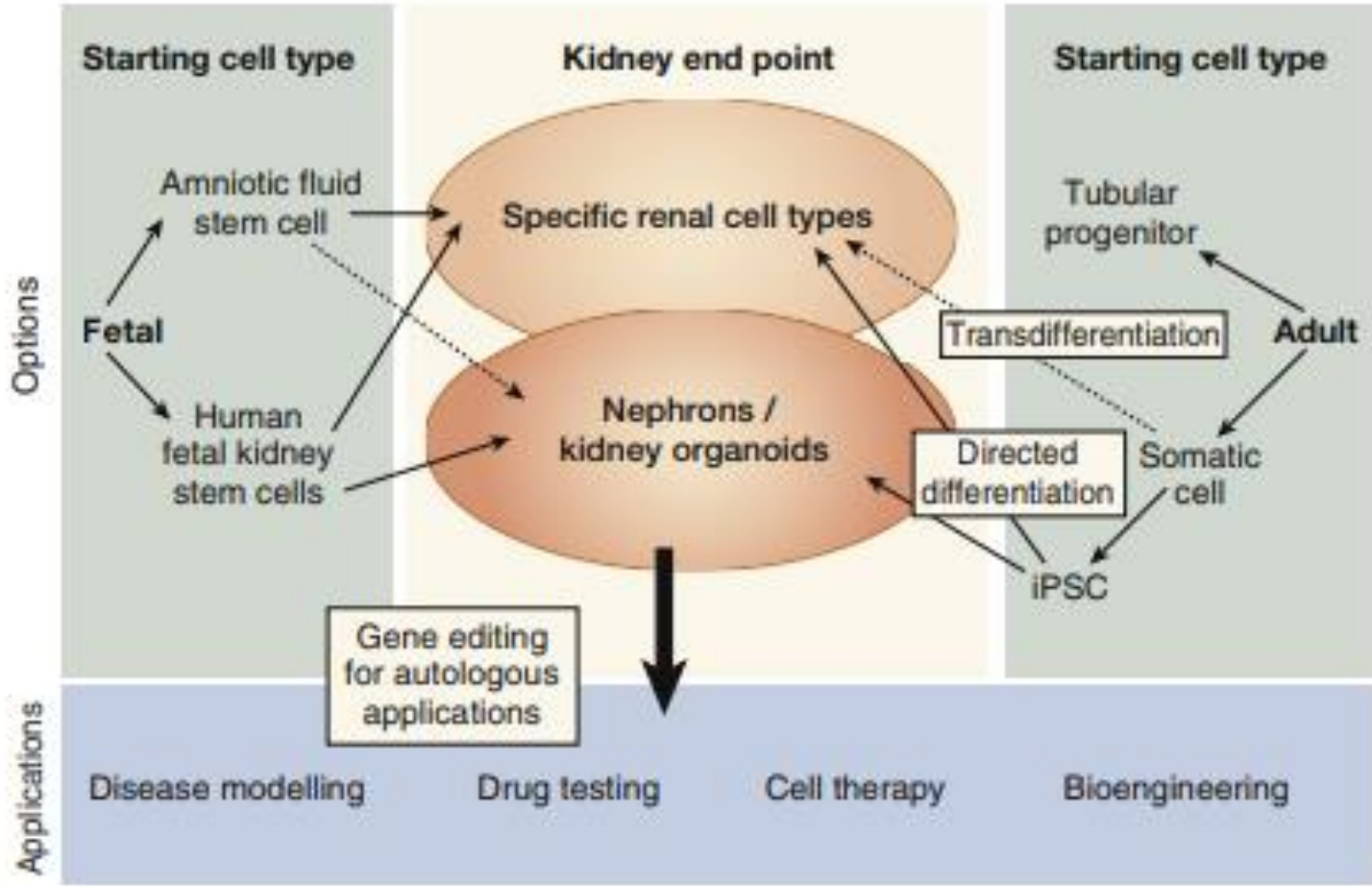


Figure 3 | Options for the regeneration of kidney tissue. The available cell options from which specific renal cell types or kidney organoids can be generated include those isolated from human fetal material, including amniotic fluid stem cells and fetal kidney stem cells, or adult tissue, including kidney tubular progenitors or any adult somatic cell type. The latter can be reprogrammed to a pluripotent state for directed differentiation to kidney tissue or transdifferentiated directly to a renal endpoint. The generation of renal tissue in this way can be applied in disease modeling, drug testing, cell therapy, and bioengineering applications. The capacity to edit the genome of a patient cell type, when an underlying genetic mutation is known, provides the possibility of autologous cell therapies. iPSC, induced pluripotent stem cells.

- Kidney International (2018) 93, 27–40; https://doi.org/10.1016/ j.kint.2017.07.030

- The most commonly used experimental model of AKI is the induction of **transient ischemia** via clamping of the renal artery.

- Performed unilaterally (provides a capacity to investigate the contralateral organ as a control) or bilaterally, this mimics clinical **ischemia reperfusion** injury.4

- This injury model in mice is known to result in a **rapid inflammatory** response with substantial epithelial cell death followed by a proliferation repair response that, over **a 7-day period**, results in a return to **normal histology**.

- A more chronic injury, unilateral ureteral ligation, results in **tubular atrophy**, interstitial expansion, and loss of renal parenchyma.

- However, once the obstruction is **removed, the tissue can** remodel to repair damaged tubules without forming new nephrons. This repair is accompanied by **substantial cell proliferation** within the epithelium.

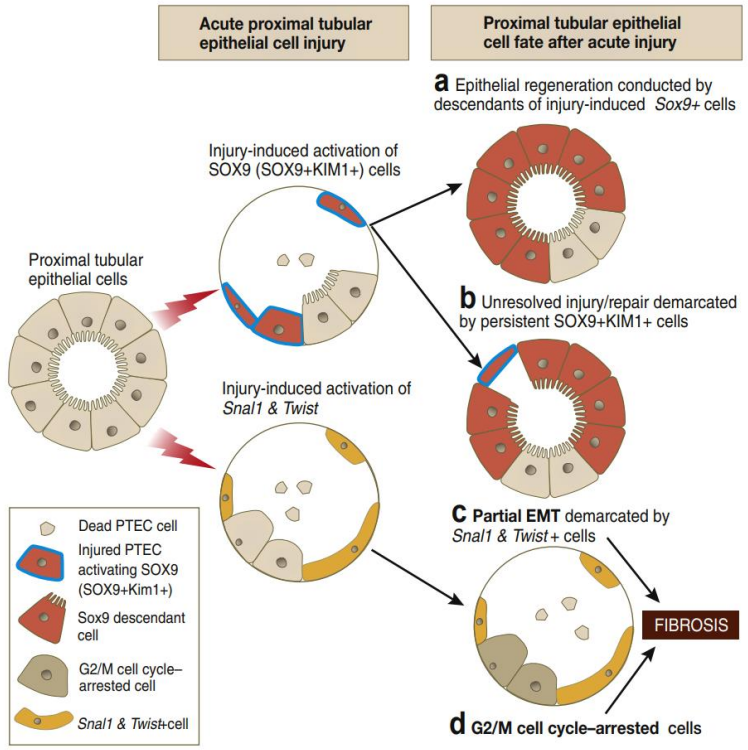


Figure 2| Proximal tubular epithelial cell (PTEC) fate after acute kidney injury. After acute kidney injury, a subset of PTECs undergo necrotic and apoptotic cell death. (**a**) Early injury/repair response: epithelial injury induces Sox9 (Sox9+Kim1+ PTEC subtype) that regenerates the injured epithelium.[29] (**b**) Chronic injury/repair response (28 days after mice ischemia reperfusion injury): Epithelial cells on successful regeneration shut down Sox9; however, the region of unresolved injury delineated by PTECs that express Kim1 continues to mount an SOX9 response in an attempt to regenerate itself. The majority of the proliferating Ki67+ PTECs at this stage are Sox9+; therefore, in the chronic phase, Sox9+Kim1+ cells demarcate "unresolved injury/repair" PTEC subtype.[29] (**c**) Injury-induced activation of *Snai1* and *Twist1* confers a partial epithelial to mesenchymal state.[38,39] A partial epithelial-mesenchymal transition (EMT) state is associated with acquisition of a pathologic proinflammatory and profibrotic secretome. A subset of such cells has been suggested to be cell-cycle arrested. (**d**) In the setting of severe acute kidney injury mice models (unilateral ischemia reperfusion injury, severe bilateral ischemia reperfusion injury, and unilateral ureteral obstructive model) and predominant fibrotic models (aristolochic acid nephropathy), G2/M cell cycle–arrested epithelial cells contribute to fibrosis.[45] Kim1, kidney injury molecule-1.

- ***Cellular approaches to improving repair:***

- A number **of cell types** are considered to be able to either **contribute directly to renal repair after** injury or substantially ameliorate renal injury **without directly** contributing the renal epithelium.

- Experimental nephrology has **focused on 4 possible origins** for cells contributing to **postnatal renal repair**:
- (i) **interstitial cell** transdifferentiation to **epithelium**,
- (ii) recruitment of cells from the **bone marrow**,
- (iii) **tubular cell** dedifferentiation and proliferation in response to injury, and
- (iv) repopulation of the renal tubules by an adult **resident kidney stem/progenitor** cell population.
- Options (i) and (ii) involve nonepithelial cells presumably transdifferentiating into renal epithelium whereas options (iii) and (iv) propose a repair process involving epithelial cel within the renal epithelium itself.

- After many years of careful studies, **there is little evidence for the first 2 options** occurring.

- The most definitive proof that renal repair involves cells within the renal epithelium of t nephron showing no evidence of dilution of the cap mesenchyme derived (Six2þ) tubula epithelium with a nonepithelial cell source.

- This did not resolve whether any cell within the epithelium can contribute to repair or whether repair relied on a resident stem cell population within the tubules.
- This debate has become a major focus over recent years.

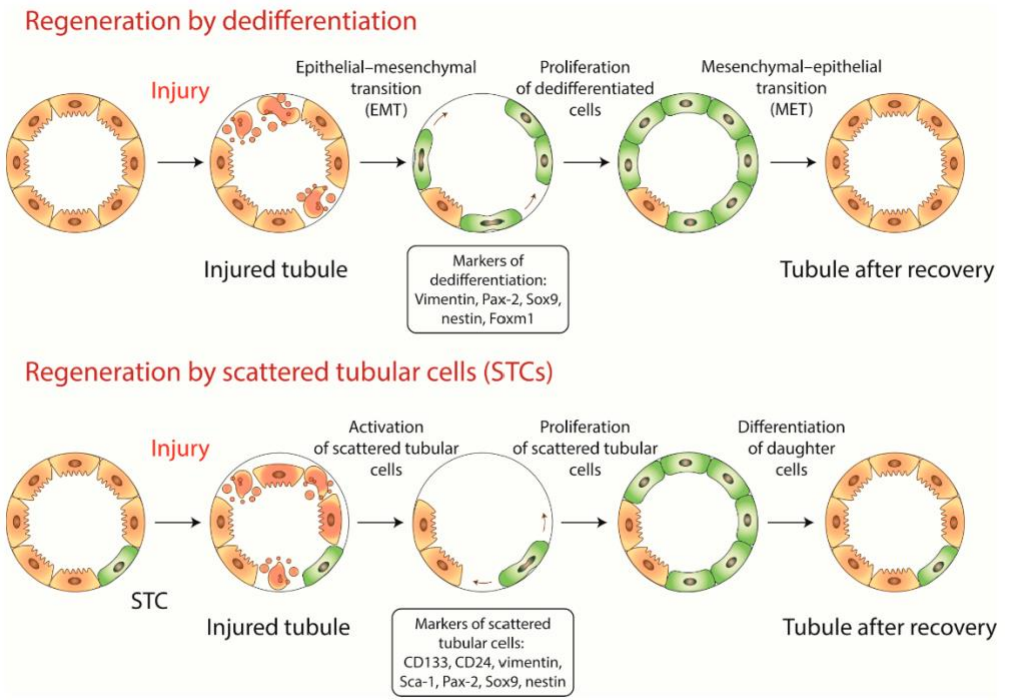


Figure 1. Two major putative mechanisms of kidney tissue regeneration: dedifferentiation of tubular epithelial cells and proliferation of resident renal progenitors with subsequent differentiation.

KI

two models have been proposed to explain the capacity for the tubular epithelium to **repair in response to acute injury**.

