Harnessing the mesenchymal stem cell secretome for regenerative urology

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Abstract | The extensive arsenal of bioactive molecules secreted by mesenchymal stem cells (MSCs), known as the secretome, has demonstrated considerable therapeutic benefit in regenerative medicine. Investigation into the therapeutic potential of the secretome has enabled researchers to replicate the anti-inflammatory, pro-angiogenic and trophic effects of stem cells without the need for the cells themselves. Furthermore, treatment with the MSC secretome could circumvent hurdles associated with cellular therapy, including oncogenic transformation, immunoreactivity and cost. Thus, a clear rationale exists for investigating the therapeutic potential of the MSC secretome in regenerative urology. Indeed, preclinical studies have demonstrated the therapeutic benefits of the MSC secretome in models of stress urinary incontinence, renal disease, bladder dysfunction and erectile dysfunction. However, the specific mechanisms underpinning therapeutic activity are unclear and require further research before clinical translation. Improvements in current proteomic methods used to characterize the secretome will be necessary to provide further insight into stem cells and their secretome in regenerative urology.

Innovations in stem cell research have unlocked the potential for stem cell therapy in regenerative urology. Stem cells are a unique cell population owing to their ability for self-renewal, unlimited proliferation and differentiation into multiple terminal cell types[1]. Furthermore, stem cells possess unique antifibrotic, pro-angiogenic and anti-apoptotic properties that might improve treatment of urological diseases for which pharmacological or surgical therapies are lacking. For example, the regenerative properties of stem cells have shown promise in reversing the smooth muscle damage associated with stress urinary incontinence (SUI)[2,3] or inhibiting the fibrotic processes associated with development of chronic kidney disease (CKD)[4,5]. Preclinical research has shown much promise, but clinical translation of stem cell therapy into the realm of regenerative urology has lagged somewhat[6]. To date, stem cells have been classified into four main categories — embryonic stem cells (ESCs), amniotic fluid stem cells (AFSCs), induced pluripotent stem cells (iPSCs) and adult stem cells (ASCs)[1]. ESCs, which are derived from a human blastocyst[7], are the most undifferentiated form of stem cell and have the greatest therapeutic potential, as they can infinitely self-renew and theoretically differentiate into any human cell type[8]. However, the use of ESCs is limited owing to ethical concerns, potential allogenicity and concerns about oncogenesis[9]. AFSCs are isolated from the amniotic fluid or the placental membrane of a developing fetus[10] and possess an intermediate potential for proliferation and differentiation (between that of ESCs and ASCs)[11]. In 2007, researchers determined that differentiated somatic cells could be reprogrammed into a pluripotent state[12]; these iPSCs possess the differentiation potential of ESCs without the necessity of using an embryo. However, owing to concerns regarding oncogenesis and the genetic stability of iPSCs, their application for regenerative urology requires further investigation[13]. ASCs, also known as somatic stem cells, are tissue-specific progenitor cells and are the most limited stem cell type along the spectrum of differentiation[14]. ASCs reside among differentiated cells throughout the body and possess the ability to repair damage and restore function locally. However, two unique populations of ASCs — haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) — challenge these conventional characteristics. Originating in bone marrow, HSCs are myeloid and lymphoid precursors that exert systemic effects by traversing the circulatory system in their path to become mature blood cells[15]. HSC transplantation (also known as bone marrow transplantation) is currently used to treat a variety of haematological diseases (such as leukaemia) and involves the intravenous infusion of HSCs as a source of blood progenitor cells[16]. MSCs, another class of mature stem cell found in the

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Key points

- Stem cells possess anti-inflammatory, pro-angiogenic and anti-apoptotic properties that might have therapeutic benefit in urological diseases for which conventional therapies are lacking.
- The acellular secretome of mesenchymal stem cells (MSCs) exerts similar therapeutic benefits to that of traditional cell-based therapy.
- The MSC secretome avoids problems associated with traditional stem cell therapy, including oncogenic transformation, immunoreactivity and cost.
- The MSC secretome exerts therapeutic benefits in preclinical models of stress urinary incontinence, acute and chronic renal disease, bladder dysfunction and erectile dysfunction.
- The specific mechanisms through which the MSC secretome exerts its therapeutic effects require further investigation, but they probably involve multiple MSC-derived bioactive cytokines that function synergistically.
- Proteomic strategies have been used to characterize the active components of the MSC secretome, which probably include MSC-derived extracellular vesicles in addition to bioactive cytokines.

bone marrow, have been thoroughly investigated for their tissue regenerative capabilities. MSCs are thought to maintain a microenvironment for HSCs and regulate haematopoiesis[17]. However, unlike tissue-specific ASCs, MSCs possess a broad differentiation potential. MSCs were originally thought to engraft or differentiate into injured host tissue, but MSCs are now known to also secrete soluble factors that function in a paracrine manner to provide a therapeutic regenerative effect[2,18–20]. Using proteomic approaches, researchers have started to characterize the bioactive cytokines secreted by stem cells, and increasing evidence suggests that some of these molecules are contained within extracellular vesicles (EVs) called exosomes or microvesicles[21–24].

In this Review, we discuss the rationale, mechanisms and current understanding of the MSC secretome in regenerative urology, highlighting preclinical studies that have evaluated its therapeutic potential in SUI, renal disease, bladder dysfunction and erectile dysfunction (ED). Finally, we describe proteomic methods used to characterize the secretome and provide insight into future perspectives regarding stem cells and their secretome.

MSCs

MSCs are a unique subset of ASCs that were first described by Friedenstein in 1974 (REF.[25]). Unlike tissue-specific ASCs, MSCs possess the ability to differentiate into endodermal, mesodermal and ectodermal lineages[26]. These properties are comparable to those of ESCs, but MSCs lack the ethical problems or potential for oncogenesis associated with ESCs. MSCs were traditionally derived from the bone marrow stroma but have also been isolated from muscle tissue, adipose tissue, skin, cartilage, bone, fallopian tissue, umbilical cord blood and menstrual blood[27,28]. In fact, MSCs are found in almost all well-vascularized tissues and are phenotypically related to pericytes, a type of vascular smooth muscle cell[29]. Although MSCs isolated from different tissues might possess different cell surface markers and gene expression profiles, all MSCs have considerable proliferative ability and multilineage differentiation potential[30,31].

Much of the clinical urology literature has focused on MSCs derived from muscle tissue, termed muscle-derived stem cells (MDSCs)[28]. In fact, the first major clinical trials investigating the use of stem cells for SUI evaluated periurethral injections of autologous MDSCs[32–34]. Although MDSCs are more accessible than bone marrow-derived MSCs, the harvesting process requires an invasive biopsy followed by a time-consuming cell expansion process[35]; a more abundant source of MSCs is adipose tissue. Adipose-derived stem cells (ADSCs) can be obtained in high numbers by clonally expanding cell populations isolated from excess fatty tissue from liposuction procedures[16]. Researchers continue to find novel sources of stem cells, including urine-derived stem cells, which might have interesting urological applications[36] (BOX 1).

Mechanisms of action. The mechanism by which bone marrow-derived MSCs localize to sites of injury is termed homing[37] — in response to chemical signals released by damaged cells, MSCs travel from the bone marrow through the circulatory system and localize to the target tissue via cytokine gradients. Although the precise mechanism is still unclear, homing is thought to involve an interaction between homing cytokines and their respective receptors on the MSC surface. For example, stromal-derived factor 1 (SDF1; also known as CXCL12), a cytokine expressed by endothelial cells after injury, has been shown to attract MSCs via its receptor, CXC-chemokine receptor 4 (CXCR4), which is present on the MSC surface[38,39]. Lin et al.[40] induced SUI in female rats with vaginal dilation and bilateral ovariectomy and subsequently injected labelled autologous ADSCs either directly into the urethra or intravenously via the tail vein. One month after treatment, both groups had markedly improved voiding patterns compared with controls (saline injection). On histological examination, labelled ADSCs and overexpression of SDF1 were found in the urethral tissues of animals in the tail vein group, suggesting that SDF1 promoted systemic migration of intravenously injected ADSCs to injured tissue.

A multitude of other cytokines that influence MSC homing have been discovered, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGFβ) and tumour necrosis factor (TNF)[41,42]. After reaching their destination in the bloodstream, MSCs must also transmigrate through endothelial cell layers to reach their target tissue, a process that involves adhesion molecules such as selectins and integrins[43].

Once MSCs reach their target tissue, they function through several interrelated mechanisms (FIG. 1). Traditionally, stem cells were thought to restore tissue function via transdifferentiation into different cell types or even engraftment into injured tissue[44]. Indeed, several preclinical studies in the urological literature support this hypothesis. Chermansky and colleagues[45] induced intrinsic sphincter deficiency in female rats using electrocauterization and periurethrally injected labelled autologous MDSCs 1 week after injury. Rats treated with MDSCs had substantial increases in leak point pressure (LPP) compared with rats treated with

Transdifferentiation
The conversion of a cell of one tissue lineage into a cell of a different lineage.

Engraftment
The process by which stem cells integrate into host tissue.

Box 1 | **Urine-derived stem cells**

Urine-derived stem cells (UDSCs) offer an exciting alternative to traditional autologous sources of mesenchymal stem cells (MSCs) such as muscle or adipose tissue, which require invasive procedures and can cause donor-site morbidity. Zhang and colleagues[142] discovered that a population of cells isolated from urine exhibit MSC-like features and possess trilineage differentiation capacity[143]. These cells express MSC and/or pericyte markers and are probably derived from the glomerular parietal epithelium[144]. UDSCs might offer an advantage over other multipotent stem cells for urological applications given that they can be easily and safely obtained from a urine sample, exhibit robust proliferative capacity and can differentiate into urothelial cells with higher efficiency[143,145]. Moreover, UDSCs can be effectively manipulated to become induced pluripotent stem cells (iPSCs), which have broad therapeutic applications such as personalized regenerative medicine or as vectors for gene delivery[36].