(a) **Tubular stem cell/progenitors** exist within the mature tubular epithelium.

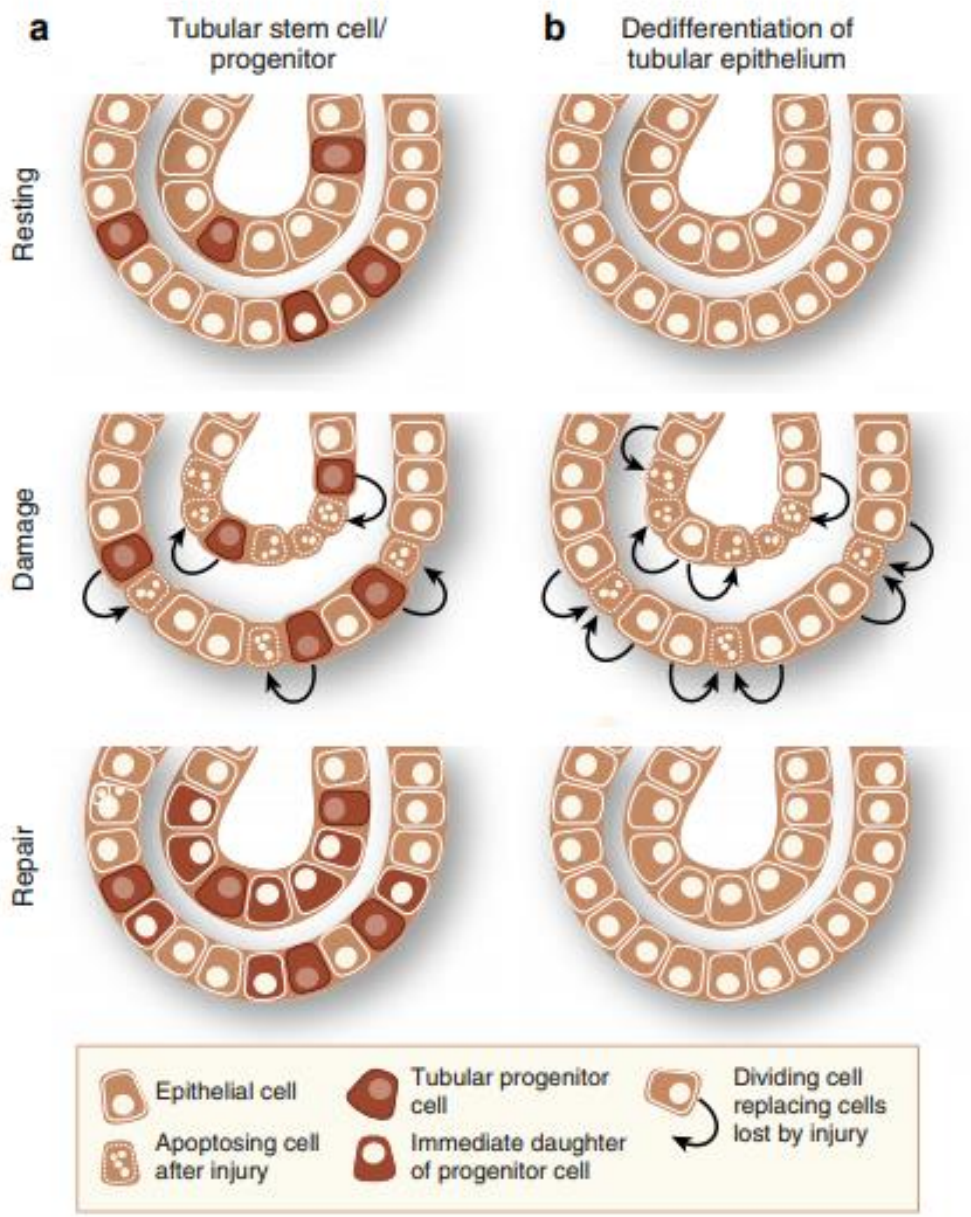
These preferentially respond to damage via proliferation to replace cells from the epithelium.

As a result, an increasing proportion of the resulting repaired epithelium will have been derived from a progenitor.

It is not clear whether these also retain progenitor status.

(b) Acute injury results in **epithelial apoptosis** but also promotes the **proliferation of any mature epithelial cell within the** tubule.

These proliferating cells contribute new epithelial cells to elicit repair after injury.
No cell has any preferential contribution to this process.



- **Endogenous tubular kidney progenitors:**

- the presence of a **resident tubular stem cel**l population (**CD133+ and CD24+) within the human adult kidney** has been proposed, with this viewed as a **defined subpopulation resident within the tubular compartments.**

- Over the last decade, **many studies have** investigated the **existence, location**, and contribution of these renal progenitor cells (**RPCs) to epithelial repair**.

- The delivery of such cells has also been reported to **act as** a **successful therapy for both acute and chronic animal models.**

- Based on specific markers, it has also been **possible to isolate and culture RPCs from human urine, providing a pathway for personalized disease modeling and drug screening.**

- **As well as** progenitors involved in **tubular turnover**, there is **also evidence** for progenitors with **overlapping protein signature**s that can contribute to the **turnover of podocytes within the glomerulus.**

- Some view the **RPC p**opulation of the postnatal renal tubules as potentially **representing a** retained tubular progenitor, in part based on expression of markers such as **Pax2 s**een in the developing organ.

- Although it is clear that **RPCs do not have a capacity to regenerate an entire nephron**, the **concept is that each segment might contain a distinct tubular progenitor** able to selectively contribute **to repair via differential proliferation** in response to injury.

- Indeed, there is an ongoing debate around whether RPCs correspond to a stable subpopulation within the tubular epithelium or a transient cell state that appears in response to a homeostatic imbalance within the kidney (Figure 1).

- The confusion has come in part from an **inability to directly compare between** human and mouse.

- The **CD24 epitope used** in association with CD133 to identify a specific double positive population in **humans is not present** in the mouse.

- Conversely, the capacity to lineage trace, though available in the mouse, is not in the human.
- **It is also possible that mouse and human kidneys are not using the same**

- **MSC therapy:**

- **adult stem cells**, **in particular msCs**, have now been shown to exert **complex paracrine** and **endocrine actions** when administered after organ injury, including the **secretion of growth factors** and **cytokines**, **modulation of the immune response**, **mitogenic, antiapoptotic** and **anti-inflammatory effects**, and stimulation of **vasculogenesis** and **angiogenesis.**

- msCs are fibroblast-like cells generated within the bone marrow that can be **induced to differentiate into osteocytes**, chondrocytes and adipocytes , **varying degrees** of **reproducibility,** into a variety of **different cell types**, such as **neuronal cells**, **hepatocytes, and lung cells**.

- Furthermore, msCs have prominent **immunomodulatory functions** including the **ability to suppress t-cell** and **B-cell responses** in vivo and in vitro. **of note, these cells do not express blood-group antigens, mHC class ii antigens, or co-stimulatory factors, facilitating their use in allogeneic protocols.**

- **msCs can be readily generated from a small-volume bone-marrow aspirate, which is obtained via a minimally invasive and safe procedure.**

- the cells can subsequently be isolated by their adherence to plastic dishes and can then be **readily expanded in culture on a large scale**, enabling the production of a standardized and potentially marketable cell product that is suitable for use in various potential therapeutic applications.

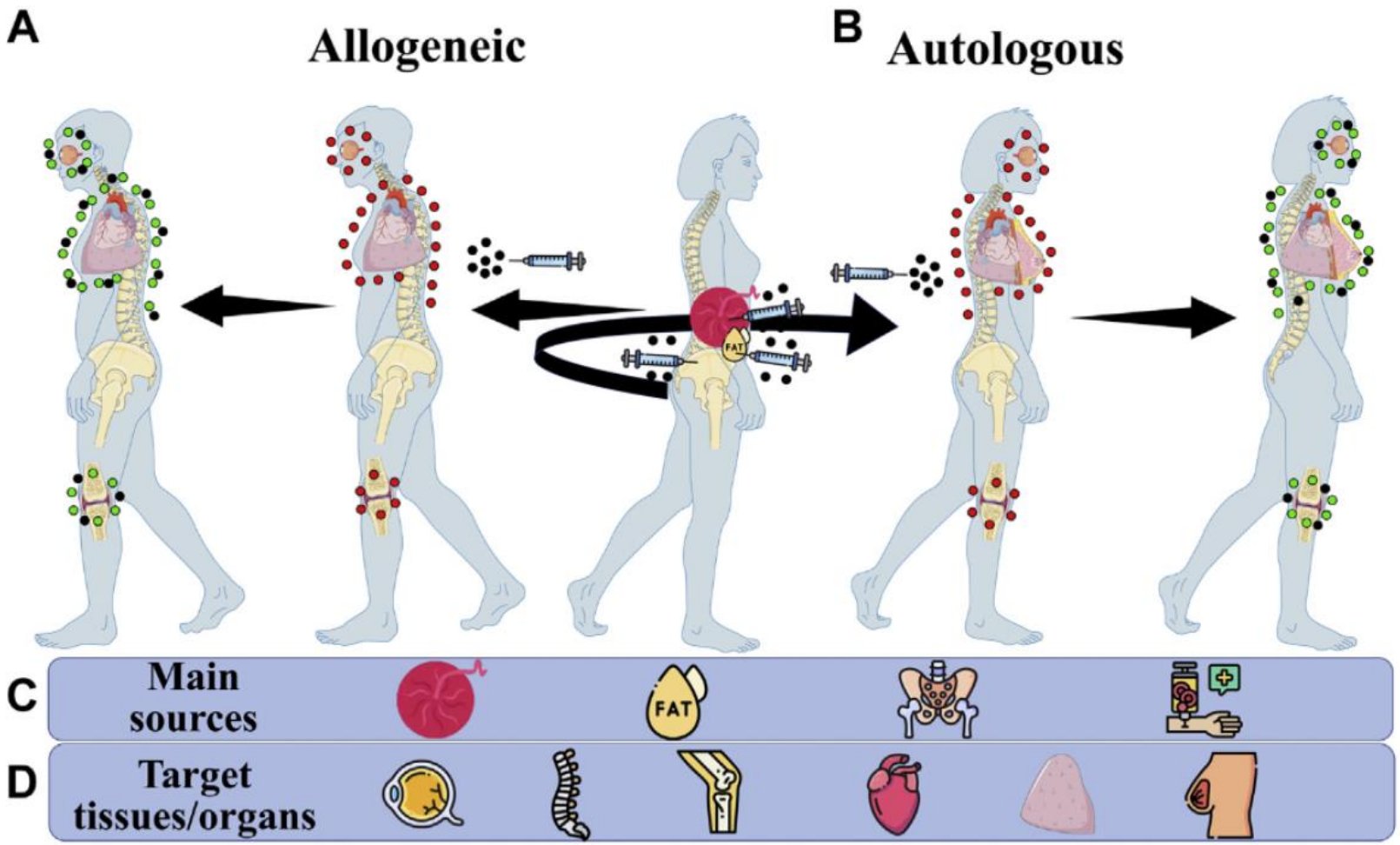


Figure 1. Treating with Medicinal Signaling Cells (MSCs). A. Therapy with allogeneic MSCs. B. Therapy with autologous MSCs. After the administration of MSCs, they migrate to the sites of inflammation and tissue damage associated with cytokine outburst, to generate a regenerative microenvironment by secreting bioactive molecules capable of stimulating local stem cells to repair injured sites. C. Main sources of MSCs: Placenta, subcutaneous adipose tissue, bone marrow, and peripheral blood. D. Target issues or organs in the reviewed clinical trials: vitreous body, spinal cord, bone marrow, cartilage, heart, lungs, and adipose tissue.

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Archives of Medical Research 52 (2021) 93–101

Archives of Medical Research

EPIDEMIOLOGICAL

Mesenchymal Stem Cells Current Clinical Applications: A Systematic Review

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Received for publication April 27, 2020; accepted August 18, 2020 (ARCMED_2020_572).

Currently, **there are multiple clinical trials(CT) using hMSCs** for therapeutic purposes in a large number of clinical settings.

Material and Methods.

The search strategy on **clinicaltrials.gov** has focused on the **key term ‘‘Mesenchymal Stem Cells’’**, and the inclusion and exclusion criteria were separated into two stages.

Stage 1, CT on **phases 1e4**: location, the field of application, phase, and status.

For stage 2, CT that have published outcome results: field of application, treat-ment, intervention model, source, preparation methods, and results.