Similar to MSCs, the regenerative properties of UDSCs have shown promise in animal models of urological disease. In a rat model of diabetic erectile dysfunction (ED), intracavernous injections of UDSCs or UDSCs transfected with fibroblast growth factor 2 (FGF2; a potent angiogenic protein) upregulated the expression of pro-angiogenic factors, such as endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF), and smooth muscle markers in penile tissues[146]. FGF2 transfection increased the expression of these factors compared with UDSC treatment only. In addition, UDSC-treated animals exhibited improved functional erectile responses without detection of cells at the injection sites 4 weeks after injection, and FGF2 transfection also markedly enhanced these responses. These findings support the paracrine hypothesis of stem cell action discussed in this Review. Indeed, exosomes secreted by UDSCs decreased urine microalbuminuria, reduced tubular epithelial damage and enhanced endothelial cell proliferation when injected intravenously in a rat model of diabetic nephropathy[147]. Thus, UDSCs might be a desirable alternative for urological pathologies owing to their ease of harvest, proliferation potential and phenotypic similarity to the urinary system.

vehicle at 2, 4 and 6 weeks, and LPP in MDSC-treated rats was comparable to that of non-injured rats 4 weeks after injury. Histological analysis at 4 weeks showed intact striated muscle in the MDSC group compared with disrupted muscle fibres in the control group. Furthermore, labelled MDSCs had integrated with the muscle fibres in the urethra. In a similar study, labelled MDSCs were injected into the detrusor muscle of rats subjected to bladder cryoinjury[46]. The study showed that, 2 weeks after injury, treated animals had improved bladder contractility compared with saline-treated controls. The MDSCs survived for up to 8 weeks after injury and expressed α-smooth muscle actin (αSMA), suggesting that the MDSCs had differentiated into smooth muscle lineages.

In contrast to these studies, other investigators have demonstrated that the therapeutic effects of MSCs can persist despite disappearance or limited numbers of cells. Sadeghi and colleagues[18] administered labelled human MSCs either periurethrally or intravenously to rats immediately after vaginal distension. Treatment groups had comparable LPPs to non-injured controls after 4, 10 and 14 days, but the MSCs could not be visualized histologically by 4 days. Many studies outside of the urological literature support this finding. Indeed, in a mouse model of myocardial infarction, systemically injected MSCs improved cardiac function and fibrosis, but no evidence of MSC engraftment was observed in heart tissue at 3 weeks after injury[47]. In another study, rats received intravenous MSC injections or liver fibroblasts 1 week after middle cerebral artery occlusion[48]. Treated rats had markedly improved neurological outcomes and decreased fibrosis compared with fibroblast-treated controls up to 4 months after injury, with only a small number of MSCs present in the brain parenchyma. Interestingly, stem cells have been found to exert their effects despite being distant from their target organ. Indeed, Shabbir and colleagues[20] reported that injection of MSCs into the hamstring muscles of hamsters with congestive heart failure improved cardiac function and attenuated pathological fibrosis. Although MSCs did not migrate outside of the hamstring, increases in circulating levels of known MSC-secreted trophic factors were observed, suggesting a paracrine rather than a local mechanism of MSC action.

Taken together, these data infer a complex mechanism of MSC action (FIG. 1). Importantly, MSCs home to sites of injury in response to cytokine gradients, and a small percentage might engraft, differentiate and integrate with host tissues, but their therapeutic benefits cannot be explained by these mechanisms alone. Indeed, over the past decade, a growing body of evidence supports the paracrine hypothesis of stem cell action, which postulates that stem cells exert a substantial therapeutic effect by secreting bioactive paracrine factors — termed the secretome — with antifibrotic, pro-angiogenic and anti-apoptotic properties.

One crucial point in the interpretation of these findings is the limitation of single-cell labelling. In order to demonstrate MSC engraftment or differentiation, transplanted cells are commonly labelled with tracking molecules such as β-galactosidase (β-gal) or green fluorescent protein (GFP) and analysed microscopically after tissue harvesting and fixing[49]. These labels are not perfect, and histological quantification of labelled cells can be hindered by native tissue autofluorescence or loss of the imaging label, reducing the specificity or sensitivity, respectively, of cell detection[50].

The MSC secretome

The MSC secretome consists of a complex array of soluble molecules, such as growth factors, cytokines, hormones and lipid mediators, that together create a microenvironment that is suitable for cellular regeneration[51]. Specifically, the secretome promotes immune and inflammatory modulation, mediates angiogenesis and inhibits apoptosis (FIG. 1). A growing body of evidence suggests that some of these molecules are packaged in MSC-derived EVs (such as exosomes and microvesicles), enabling more efficient remote communication and targeting than soluble molecules[21–24].

The MSC secretome offers several advantages over traditional cell-based therapies for regenerative urology. First, therapeutic use of the acellular secretome might circumvent issues related to tumorigenicity, immunoreactivity and maldifferentiation associated with cell-based therapy[52–55]. Second, secretome therapy might enable cheaper and more efficient development of off-the-shelf treatments than the expansion and maintenance of individualized clonal cell populations. Finally, once the MSC secretome is further characterized, its active components could potentially be molecularly modified and tailored to the disease process of interest.

Maldifferentiation
The formation of unwanted ectopic tissue (for example, tumour cells).

MSC-conditioned culture medium
(MSC-CCM). Culture medium containing biologically active components that are secreted by mesenchymal stem cells (MSCs).

Immunosuppressive, anti-inflammatory and anti-fibrotic effects. The immune system has a crucial role in eliminating pathogens and repairing damage following tissue injury. However, at the extreme end of the spectrum, the immune defences of the body can cause more harm than good[56]. Examples of this phenomenon include septic shock or pathological remodelling of the heart following myocardial infarction. MSCs might have a role in subduing such inappropriate inflammatory responses by switching activated macrophages to an anti-inflammatory phenotype[57,58], inhibiting the activation of natural killer cells[59], suppressing dendritic cell maturation and function[60] and modulating the T helper 1 (T_H1) cell and/or T_H17 cell (pro-inflammatory) to T_H2 cell and/or regulatory T cell (anti-inflammatory) T lymphocyte balance[61–63]. The immunosuppressive effect of MSCs is thought to be mediated by secretion of soluble factors such as TGFβ1, prostaglandin E_2 (PGE_2), nitric oxide (NO), IL-6 and indoleamine 2,3-dioxygenase (IDO)[58,64–66]. Thus, given the influence of MSCs and their secretions, these signalling pathways could be an interesting future target for therapeutic modulation in the treatment of urological pathologies related to immune dysregulation, such as interstitial cystitis or chronic prostatitis[67,68].

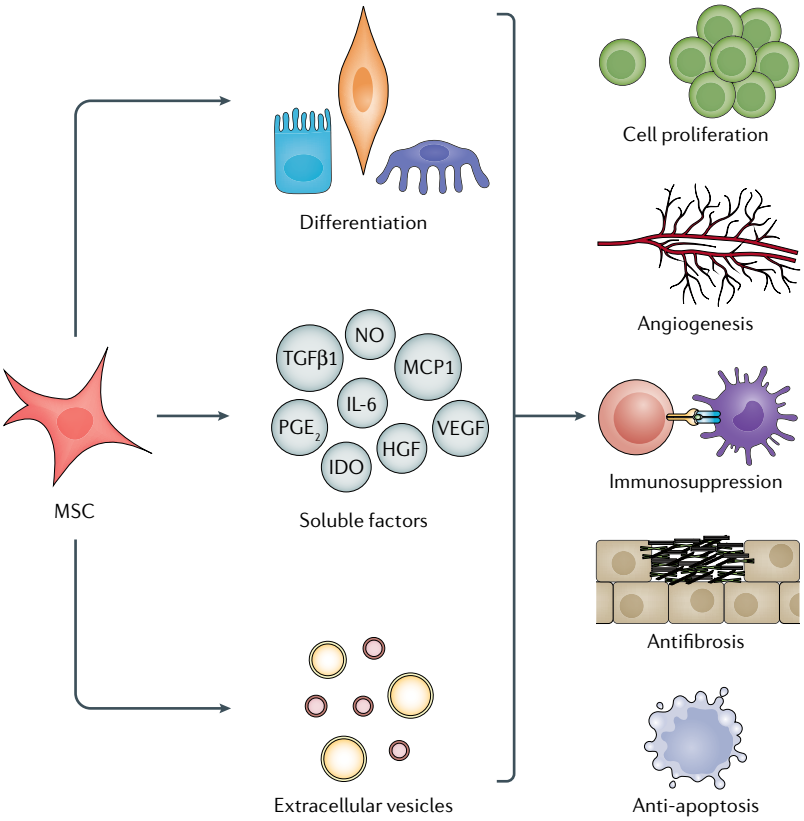

Fig. 1 | **MSC mechanisms of action.** Putative mechanisms of action of mesenchymal stem cells (MSCs) are depicted. MSCs might migrate to the site of tissue injury and restore function via differentiation or engraftment, a hypothesis that is supported by studies reporting that transplanted MSCs reside in injured tissue weeks or even months following treatment[45,46]. Conversely, other studies have shown that injected MSCs exert a therapeutic effect despite disappearance or reduced numbers of the cells at the site of injury[18,20,82,84], suggesting that MSCs might function via local or systemic secretion of soluble factors. This paracrine hypothesis is reinforced by studies demonstrating the therapeutic benefit of MSC-conditioned culture medium (MSC-CCM) alone[2–4,99,110]. Some of the soluble factors secreted by MSCs might be contained in extracellular vesicles[21–23,133,134]. Collectively, MSCs exert trophic, pro-angiogenic, immunosuppressive, antifibrotic and anti-apoptotic effects that, in turn, promote the regeneration of injured tissues. HGF, hepatocyte growth factor; IDO, indoleamine 2,3-dioxygenase; MCP1, monocyte chemotactic protein 1; NO, nitric oxide; PGE_2, prostaglandin E_2; TGFβ1, transforming growth factor-β1; VEGF, vascular endothelial growth factor.