Results. By **July 2020**, there were a total **of 1,138** registered CT.

Most studies belong to either phase 2 (61.0%) or **phase 1 (30.8%);** most of them focused in **the fields of traumatology**, **neurology, cardiology**, and immunology.

Only 18 clinical trials had published results:

the **most common source of isolation was bone marrow;**

all of them have **similar preparation methods**;

all of them have **positive results** with **no serious adverse** effects.

Conclusions. There appears to be a **broad potential for the clinical** use of hMSCs with no reported serious adverse events.

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Table 1. Distribution of clinical trials registered at *clinicaltrial.gov* by the medical field

Medical speciality	# of CT	%	Phase1	Phase2	Phase3	Phase4	Total
Traumatology	234	25.9	72	137	23	2	234
Pneumology	99	11.0	43	53	3	0	99
Neurology	97	10.7	31	61	4	1	97
Cardiology	83	9.2	21	50	12	0	83
Immunology	78	8.6	14	50	14	0	78
Hepatology	53	5.9	11	39	2	1	53
Endocrinology	47	5.2	13	30	4	0	47
Dermatology	31	3.4	9	22	0	0	31
Gastroenterology	30	3.3	9	20	1	0	30
Nephrology	27	3.0	12	15	0	0	27
Hematology	25	2.8	9	13	2	1	25
Oncology	24	2.7	10	13	1	0	24
Gynecology	16	1.8	5	11	0	0	16
Psychiatry	16	1.8	2	13	0	1	16
Urology	16	1.8	9	6	1	0	16
Ophthalmology	11	1.2	3	7	1	0	11
Odontology	10	1.1	2	8	0	0	10
Other	6	0.7	3	3	0	0	6
Total	903	100	278	551	68	6	903

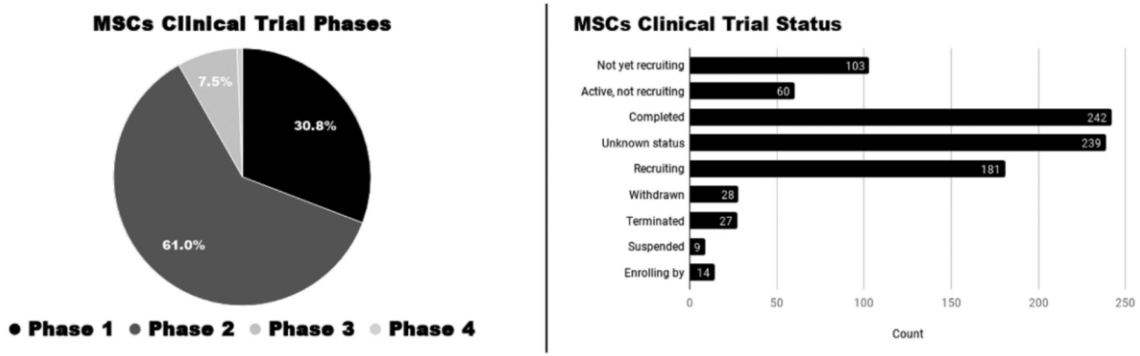

Figure 3. Distribution of the MSCs registered clinical trials by phase and progress.

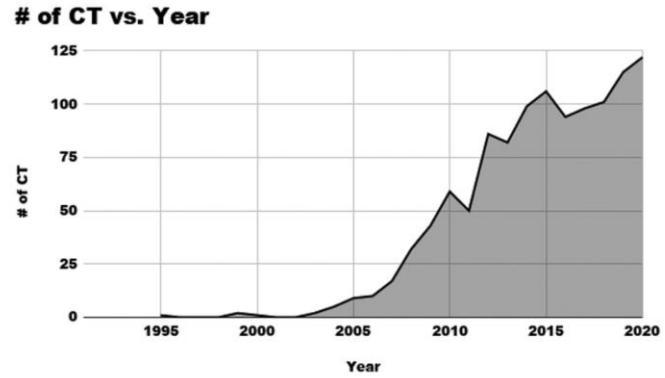

Figure 2. Statistics of registered clinical trials testing MSCs according to evolution of yearly registrations since the first clinical trial began.

Table 2. Listed MSC Clinical Trials around the world

Country	Total	Percentage	Phase1	Phase2	Phase3	Phase 4
China	228	25.25	60	154	13	1
United States	186	20.60	83	88	13	2
Spain	69	7.64	9	59	1	0
South Korea	62	6.87	20	32	10	0
Iran	44	4.87	25	15	4	0
Brazil	21	2.33	6	12	3	0
France	20	2.21	2	16	2	0
Jordan	20	2.21	8	12	0	0
India	19	2.10	4	13	1	1
Egypt	16	1.77	4	7	4	1
Taiwan	13	1.44	9	4	0	0
Russia	12	1.33	2	8	2	0
Poland	11	1.22	4	4	3	0
Belgium	11	1.22	1	9	1	0
Denmark	10	1.11	3	6	1	0
Belarus	10	1.11	0	10	0	0
Germany	10	1.11	2	8	0	0
Netherlands	9	1.00	2	6	1	0
Indonesia	9	1.00	3	6	0	0
Israel	8	0.89	1	7	0	0
Malaysia	8	0.89	1	7	0	0
Sweden	8	0.89	3	5	0	0
Canada	8	0.89	4	4	0	0
Italy	7	0.78	2	4	0	1
Turkey	7	0.78	1	4	2	0
Panama	7	0.78	0	7	0	0
Vietnam	7	0.78	1	6	0	0
Pakistan	6	0.66	2	4	0	0
United Kingdom	6	0.66	2	4	0	0
Japan	6	0.66	4	2	0	0
Chile	5	0.55	0	4	1	0
Greece	4	0.44	0	3	1	0
Czech Republic	4	0.44	1	3	0	0
Mexico	3	0.33	1	1	1	0
Colombia	3	0.33	0	3	0	0
Saudi Arabia	3	0.33	1	2	0	0
Australia	3	0.33	2	1	0	0
Austria	2	0.22	0	1	1	0
Kazakhstan	2	0.22	0	1	1	0
Singapore	2	0.22	0	2	0	0
Trinidad and Tobago	2	0.22	0	2	0	0
Switzerland	2	0.22	1	1	0	0
Norway	2	0.22	2	0	0	0
Ecuador	1	0.11	0	0	1	0
Lebanon	1	0.11	0	0	1	0
Finland	1	0.11	0	1	0	0

msCs effectively improve outcome after aKi in different experimental animal models.

- infusion of msCs improved **recovery after**
- **cisplatin-induced aKi**,
- after **aKi induced by ischemia–reperfusion injury**, and
- after **glycerol-induced aKi**.

- **msCs that were administered** into the **suprarenal aorta** or **intravenously** were subsequently found predominantly in **glomerular and peritubular capillaries** and **disappeared** from the kidney and other organs within **72 h after infusion**.

Am J Stem Cells 2016;5(1):1-10
www.AJSC.us /ISSN:2160-4150/AJSC0026274

Review Article

Comparison of stem cell therapies for acute kidney injury

Carol J Barnes[1*], Casey T Distaso[1*], Kristin M Spitz[1*], Valerie A Verdun[1*], Aviad Haramati[2]

[1]*Georgetown University School of Medicine, Washington, DC, USA;* [2]*Department of Biochemistry and Molecular and Cellular Biology, Georgetown University School of Medicine, Washington, DC, USA.* [*]*Equal contributors.*

Received February 19, 2016; Accepted April 28, 2016; Epub May 15, 2016; Published May 30, 2016

Stem cell therapy for AKI

Table 2. Comparison of iPSCs, SSCs, and MSCs for treatment of AKI

	Harvest Method	Reprogramming Factors	Differentiation into Kidney Progenitors	Pre-clinical Benefits	Clinical Trial Results
Induced Pluripotent Stem Cells	Skin biopsy (provides keratinocytes and fibroblasts)	OCT-4, SOX-2, KLF-4, and/or c-MYC	Ureteric bud cells and podocyte	●Reduced severity of AKI in rats ●Reduced macrophage proliferation in kidney ●Reduced oxidative stress in kidney	None
Spermatogonial Stem Cells	Testis biopsy	Not Applicable	Epithelial cells of the renal tubule and podocytes (via human embryonic like cells)	●Differentiate into functional renal tubular like cells ●Administration to AKI rats reduced serum creatinine levels and reduced necrotic tubules	None
Mesenchymal Stem Cells	Biopsy from bone marrow, cord blood, or fetal membrane	Not Applicable	Renal parenchymal cells, glomerular and tubular epithelial cells, glomerular and tubular interstitial cells	●Immunomodulatory and anti-inflammatory effects ●Anti-apoptotic ●Mitogenic ●Lower kidney injury score compared to control	●Reduced hospital stay ●Decreased readmission rates ●Prevention of further renal damage

A summary of the present research regarding stem cell-based therapies for acute kidney injury (AKI). Although many positive outcomes have been observed in pre-clinical settings for the use of spermatogonial stem cells (SSCs) and induced pluripotent stem cells (iPSCs) in the treatment of AKI, the current body of research on mesenchymal stem cell (MSC) treatments is more robust. MSC-based therapies are the only stem cell therapies currently in Phase I clinical trials for the treatment of AKI.

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Table 1. Survival rates for saline (control), bone marrow-MSC, and cord blood-MSC following cisplatin injection and subsequent damage. Saline treated cells had a 0 survival rate 7 days after cisplatin injection. Bone marrow-MSC treated cells had a 50 survival rate, and cord blood-MSC had an 86 survival rate. Table is adapted from Morigi 2013 [29]

Mesenchymal Stem Cells and Kidney Repair Comparative effect of stem cells of different origin in experimental ARF			
Treatment	Blood Urea Nitrogen (mg/dL)	Renal Histology[a]	Survival[b]
Saline	>140	Damaged	0
Bone marrow-MSC	63 ± 5	Preserved	50
Cord blood-MSC	58 ± 7	Preserved	86

[a]On day 4 from cisplatin injection. [b]On day 7 from cisplatin injection.

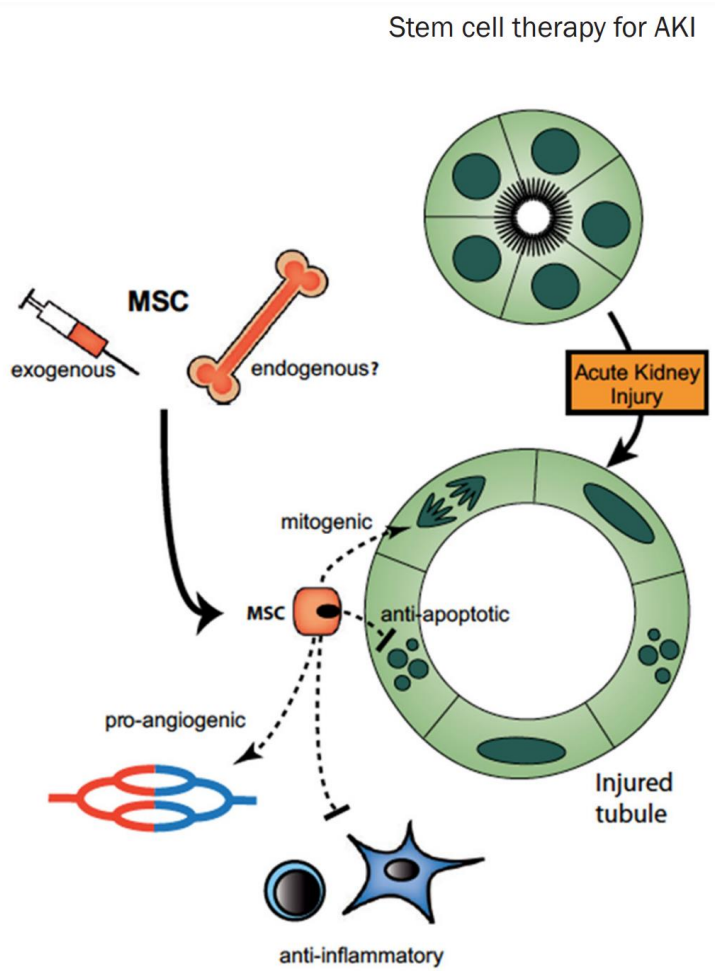


Figure 1. Model for paracrine actions of MSCs on the injured tubule. After an acute kidney injury, injected MSCs home to injury sites and may be recruited from endogenous niches (bone marrow or kidney) as well. MSCs bind to glomerular and/or peritubular capillary endothelium and both protect the kidney from further injury and accelerate repair. Paracrine mediators play important roles in repair, including VEGF, IGF, HGF, PGE_2, and other soluble factors that exert mitogenic, antiapoptotic, proangiogenic and anti-inflammatory effects [17].