Humans generally heal by fibrosis (generating scars) in response to tissue injury rather than by tissue regeneration (as occurs in some other eukaryotic organisms)[69]. However, humans possess some of these regenerative abilities in utero, but they are lost postpartum, with a few notable exceptions[70]. For example, both the adult liver and epidermis demonstrate regenerative potential, which is thought to be caused by stem cells residing in these tissues that might either differentiate to replace injured cells or secrete factors to enhance healing[71,72]. Similarly, MSCs, through their paracrine secretions, could have a role in shifting tissue healing in other organs towards a regenerative, rather than a fibrotic, process after injury or surgery[73]. To this end, MSC-conditioned culture medium (MSC-CCM) has been shown to enhance cutaneous wound healing in mice through trophic and anti-inflammatory cytokines, which induce migration of keratinocytes and endothelial cells to the area of injury[74]. Thus, treatment with MSC-CCM after trauma or surgery might facilitate tissue regeneration and attenuate fibrosis and scarring.

Inflammation also has a role in the fibrotic response of the kidney after ischaemic injury, which ultimately results in CKD[75]. One of the major mechanisms involved in this fibrotic response is the epithelial–mesenchymal transition (EMT) of proximal tubular epithelial cells (PTECs), which is mediated by the pro-inflammatory cytokine TGFβ[76]. In an in vitro model of EMT, incubation of human PTECs (HK-2) with MSC-CCM inhibited the morphological changes associated with EMT[76] (FIG. 2). Likewise, in a subtotal nephrectomy model of CKD in rats, Semedo et al.[77] showed that intravenous injections of MSCs improved renal function and markedly reduced fibrosis and glomerulosclerosis. By analysing mRNA expression in the kidney, the investigators demonstrated that kidney tissues from MSC-treated animals had upregulated expression of certain genes encoding anti-inflammatory cytokines, such as haem oxygenase 1 (HO1) and hepatocyte growth factor (HGF), and downregulated expression of pro-inflammatory genes, such as those encoding IL-6 and TNF[77]. These data, along with numerous experiments involving other tissues and organ systems[78–80], establish that MSCs secrete factors that can suppress inflammation systemically in response to injury. This finding has implications for the future treatment of urological diseases associated with fibrosis, such as urethral stricture and Peyronie disease (BOX 2).

Pro-angiogenic effects. Angiogenesis, the formation of new blood vessels from existing ones, is crucial for tissue regeneration and viability, as it provides a source of oxygen and nutrients to injured tissue. MSC-CCM contains a substantial amount of vascular endothelial growth factor (VEGF), which has a major role in angiogenesis[81], and other pro-angiogenic cytokines such as basic fibroblast growth factor (bFGF), placental growth factor (PGF) and monocyte chemoattractant protein 1

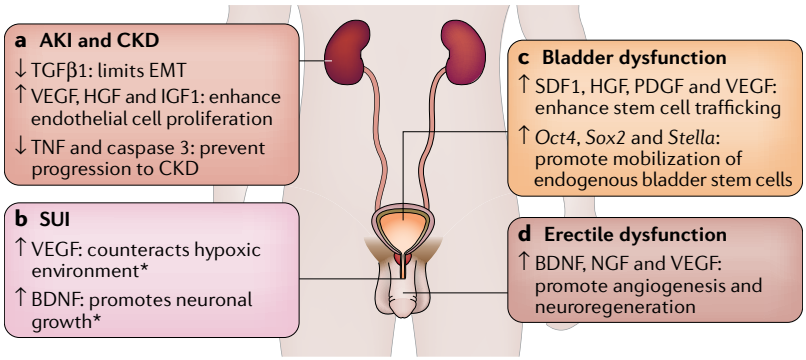

Fig. 2 | **The MSC secretome in regenerative urology.** The figure summarizes the therapeutic effects of the mesenchymal stem cell (MSC) secretome on urological pathologies, as demonstrated in preclinical studies. **a** | MSC-conditioned culture medium (MSC-CCM) prevented acute kidney injury (AKI) and limited chronic kidney disease (CKD) by increasing angiogenesis through the action of vascular endothelial growth factor (VEGF)[84], decreasing expression of pro-inflammatory cytokines such as tumour necrosis factor (TNF)[5] and transforming growth factor-β1 (TGFβ1)[76] and inhibiting apoptosis by reducing caspase 3 levels[5]. **b** | In stress urinary incontinence (SUI) models, MSC-CCM facilitated recovery after simulated childbirth injury by promoting periurethral elastogenesis and neuronal growth[2,3]. The specific cytokines involved have not been elucidated but might involve pro-angiogenic (for example, VEGF) and neurotrophic factors (for example, brain-derived neurotrophic factor (BDNF)). **c** | MSCs improved bladder function in a rat model of overactive bladder by upregulating trophic and pro-angiogenic cytokines[19] without engraftment of cells in bladder tissue. MSC treatment also upregulated expression of *Oct4*, *Sox2* and *Stella* in bladder tissue, which might indicate mobilization of endogenous stem cells. **d** | MSC-CCM prevented erectile dysfunction through the action of angiogenic (VEGF) and neurotrophic (BDNF and nerve growth factor (NGF)) cytokines[110]. Speculative mechanisms of action that require further investigation are indicated with asterisks. EMT, epithelial–mesenchymal transition; HGF, hepatocyte growth factor; IGF1, insulin-like growth factor 1; PDGF, platelet-derived growth factor; SDF1, stromal-derived factor 1.