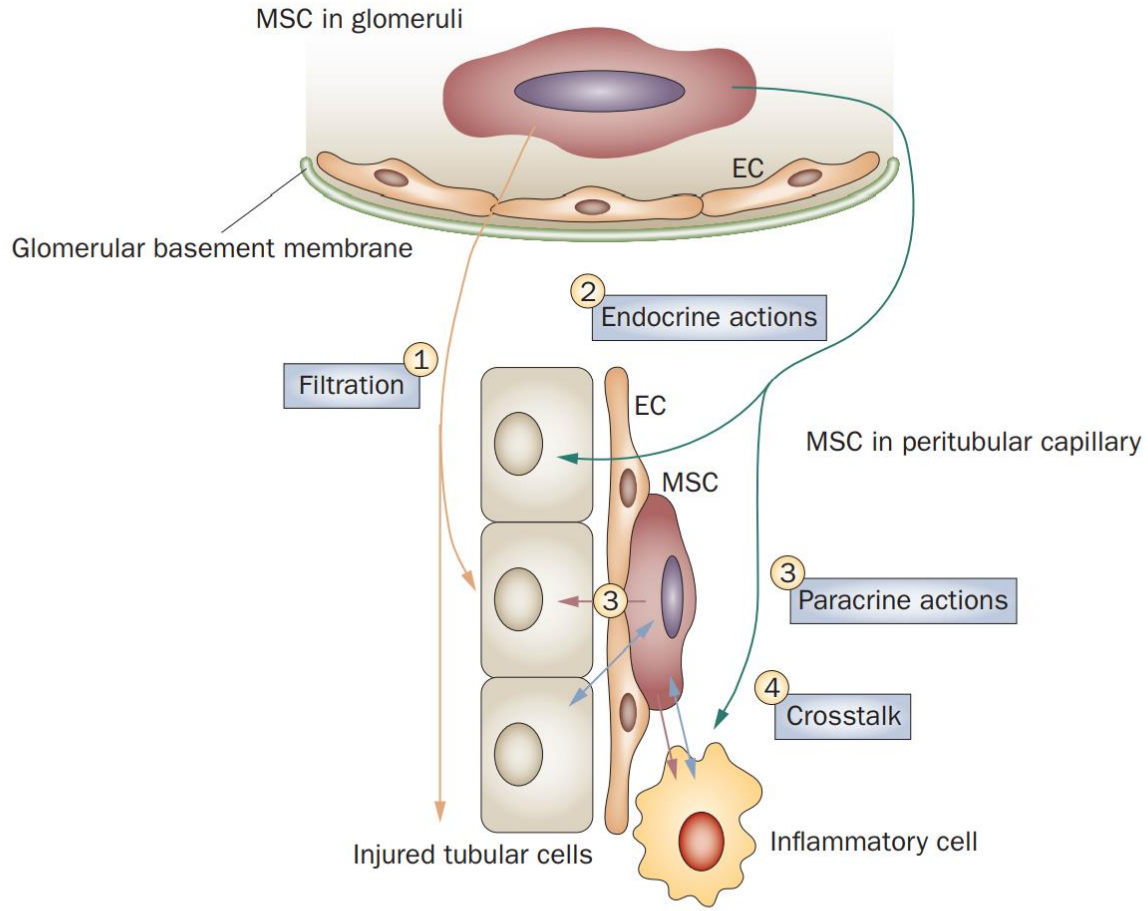


Figure 1 | Intrarenal actions of MSCs in acute kidney injury. Infusion of MSCs results in their homing to sites of renal injury and temporary adhesion to glomerular and postglomerular capillaries. Growth factors and cytokines are secreted and delivered to the luminal aspects of injured proximal tubules by filtration across the glomerular basement membrane (1). These factors are also released into postglomerular capillaries (2), thereby reaching proximal tubular cells from their basolateral side. Growth-factor-receptor expression is upregulated in viable tubular cells, and receptor distribution in injured, proximal tubular cells is both luminal and basolateral. Adherent MSCs act in a paracrine fashion (3) in glomeruli and in the postglomerula circulation, targeting glomerular, microvascular endothelial, and inflammatory cells.
(4) 'Crosstalk' (blue arrows) between MSCs and adjacent renal and inflammatory cells causes beneficial changes in the respective gene expression profiles. Abbreviations: EC, endothelial cell; MSC, mesenchymal stem cell.

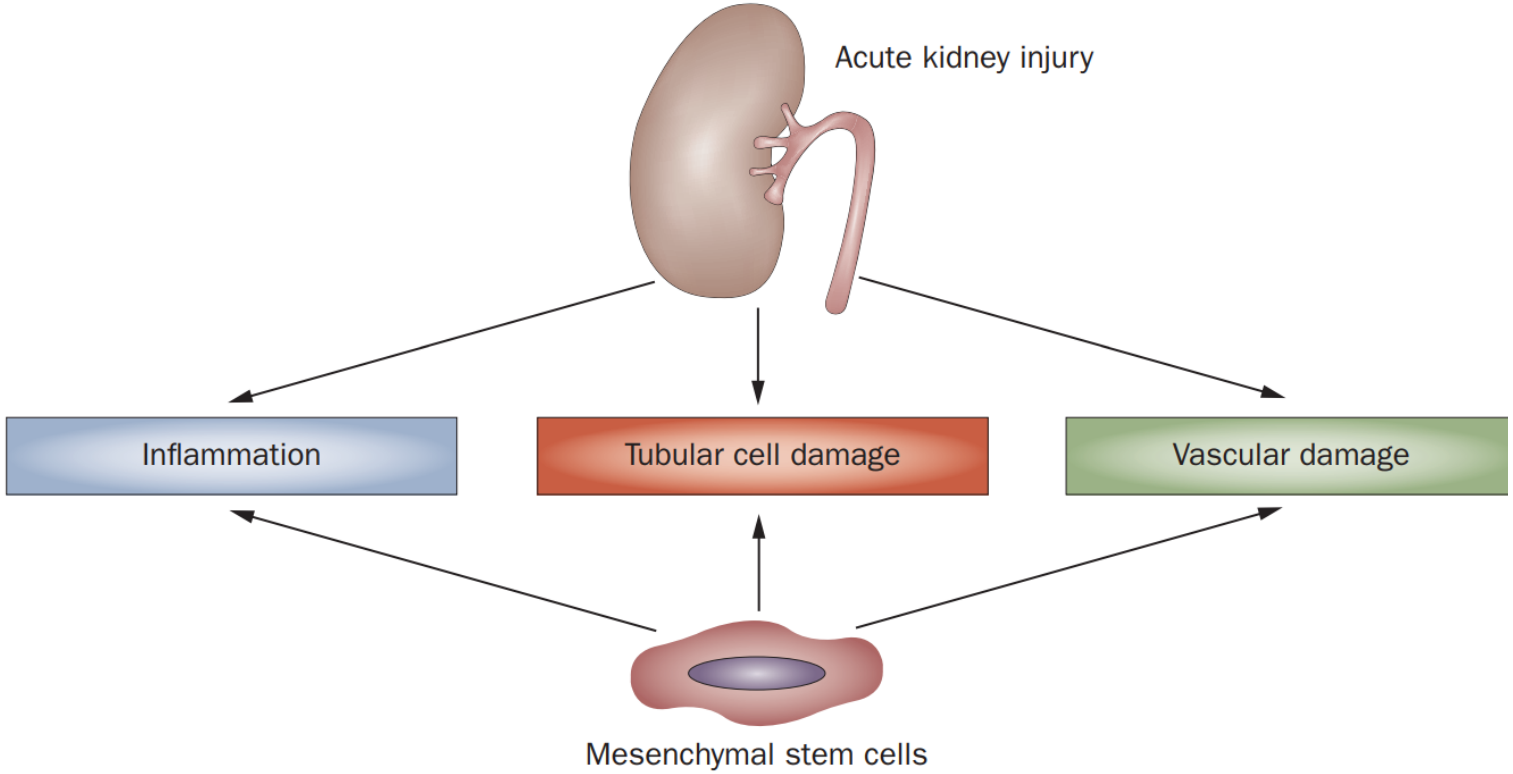


Figure 2 | Mesenchymal stem cells (MSCs) target all pathophysiological components of acute kidney injury (AKI). Major axes of the pathophysiology of AKI include inflammation, vascular and tubular damage. Inflammation is triggered by ischemia–reperfusion injury, inflammatory cytokines, and immune cell attachment and migration. Vascular damage is caused by ischemia-reperfusion. Endothelial injury and further microcirculatory impairment aggravates tubular cell damage and increases inflammation. Tubular cell injury is caused by hypoxia and by the generation of reactive oxygen species during reperfusion. MSCs reduce inflammation by direct (cell–cell contact) and indirect (paracrine) actions, by modulating immune responses, by suppression of T-cell, B-cell and natural-killer-cell proliferation, and by protecting endothelial cells, thereby reducing vascular damage and improving microcirculation.[12] Tubular and microvascular damage are reduced by the secretion of growth factors (for example, EGF, HGF, VEGF and IGF-I), which collectively decrease apoptosis and enhance proliferation of critically damaged tubular and endothelial cells.

Signal Transduction and Targeted Therapy (2020) 5:9

Table 1. Growth factors may contribute to different types of AKI.

Model of AKI	Growth factor
Ischemia-reperfusion Injury	BMP-7/EGF/FGF-2/HGF/ IGF-1/TGF-β1/VEGF/PDGF
Folic acid-induced AKI	BMP-7/EGF/FGF-23/HGF/ TGF-β1/VEGF/IGF-1
Cisplatin-induced AKI	BMP-7/TGF-β1/VEGF/ FGF-21/FGF-10/EGF/IGF-1/HGF
Lipopolysaccharide-induced AKI	BMP-7/FGF-2/TGF-β1/HGF/EGF
Mercuric chloride-induced AKI	EGF/IGF-1
Glycerol-induced AKI	HGF/TGF-β1
Colistin-induced AKI	TGF-β1/EGF
Gentamicin-induced AKI	IGF-1/TGF-β1/EGF/PDGF/VEGF

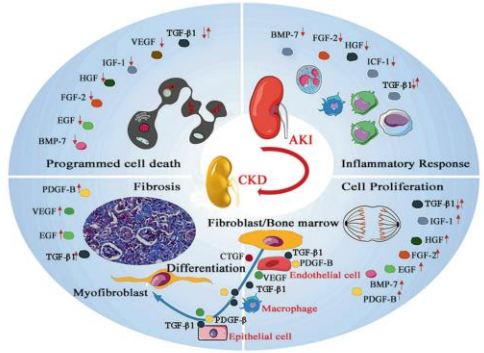
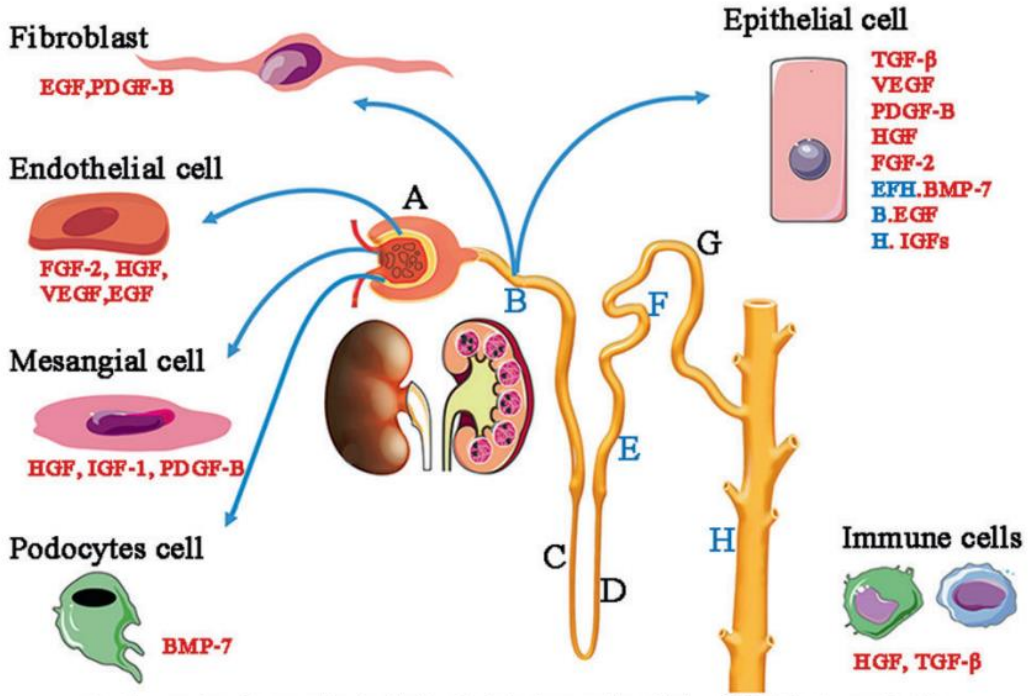