(MCP1; also known as CCL2)[82]. Indeed, MSC-CCM enhances endothelial cell proliferation in vitro through the action of these cytokines, and its effect is partially inhibited by co-treatment with anti-VEGF or anti-bFGF antibodies[82]. In a mouse model of hindlimb ischaemia, intramuscular injection of MSCs improved blood flow, collateral formation and functional outcomes without evidence of MSC incorporation into target tissues[82]. These beneficial effects were replicated with injection of MSC-CCM but not with control medium, suggesting that the therapeutic effect of MSCs in this model occurred via a paracrine pathway that can be reproduced by treatment with the secretions only[83].

The vasculogenic properties of the MSC secretome contribute to the recovery of renal function after acute kidney injury (AKI). Togel and colleagues[84] showed that MSC-CCM stimulates the proliferation of aortic endothelial cells in culture through the action of VEGF and other renotropic cytokines (FIG. 2). In addition, intra-arterial injections of MSCs after a 60 minute bilateral renal hilum clamp were performed. MSCs homed to the kidney but rarely engrafted into peritubular capillaries (<1 cell per whole kidney section 24 hours after injury). Moreover, areas of the kidney where MSCs did persist showed less apoptosis than areas without stem cells[84]. Unfortunately, the angiogenic potential of MSCs might also be harnessed by cancer cells to support growth. Indeed, the human prostate cancer cell line DU145 exhibited a substantial increase in growth rate when co-cultured with MSCs or treated with MSC-CCM compared with fibroblast co-culture[85]. Moreover, treatment of DU145 cells with MSC-CCM induced the formation of capillary tubes, an indicator of angiogenesis[85]. Increased tumour cell proliferation and angiogenesis were also observed in excised xenograft tumours from nude mice co-injected with DU145 cells and MSCs. In addition, the cross-sectional area of blood vessels was increased by MSC treatment. These findings suggest that the vasculogenic effects of MSCs are influenced by the local microenvironment and might not be entirely beneficial in the setting of malignancy. The pro-angiogenic effects of MSCs and their secretions might be harnessed in future urological therapies (such as preventing kidney injury during partial nephrectomy), but further research on MSC interactions with tumour cell populations is necessary before their clinical use.

Anti-apoptotic effects. Data from a wide variety of pathologies indicate that MSCs secrete bioactive factors that promote cytoprotection and prevent apoptosis. The cytoprotective effects probably stem from the aforementioned immune and angiogenic effects of the MSC secretome but also from direct inhibition of apoptosis. Takahashi et al.[86] detected PDGF and insulin-like growth factor 1 (IGF1), along with other common cytokines (such as VEGF and IL-1β), in the supernatant of MSCs (that is, MSC-CCM). Using terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assays, they showed that these cytokines inhibited apoptosis of cardiomyocytes in vitro. Furthermore, in a rat model of myocardial ischaemia, intraperitoneal plus intramyocardial injections of MSC supernatant, but not intramyocardial injections alone, improved contractile function[86]. The authors speculated that this finding was a result of the additional dose of cytokines provided by intraperitoneal injections or that the intramyocardial injections of cytokines actually harmed the cardiac muscle tissue through anti-inflammatory effects. Using transwell co-culture experiments, Li and colleagues[87] found that MSCs secrete factors that prevent apoptosis of alveolar macrophages. Specifically, MSCs decreased the expression of the pro-apoptotic proteins caspase 3 and apoptosis regulator BAX and increased levels of apoptosis regulator BCL-2, an anti-apoptotic protein, in alveolar macrophages. In a rat model of ED after bilateral cavernous nerve ablation, Fall et al.[88] demonstrated that local injection of bone marrow mononuclear cells (which contain MSCs) partially restored erectile responses. Furthermore, cell therapy increased expression of endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) and markedly reduced the number of apoptotic cells in the erectile tissue.