Fig. 2 Effect of growth factors on AKI and AKI-CKD progression. Many growth factors, such as BMP-7, EGF, FGF-2, HGF, IGF-1, VEGF, and TGF-β1, are involved in the programmed cell death of endothelial or epithelial cells in the acute injury phase. BMP-7, FGF-2, HGF, TGF-β1, and IGF-1 participate in the regulation of the inflammatory microenvironment that is responsible for cytokine production and immune cell recruitment. TGF-β1 is a double-edged growth factor. In addition, TGF-β1 exerts anti-inflammatory effects, and TGF-β1 overproduction leads to acute tubular injury. After injured epithelial cells fail to regenerate through differentiation, fibrosis is induced as a self-limiting repair process to limit damage. In this stage, overproduction of growth factors such as TGF-β1, PDGF, and FGF induces fibroblast/pericyte proliferation, transdifferentiation of tubular epithelial cells, endothelial cells, and macrophages, and extracellular matrix production, leading to CKD. Concurrently, abnormal synthesis of PDGF-B, VEGF, EGF, and TGF-β1 has a negative impact on endothelial integrity and causes capillary rarefaction, accelerating renal fibrosis.



A, glomerulus; B, proximal tubule; C, thin descending limb of Henle's loop; D, thin ascending limb; E, medullary thick ascending limb of Henle's loop; F, cortical thick ascending limb; G, distal convoluted tubule; H, collecting duct.

- ***Cell-based therapies in human AKI – perspectives and current limitations:***

- The first problem that needs to be addressed is related to the **exact timing of cell administration.**

- In an **ideal situation**, cells would be applied at the **moment of AKI onset**, e.g. **shortly after renal** ischemia.

- Unfortunately, acute kidney dysfunction does neither cause any typical symptoms, comparable to cardiac ischemia for instance nor are any marker molecules available that allowed a fast and early detection of AKI.

- From the clinicians´ perspective it is **impossible to define** the **exact moment at** which AKI evolves. Even if it became possible to **predict the** moment, cells for therapeutic administration should be available more or less immediately.

frontiers in Immunology

REVIEW
published: 03 June 2021
doi: 10.3389/fimmu.2021.684496

Mesenchymal Stem Cell-Derived Extracellular Vesicles: A Potential Therapeutic Strategy for Acute Kidney Injury

Jia-Kun Li[1†], Cheng Yang[2†], Ying Su[1], Jing-Chao Luo[1], Ming-Hao Luo[3], Dan-Lei Huang[3], Guo-Wei Tu[1] and Zhe Luo[1,4*]*

- **MSC-EVS ARE SUPERIOR TO MSCS AND CAN BE MODIFIED ARTIFICIALLY AS MEDICATION CARRIERS, WITH RARE ADVERSE REACTIONS**
-

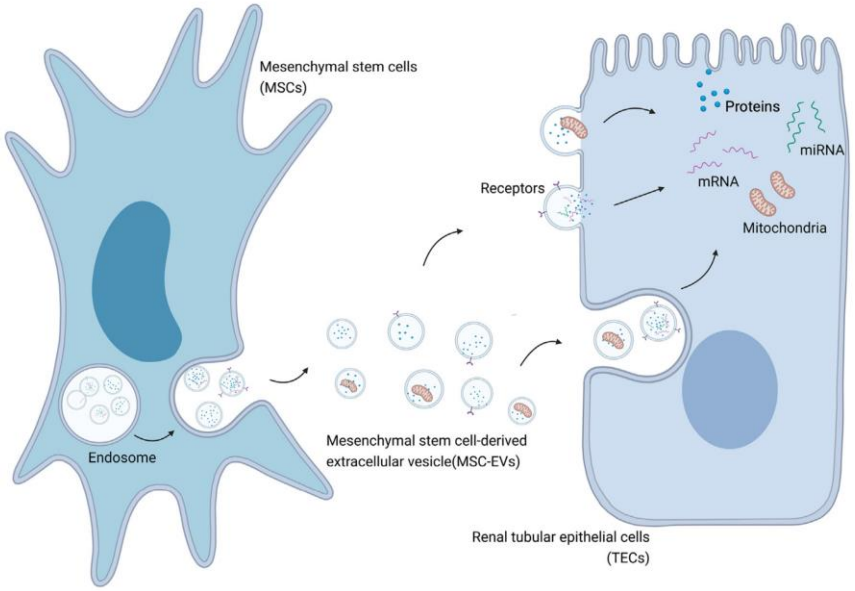


FIGURE 1 | MSC-EVs mediate transportation of biological modules to injured cells in AKI. Created with BioRender.com.

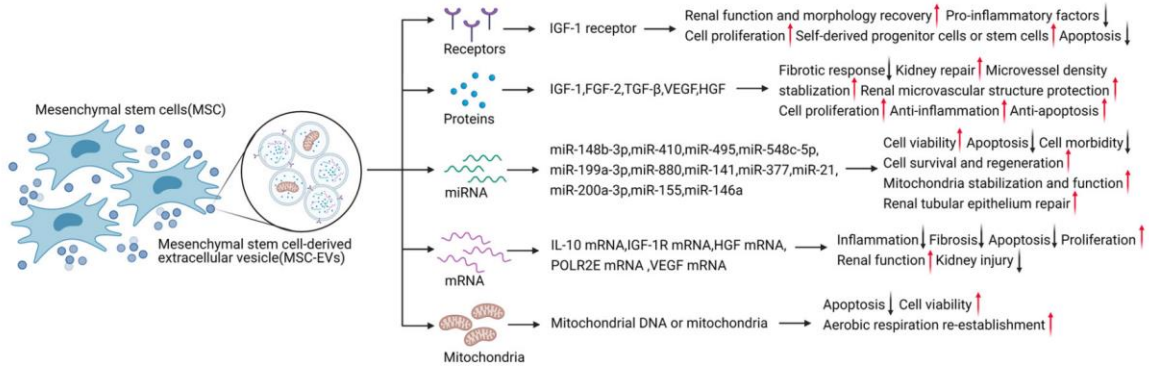


FIGURE 2 | MSC-EVs carry various contents to exert therapeutic effects in AKI. Created with BioRender.com.

- **HOW TO ADMINISTER MSC-EVS:**

- When administered via **peripheral intravenous injection**, **most MSCs or MSC-EVs distribute to the lung, spleen, or celiac lymph nodes, thus, reducing their therapeutic efficacy** .

- Moreover, the **homing ability of EVs is lower than that of MSCs**.

- However, a **recent study showed that renal artery administration could transport more EVs and generate better therapeutic effects to injured kidney tissue with greater precision compared with other administration routes** .

- However, although EV injection via the renal artery provides a possible approach, this administration route is **more difficult** and is associated with **ethical concerns** in clinical practice .

- Bruno et al. utilized MSC-derived microvesicles, a kind of EVs, in lethal cisplatin-induced AKI and showed that increased administration times improved the therapeutic effects due to anti-apoptosis in AKI.

- They also found that using **multiple injections** of EVs significantly reduced the mortality of mice, and mice **surviving at day 21** showed normal **histology and renal function**

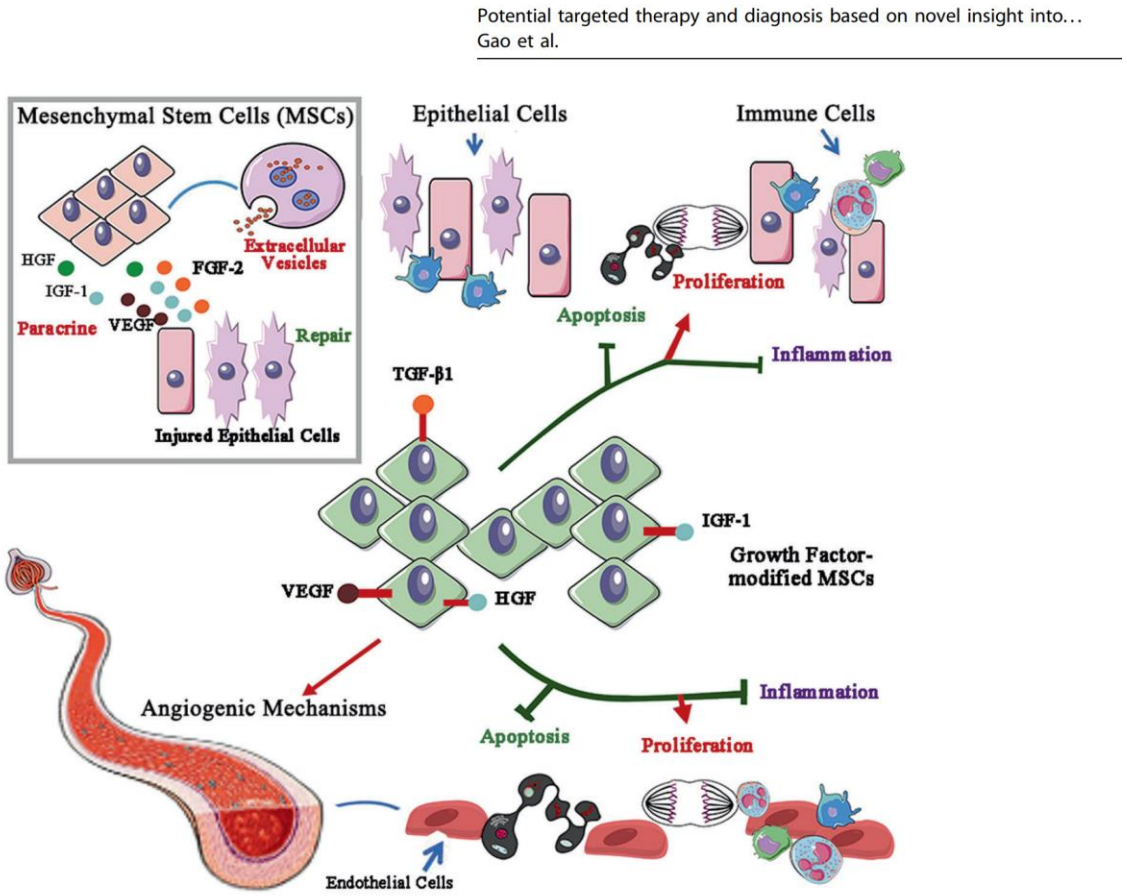


Fig. 3 Growth factors and stem cell-based AKI therapy. Extracellular vesicle (EV)-delivered and paracrine factors such as HGF, IGF-1, VEGF, and FGF-2 from mesenchymal stem cells contribute to repair after renal injury. More importantly, stem cells modified by growth factors, including VEGF, TGF-β1, and IGF-1, efficiently protect against AKI by decreasing apoptosis and the inflammatory response and promoting tubular epithelial and endothelial cell proliferation. VEGF-modified stem cells change capillary density via angiogenic mechanisms to attenuate renal ischemia-reperfusion injury.

- A **meta-analysis using serum** creatinine (Scr) as an indicator of efficacy compared the timing of administration in various studies (between **1 h and 3 days** after the occurrence of AKI), and showed a **better treatment effect** following administration of MSC-EVs **within 1 h after** the occurrence of AKI, suggesting that they should be administered **as early as possible** .

- Current research mainly focuses on **EVs secreted by MSCs derived from adipose** tissue, bone marrow, and cord blood. However, the **source tissue also has an impact** on MSCEVs .

- For example, **compared with cord blood-derived MSCs**, signals mediated by EVs derived from **bone marrow MSCs** had **greater effects on bone growth and differentiation** .