In preclinical models of toxic kidney injury, the apoptosis of renal tubular epithelial cells has a major role in permanent renal dysfunction[89]. Imberti and colleagues[90] found that co-culture of PTECs with MSCs prevents the rapid death of PTECs that is usually observed after exposure to cisplatin. In addition, they demonstrated that this effect was facilitated by the anti-apoptotic cytokine IGF1, which was highly expressed and secreted by the

Box 2 | **MSC therapy for fibrotic penile disease**

The pathophysiologies of urethral stricture and Peyronie disease are related in that both are chronic fibrotic processes of the penile shaft that might be driven by the profibrotic transforming growth factor-β1 (TGFβ1) signalling pathway[50]. Thus, both diseases are interesting candidates for mesenchymal stem cell (MSC) therapy and have been investigated in preclinical studies.

In a 2016 study[148], Castiglione and colleagues induced urethral stricture in rats with urethral incisions followed by recombinant TGFβ1 injection. Four weeks after injury, these animals demonstrated urodynamic evidence of obstruction, and histological analysis showed disorganized collagen formation. Local injection of adipose-derived stem cells (ADSCs) 1 day after injury markedly improved voiding function, preserved corpus spongiosum architecture and increased tissue levels of inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) compared with untreated animals.

Similarly, TGFβ1 injection into the tunica albuginea has been used to create a rat model of Peyronie disease. Several studies using this model have demonstrated that local injection of ADSCs reduces tunica albuginea fibrosis and improves erectile function 5–6 weeks after treatment[149,150].

MSCs; blocking IGF1 attenuated the protective effect. In a model of acute renal failure caused by cisplatin in immunodeficient non-obese diabetic (NOD)–severe combined immunodeficient (SCID) mice, intravenous MSC injections prevented renal failure and preserved the integrity of the tubular epithelium[91]. Using TUNEL assays, the researchers demonstrated that animals treated with MSCs had substantially fewer apoptotic cells in renal tissue than animals treated with saline or fibroblasts. Similar to previously discussed studies[18,20,82,84], the authors demonstrated very low levels of labelled MSCs in the renal tissue, suggesting a paracrine effect. Remarkably, the mice treated with MSCs demonstrated markedly lower mortality than saline-treated animals, offering hope that the renotropic effect of MSCs could eventually be used therapeutically for renal failure.

Taken together, these preclinical studies demonstrate that MSCs secrete factors that inhibit apoptosis after tissue injury. This cytoprotective effect could be utilized in future therapies directed at kidney injury or ED.

The secretome in regenerative urology

The anti-inflammatory, antifibrotic, pro-angiogenic and anti-apoptotic properties of the MSC secretome could offer therapeutic benefits in urological diseases for which medical or surgical treatments are inadequate. The hypothesis that the paracrine activity of MSCs is responsible for their therapeutic action is supported by several preclinical studies demonstrating its effectiveness in animal models of SUI, renal disease, bladder dysfunction and ED (FIG. 2; TABLE 1).

Stress urinary incontinence. SUI, the involuntary loss of urine with exertion, affects up to 25% of women in the United States and accounts for nearly US$12 billion in annual health-care costs[92]. Treatments for SUI include pelvic floor muscle training, pharmacological therapy (in Europe), vaginal pessary devices, periurethral bulking agents and vaginal sling surgery[92]. Surgical therapy, the gold standard for treatment of SUI, is effective but serves as only a symptomatic treatment and is associated with complications, including pain, infection, urinary retention, bladder or urethral injury or mesh erosion[93]. Thus, stem cells and their secretome might offer an effective and safe therapy that targets the pathophysiological mechanisms underpinning SUI.

In a study by Dissaranan and colleagues[2] (TABLE 1), simulated childbirth injury was performed in rats using serial vaginal dilation, after which animals were treated with GFP-labelled MSCs via intravenous injection or MSC-CCM via periurethral administration. One week after injury, labelled MSCs preferentially homed to the urethra, vagina and spleen of injured animals compared with sham-injured animals. Moreover, LPP was markedly improved in MSC-treated and MSC-CCM-treated animals compared with animals treated with non-conditioned medium (NCM), although external urethral sphincter function (as measured by electromyography) was not improved. Both rats treated with MSCs and rats treated with MSC-CCM demonstrated increased elastin fibre density in periurethral smooth muscle tissues compared with control animals. Thus, this study demonstrated that MSC-CCM provided a therapeutic benefit in the absence of the MSCs themselves. However, the comparison of intravenous MSC therapy with local MSC-CCM therapy in this study makes it difficult to infer whether or not the MSC secretome functions systemically.

In a similar study (TABLE 1), the same group demonstrated that systemic administration of MSC-CCM restored urethral function in rats after simulated childbirth injury[3]. For this study, the investigators developed a dual-injury model consisting of vaginal distension and pudendal nerve crush, which theoretically mimics human childbirth injury more closely than serial vaginal dilation, as it elicits multiple mechanisms of injury (nerve and muscle). Treatment groups consisted of intravenous MSCs or intraperitoneal MSC-CCM administration 1 hour after injury. LPP was markedly decreased 3 weeks after treatment with NCM, but rats receiving MSCs or MSC-CCM had LPPs that were not substantially different from that of sham-injured rats. In rats receiving MSCs, stem cells were undetectable in the urethra or vagina after 3 weeks. Similar to the previous findings of the Dissaranan et al. study[2], both stem cell and acellular secretome treatments were associated with increased periurethral elastin fibre density in injured animals[3]. Moreover, pudendal nerve morphology and sensory branch potentials were also preserved with MSC or MSC-CCM treatment, suggesting that factors secreted by MSCs can have different therapeutic effects in different tissue microenvironments and injury paradigms.