- In addition, **adipose-derived MSCs** had **similar immune regulation** effects to bone marrow-derived MSCs .
- The **EV source** should be **selected flexibly** according to the **type of kidney** injury and treatment needs.

- Meanwhile, because **MSC from different** sources have **different characteristics**, the **EVs secreted** by them will **also differ**.

- The therapeutic effects of EVs **from other sources** of MSCs in AKI are still **unclear**, and there is **much need** for further research,

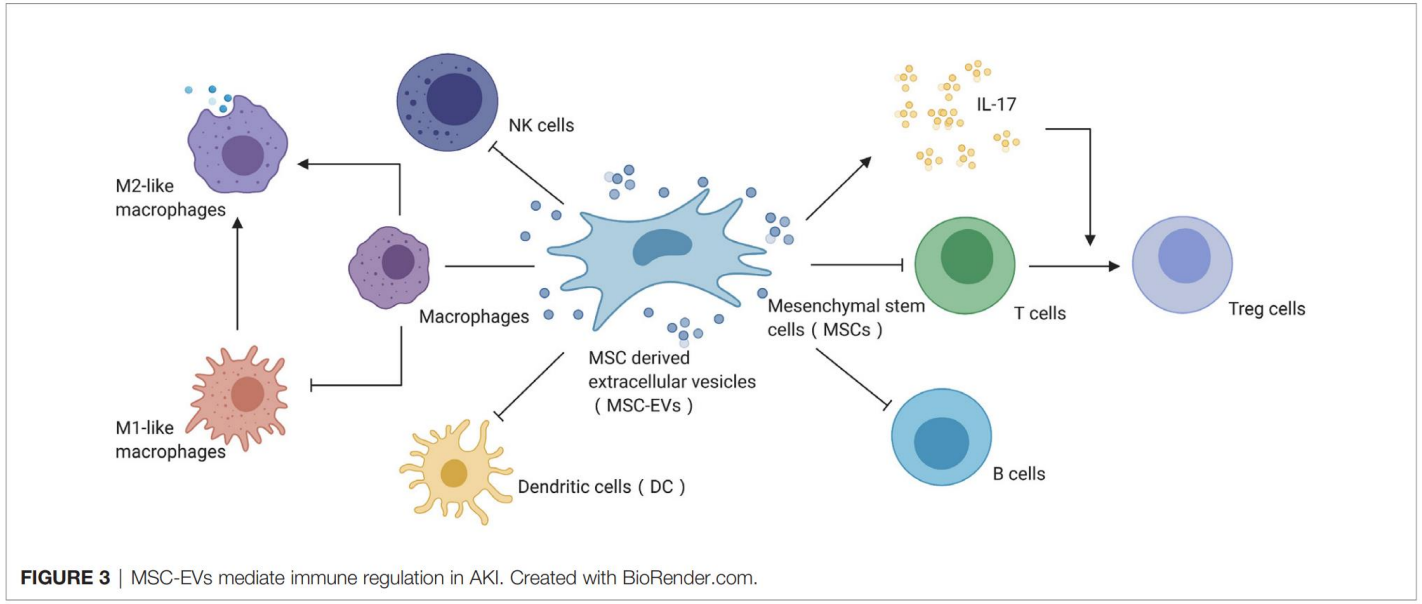


FIGURE 3 | MSC-EVs mediate immune regulation in AKI. Created with BioRender.com.

TABLE 1 | Recent research into the therapeutic effects of MSC-EVs.

Authors	Title	Year	EVs source	AKI model	Intervention	Effects
Lee, JH et al. (128)	Reproducible large-scale isolation of exosomes from adipose tissue-derived mesenchymal stem/stromal cells and their application in acute kidney injury	2020	Adipose tissue-derived MSCs	Cisplatin-induced AKI	Produce ASC-EVs with tangential flow filtration	EV yield↑; EV quality↑
Cao, J et al. (127)	Three-dimensional culture of MSCs produces exosomes with improved yield and enhanced therapeutic efficacy for cisplatin-induced acute kidney injury	2020	Fresh human umbilical cord-derived MSCs	Cisplatin-induced AKI	Produce MSC-EVs with a hollow fiber bioreactor-based three-dimensional culture system	EV yield↑; EV quality↑; therapeutic efficacy↑; collection efficiency↑; efficiency of TECs uptake↑
Ullah, M et al. (129)	Reversing acute kidney injury using pulsed focused ultrasound and MSC therapy: a role for HSP-mediated PI3K/AKT signaling	2020	Bone marrow-derived MSCs	Cisplatin-induced AKI	Combine pFUS pretreatment of the kidney with MSC-derived EVs	No significant improvement in homing ability of EVs; kidney injury markers↓; renal function↓; inflammation↓; apoptosis↓; cell proliferation↑
Ullah, M et al. (130)	HSP70-mediated NLRP3 inflammasome suppression underlies reversal of acute kidney injury following extracellular vesicle and focused ultrasound combination therapy	2020	Bone marrow-derived MSCs	Cisplatin-induced AKI	Combine pFUS pretreatment of the kidney with MSC-derived EVs	HSP70↓; NLRP3 inflammasome↓;IL-1↓; IL-18↓; therapeutic effects of MSC-EVs↑; anti-inflammation↑; cell regeneration↑
Ullah, M et al. (131)	Pulsed focused ultrasound enhances the therapeutic effect of mesenchymal stromal cell-derived extracellular vesicles in acute kidney injury	2020	Bone marrow-derived MSCs	Cisplatin-induced AKI	Combine pFUS pretreatment of the kidney with MSC-derived EVs	MAPK/ERK↑; PI3K/Akt↑; eNOS↑; SIRT3↑; kidney injury markers↓; renal function↓; inflammation↓; apoptosis↓; cell proliferation↑; survival↑
Zhang, C et al. (77)	Supramolecular nanofibers containing arginine-glycine-aspartate (RGD) peptides boost therapeutic efficacy of extracellular vesicles in kidney repair	2020	Human placenta-derived MSCs	Ischemic reperfusion injury-induced AKI	Precondition EVs with RGD peptides	Stability and retention of MSC-EVs↑; anti-fibrosis in the chronic phase↑; kidney injury↓; cell proliferation↑; EV integrin-mediated loading↑
Liu, Y et al. (132)	Enhanced therapeutic effects of MSC-derived extracellular vesicles with an injectable collagen matrix for experimental acute kidney injury treatment	2020	Human placenta-derived MSCs	Ischemic reperfusion injury-induced AKI	Precondition EVs with collagen matrix	Angiogenesis↑; apoptosis↓; stability and retention of MSC-EVs↑; therapeutic efficacy↑
Alzahrani, FA et al. (133)	Melatonin improves therapeutic potential of mesenchymal stem cells-derived exosomes against renal ischemia-reperfusion injury in rats	2019	Bone marrow-derived MSCs	Ischemic reperfusion injury-induced AKI	Precondition EVs with melatonin	Kidney damage↓; inflammation; renal regeneration↑; angiogenesis↑; anti-oxidation↑; oxidative stress↓
Zhang, ZY et al. (134)	Oct-4 enhanced the therapeutic effects of mesenchymal stem cell-derived extracellular vesicles in acute kidney injury	2020	Human umbilical cord-derived MSCs	Ischemic reperfusion injury-induced AKI	Overexpress Oct-4 by lentiviral vector transduction	Apoptosis↓; Scr↓; BUN↓; renal fibrosis↓; renal tubular epithelial cell proliferation↑

EV, extracellular vesicle; MSC, mesenchymal stem cell; AKI, acute kidney injury; pFUS, pulsed focused ultrasound; HSP70, heat shock protein 70; NLRP3, NLR Family, Pyrin Domain Containing Protein 3; IL, interleukin; TEC, tubular epithelial cell; RGD, arginine-glycine-aspartate; MAPK, mitogen-activated protein kinase; ERK, extracellular regulated protein kinase; Scr, serum creatinine; BUN, blood urea nitrogen; eNOS, endothelial nitric oxide synthase.

Nephrol Dial Transplant (2016) 31: 1062–1069
doi: 10.1093/ndt/gfv317
Advance Access publication 1 September 2015

Full Review

Mitochondria: a therapeutic target in acute kidney injury

Yu Ishimoto[1] and Reiko Inagi[1,2]

discussed the possibility of MSCs relieving mitochondrial dysfunction for the treatment of AKI.

Mitochondria and their dysfunction in AKI

Mitochondria are fundamental organelles that are present in almost all eukaryotes. Mitochondria have a unique structure characterized by a highly permeable outer membrane and a highly impermeable inner membrane. The basic function of mitochondria is the generation of ATP through oxidative phosphorylation (OXPHOS), but an increasing number of studies have demonstrated that mitochondria also play essential roles in cell proliferation, the modulation of intracellular reactive oxygen species (ROS), calcium homeostasis and apoptosis [21]. The density and distribution of mitochondria vary in different tissues because they are determined by the different levels of energy demand. Due to its physiological function of blood purification, the kidney is an organ with abundant mitochondria, consuming approximately 7% of the daily ATP expend-

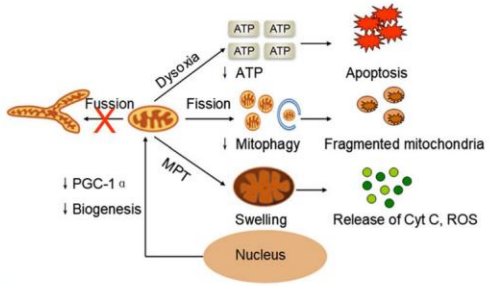


Fig. 1 Mitochondrial dysfunction during AKI. During AKI, the mitochondria tend towards fission rather than fusion. Together with the suppression of mitophagy, fragmented mitochondria are observed in the cytoplasm. Downregulation of PGC-1α inhibits the biogenesis of mitochondria. Mitochondrial swelling is regarded as a consequence of MPT, which may subsequently release Cyt C and ROS. Dysoxia is also very common, inducing a reduced generation of ATP and cell apoptosis. *PGC-1α* peroxisome proliferator-activated receptor-γ coactivator-1α, *MPT* mitochondrial permeability transition, *ATP* adenosine triphosphate, *Cyt C* cytochrome C, *ROS* reactive oxygen species

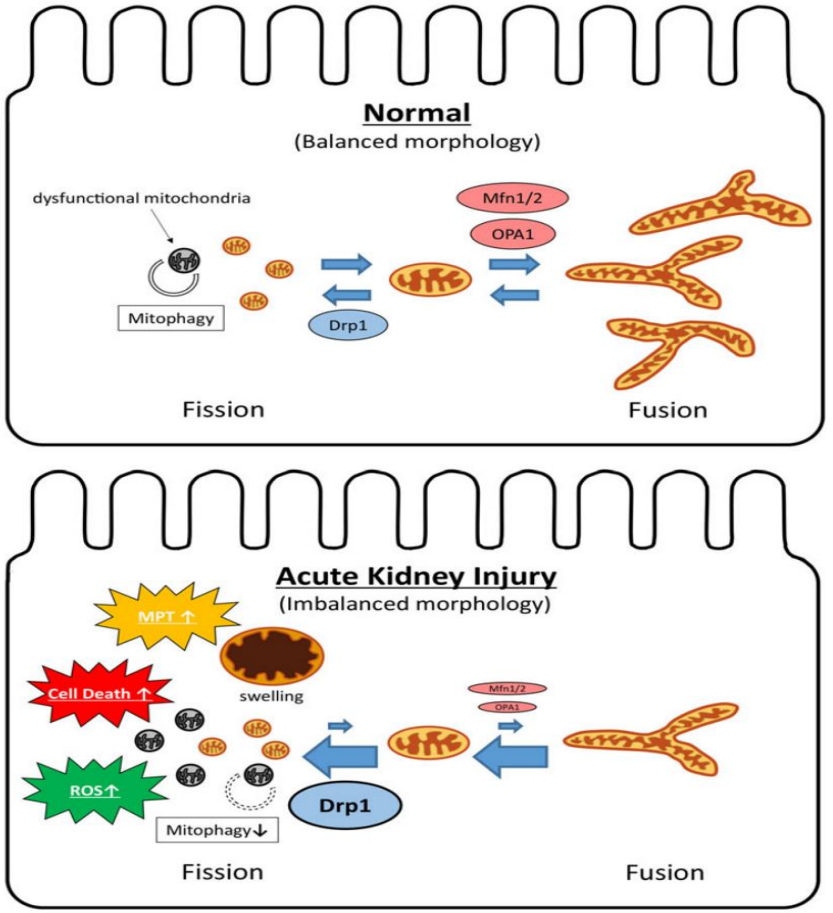


FIGURE 2: Mitochondrial dynamics and tubular injury in AKI. In the normal condition (upper panel), the frequencies of mitochondrial fusion and fission are finely tuned and well balanced to maintain mitochondrial homeostasis. Dysfunctional mitochondria, which are toxic for tubular cells, are removed by mitophagy. In the disease condition (lower panel), mitochondria become fragmented. This pathological fragmentation is the combined result of the activation of fission and suppression of fusion. Mitochondrial fragmentation has been demonstrated to contribute to mitochondrial damage and a consequent increase in ROS and cell death. MPT is induced under certain pathological conditions such Ca^{2+} overload and oxidative stress and causes mitochondrial swelling. Mitophagy is defective in the disease condition. Drp1, dynamin-related protein 1; Mfn1/2, mitofusin 1 and 2; OPA1, optic atrophy 1; ROS, reactive oxygen species; MPT, mitochondrial permeability transition.

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Full Review

Mitochondria: a therapeutic target in acute kidney injury

Yu Ishimoto[1] and Reiko Inagi[1,2]

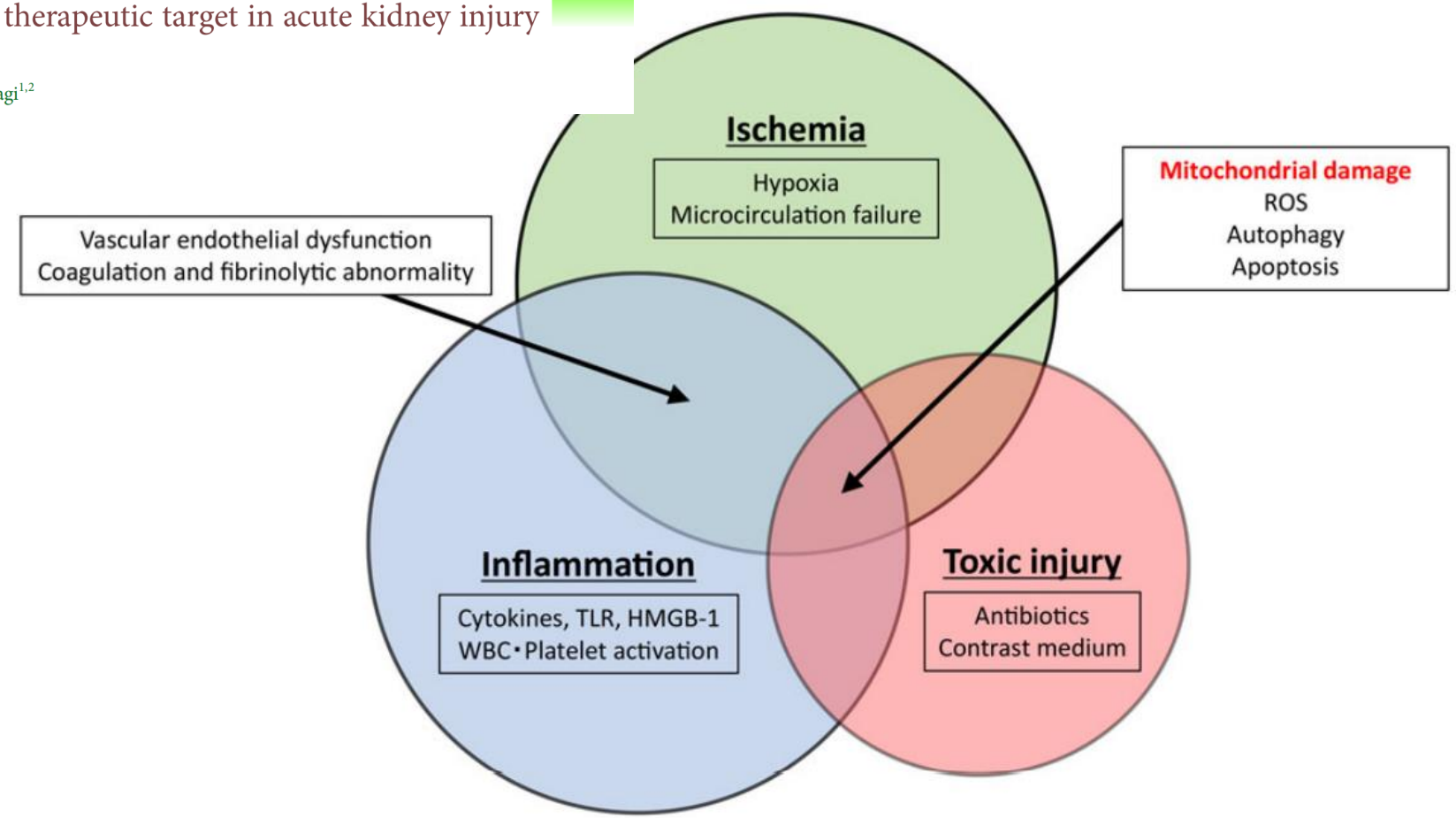


FIGURE 3: Pathogenesis of septic AKI. Septic AKI is the most common cause of AKI. Accumulating evidence from basic research revealed that the pathogenesis of septic AKI consists of three main components: (i) inflammation (excessive immunoreaction), (ii) ischemia and microangiopathy and (iii) induction of cell injury and death. Mitochondrial damage contributes to every component and plays a central role in the pathophysiology of septic AKI. TLR, toll-like receptor; HMGB-1, high-mobility group box-1; ROS, reactive oxygen species; WBC, white blood cell.

MITOCHONDRIA AS A THERAPEUTIC TARGET IN ACUTE KIDNEY INJURY

Dr. Sangeetha R , MD

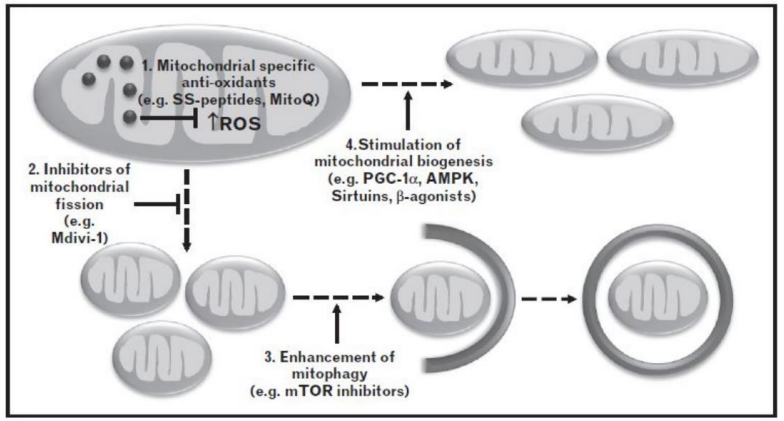


FIGURE 2. Summary diagram of current experimental strategies to target mitochondria in acute kidney injury. (1) Mitochondrial-specific antioxidants, such as SS-peptides and MitoQ, accumulate within the mitochondrial matrix and can limit the increase in reactive oxygen species (ROS) that is thought to occur in acute kidney injury (AKI), thus minimizing oxidative stress. (2) Inhibition of the pro-fission protein DRP-1 with Mdivi-1 can limit mitochondrial fragmentation and the subsequent activation of cell death pathways. (3) Damaged mitochondria are removed via mitophagy, whereby they are engulfed by autp-phagosomes, and enhancement of this process might be beneficial in AKI, but this remains controversial. (4) Stimulation methods can accelerate recovery post AKI. AMPK, AMP-activated kinase; PGC-1α, or-gamma coactivator-1 alpha.

Table 1 Associated articles demonstrating the mechanisms by which MSC therapy target mitochondrial dysfunction in AKI

Year	Animal	AKI model	MSCs source	Outcomes	References
2017	Mice	Glycerol	Bone marrow	↑ATP, ↑Activation of PI3K/Akt pathway; ↓ROS, ↓Mitochondrial-apoptosis related proteins, ↓Cell apoptosis	Geng et al. [64]
2013	Rats	Cisplatin	Umbilical cord	↓Activation of mitochondrial apoptosis signaling, ↓MDA	Peng et al. [65]
2017	Rats	Cisplatin	Bone marrow	↑PGC-1α, ↑Activation of wnt/β-catenin pathway; ↓ROS	Jiao et al. [66]
2016	Rats	I/R	Wharton Jelly	↑miR-30; ↓Mitochondrial fssion, ↓Cell apoptosis	Gu et al. [68]
2017	Mice	Cisplatin	Umbilical cord	↑ATP, ↑PGC-1α, ↑NAD+, ↑SIRT3; ↑Mitochondrial exchange ↓ROS; Normalized mitochondrial shape, density and mass	Perico et al. [70]

MSCs mesenchymal stem cells, *AKI* acute kidney injury, *I/R* ischemia/reperfusion, *ATP* adenosine triphosphate, *ROS* reactive oxygen species, *MDA* malondialdehyde, *PGC-1α* peroxisome proliferator-activated receptor-γ coactivator-1α, *SIRT3* sirtuin 3

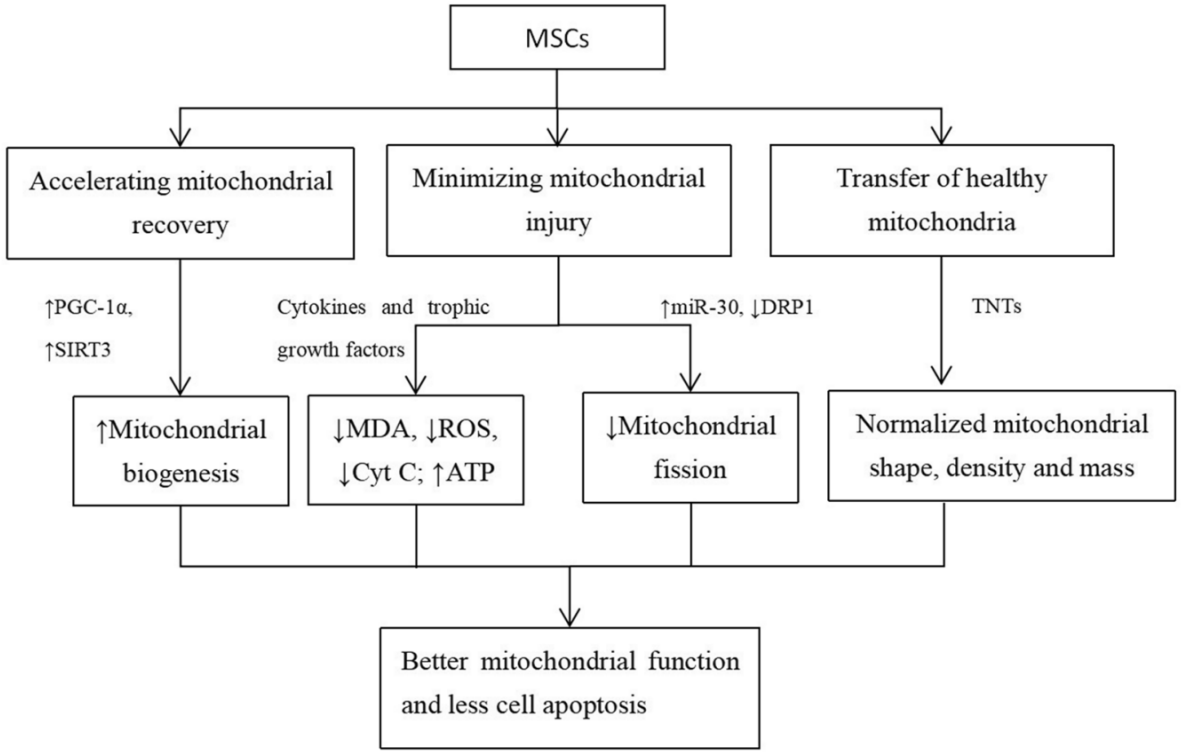


Fig. 2 The mechanism by which MSC therapy targets mitochondrial dysfunction in AKI. MSCs are able to accelerate mitochondrial recovery, minimize mitochondrial injury and transfer healthy mitochondria to injured cells. These actions result in decreased levels of MDA, ROS and Cyt C, accompanied by reduced mitochondrial fission and enhanced mitochondrial biogenesis, finally inducing improved mitochondrial function and a reduction in cell apoptosis. *MSCs* mesenchymal stem cells, *PGC-1α* peroxisome proliferator-activated receptor-γ coactivator-1α, *SIRT3* sirtuin 3, *DRP1* dynamin related protein 1, *TNTs* tunneling nanotubes, *MDA* malondialdehyde, *ROS* reactive oxygen species, *Cyt C* cytochrome C, *ATP* adenosine triphosphate

- Genome engineering is an **emerging technology** that enables the **insertions or deletions** of **DNA using engineered nucleases.**
- The present study aimed
- (i) to **generate hUC-MSCs secreting angiogenic factors VEGF** or angiopoietin-1 or **anti-inflammatory factors (erythropoietin** [EPO] or **a-melanocyte–stimulating hormone [aMSH])** via zinc finger nuclease (ZFN)–mediated genome engineering technology;

- AKI is induced by clamping of bilateral renal pedicles for 30 min and then release. During occlusion of the renal pedicle (at 20 minutes after the occlusion of both renal pedicles), cell sheets of genomeengineered hUC-MSCs are applied to the decapsulated kidney surface. At 30 minutes after renal pedicle occlusion, both clamps are removed (i.e., bilateral renal ischemia-reperfusion [I/R] injury).

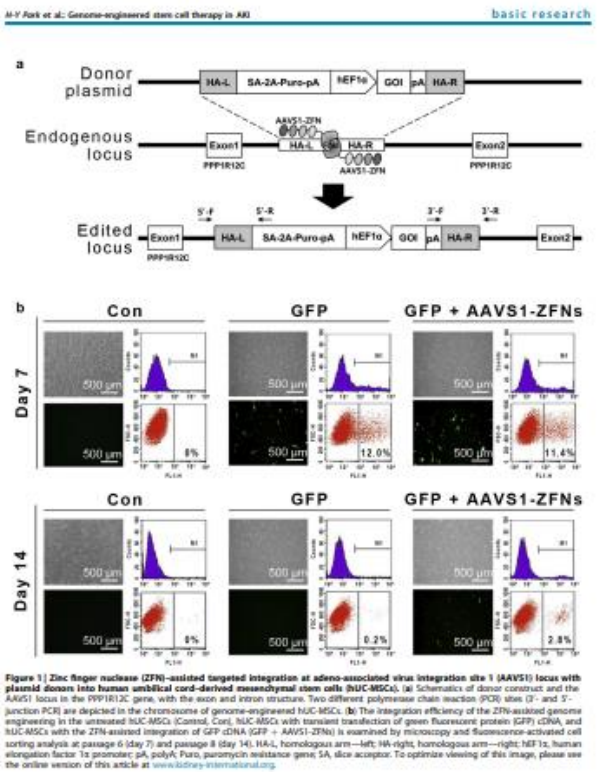


Figure 1 | Zinc finger nuclease (ZFN)–assisted targeted integration at adeno-associated virus integration site 1 (AAVS1) locus with plasmid donors into human umbilical cord–derived mesenchymal stem cells (hUC-MSCs). (a) Schematics of donor construct and the AAVS1 locus in the PPP1R12C gene, with the exon and intron structure. Two different polymerase chain reaction (PCR) sites (3′- and 5′-junction PCR) are depicted in the chromosome of genome-engineered hUC-MSCs. (b) The integration efficiency of the ZFN-assisted genome engineering in the untreated hUC-MSCs (Control, Con), hUC-MSCs with transient transfection of green fluorescent protein (GFP) cDNA, and hUC-MSCs with the ZFN-assisted integration of GFP cDNA (GFP + AAVS1-ZFNs) is examined by microscopy and fluorescence-activated cell sorting analysis at passage 6 (day 7) and passage 8 (day 14). HA-L, homologous arm—left; HA-right, homologous arm—right; hEF1α, human elongation factor 1α promoter; pA, polyA; Puro, puromycin resistance gene; SA, slice acceptor. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

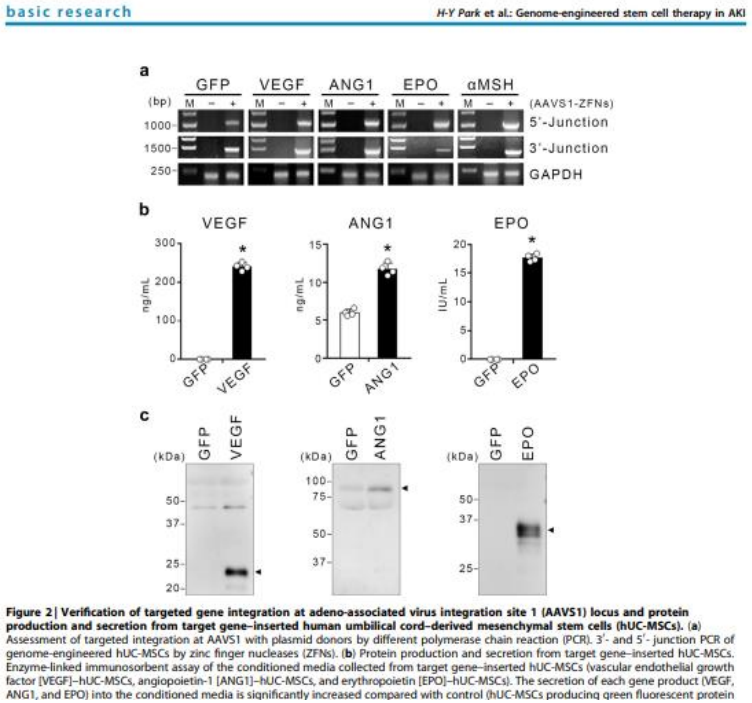
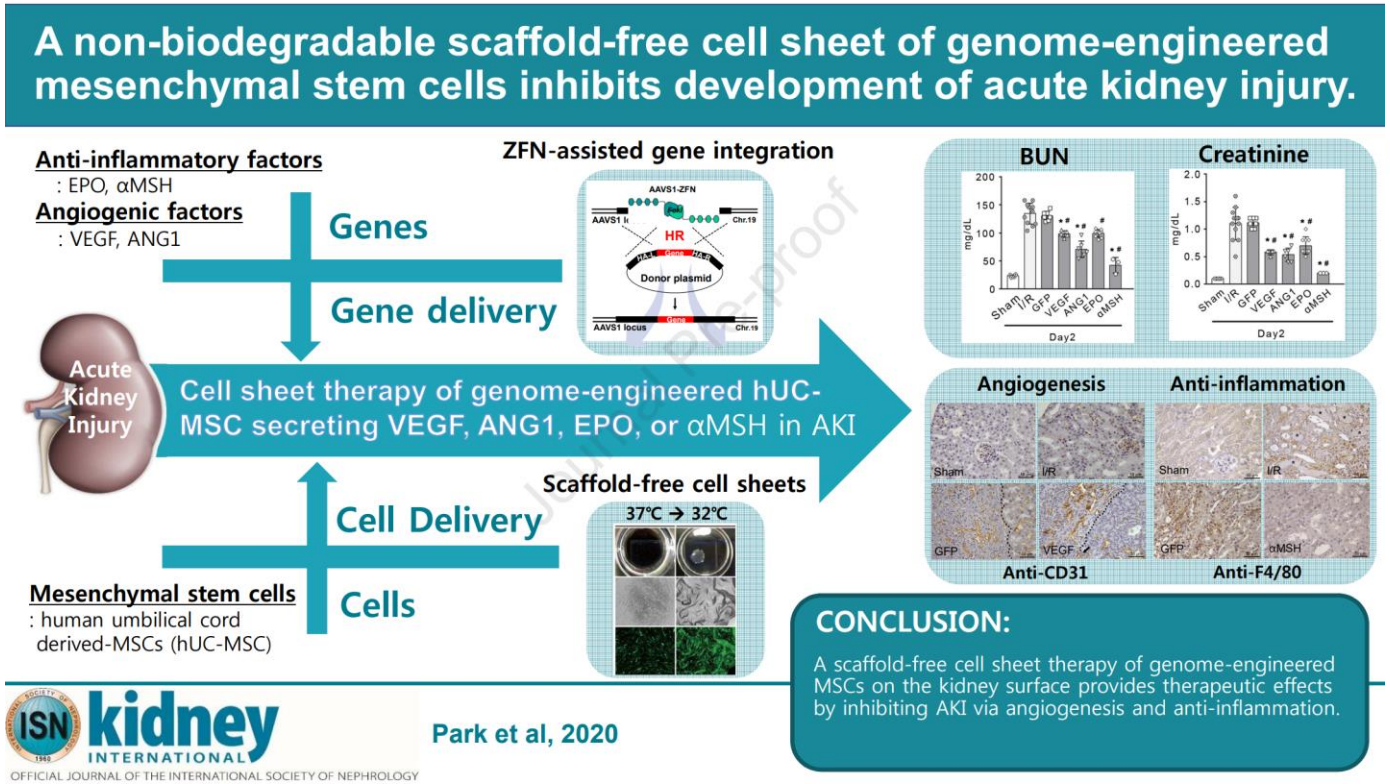


Figure 2 | Verification of targeted gene integration at adeno-associated virus integration site 1 (AAVS1) locus and protein production and secretion from target gene–inserted human umbilical cord–derived mesenchymal stem cells (hUC-MSCs). (a) Assessment of targeted integration at AAVS1 with plasmid donors by different polymerase chain reaction (PCR). 3′- and 5′- junction PCR of genome-engineered hUC-MSCs by zinc finger nucleases (ZFNs). (b) Protein production and secretion from target gene–inserted hUC-MSCs. Enzyme-linked immunosorbent assay of the conditioned media collected from target gene–inserted hUC-MSCs (vascular endothelial growth factor [VEGF]–hUC-MSCs, angiopoietin-1 [ANG1]–hUC-MSCs, and erythropoietin [EPO]–hUC-MSCs). The secretion of each gene product (VEGF, ANG1, and EPO) into the conditioned media is significantly increased compared with control (hUC-MSCs producing green fluorescent protein

This suggests that the strategy of **systemic administration** of MSCs may be inefficient in other organs, especially **in the lung**, and the number of cells reaching the kidney may not be sufficient to result in a therapeutic effect.
Thus, **local administration** of a **cell sheet** may be a **better choice** for the treatment of kidney diseases.

This approach clearly resulted **in amelioration of renal dysfunction both physiologically and histopathologically.**

however, this comes with an **increased risk of side effects**.



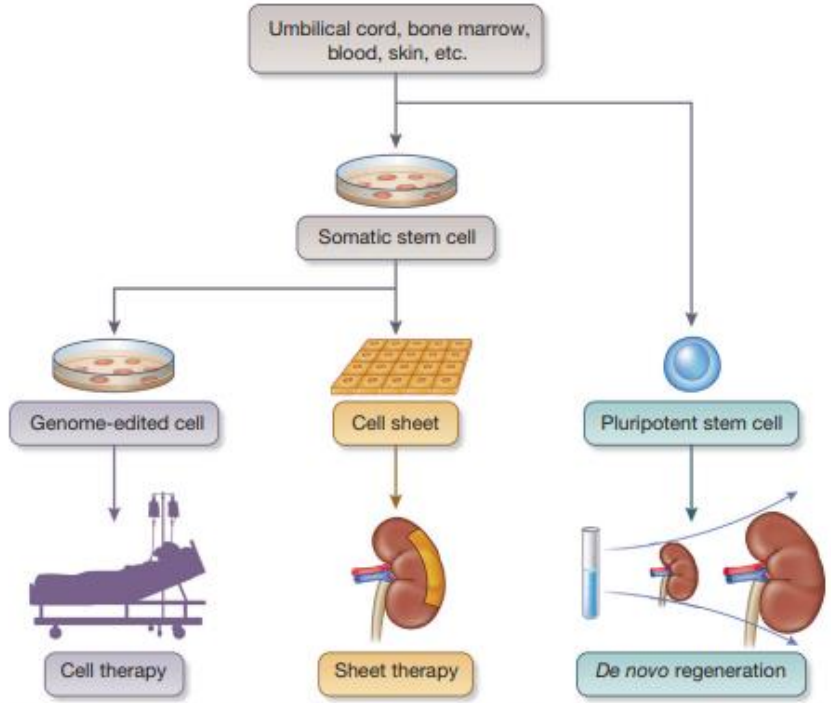


Figure 1 | Stem cell therapies for kidney diseases. Stem cell therapy can be performed with 3 main approaches. Right: in cell th

Thank you