Pro-angiogenic molecules such as VEGF are secreted by MSCs and might counteract the hypoxic microenvironment involved in the pathophysiology of childbirth-induced SUI[83] (FIG. 2). In addition, trophic factors secreted by MSCs could reverse the periurethral smooth muscle damage and pudendal nerve dysfunction associated with SUI[86,94]. Although the exact mechanisms require further investigation, these studies[2,3] (TABLE 1) demonstrate that the acellular secretions of stem cells restore urethral and nerve function in rodent SUI models. Several clinical trials have shown that local injections of stem cells are efficacious in human SUI[32–34] (BOX 3), but future clinical studies should evaluate the efficacy of MSC secretome

therapy — possibly administered systemically — for the treatment, or even prophylaxis, of SUI.

Renal disease. CKD is a global health burden. In the United States, the prevalence of CKD approaches 15%, and the incidence of end-stage renal disease (ESRD) is steadily increasing[95]. Despite the ready availability of dialysis in the United States, ESRD is a morbid state with a mortality of nearly 200 per 1,000 patient-years[96]. Furthermore, the waiting list for kidney transplantation, the gold standard of curative therapy for ESRD, includes >80,000 patients each year in the United States[95]. Thus, MSCs and their secretome offer a regenerative medicine solution that could benefit patients with chronic renal dysfunction.

Several preclinical studies have shown that MSCs are effective in preventing loss of kidney function following renal insult[77,84,91,97,98]. Importantly, in most of these studies, the incorporation or differentiation of MSCs into the native tissue at the site of injury was rare, suggesting a

Table 1 | **Preclinical studies of secretome treatment for urological diseases**

Study	Stem cell type	Study design	Major conclusions	Refs
SUI				
Dissaranan et al., 2014	MSCs and MSC-CCM	Intravenous MSC or periurethral MSC-CCM administration after vaginal dilation in rats; 1 week after injury, LPP and EUS function (via electromyography) were measured	LPP was markedly improved in both treatment groups compared with NCM-treated controls, demonstrating that local injection of MSC-CCM provided a similar benefit to systemic cell-based therapy	2
Deng et al., 2015	MSCs and MSC-CCM	Intravenous MSC or intraperitoneal MSC-CCM administration following vaginal distension and pudendal nerve crush in rats; 3 weeks after injury, LPP and pudendal nerve sensory branch potentials were measured	LPP was markedly improved in both treatment groups compared with NCM-treated controls, suggesting that systemic administration of the acellular secretome has similar efficacy to cell-based therapy	3
AKI				
Bi et al., 2007	MSCs and MSC-CCM	Systemic MSC or MSC-CCM administration after cisplatin-induced AKI in mice; renal function was measured 3 and 6 days following injury, and renal histology was assayed at day 6	When administered systemically, both MSC and MSC-CCM treatment markedly improved renal function and histology following acute renal injury compared with NCM; MSC-CCM also improved survival after renal injury	99
CKD				
Van Koppen et al., 2012	MSC-CCM	Intravenous injection of MSC-CCM or NCM in a subtotal nephrectomy model of CKD in rats; renal function and histology were analysed 6 weeks after treatment	Treatment with MSC-CCM markedly improved GFR compared with NCM treatment; MSC-CCM-treated rats had less tubular damage than controls; MSC-CCM was effective in reversing chronic kidney damage	4
Da Silva et al., 2015	MSCs and MSC-CCM	Intravenous administration of MSCs or MSC-CCM in a unilateral ureteral obstruction model of CKD in rats; inflammatory cytokines in tissue and renal histology were analysed at 7 and 14 days	In both treatment groups, levels of pro-inflammatory cytokines were markedly reduced compared with untreated rats; histological analysis showed decreased fibrosis and apoptosis in both groups	5
DBD				
Zhang et al., 2011	ADSCs	DBD was induced in rats using a high-fat diet and streptozocin; ADSCs were injected in the detrusor or via the tail vein; conscious cystometry was performed 1 month later to assess bladder function	60% of rats receiving tail vein injections and 40% of rats receiving intra-detrusor injections demonstrated bladder dysfunction, compared with 100% in PBS-treated controls; only a fraction of injected ADSCs remained in the bladder, suggesting a paracrine effect	105
OAB				
Song et al., 2013	MSCs	Intradetrusor injection of (human) MSCs, intradetrusor PBS or intravenous solifenacin administration in rats with OAB induced by urethral ligation; cystometry was performed at 2 and 4 weeks	Bladder parameters substantially improved with MSC treatment compared with PBS-treated controls and surpassed those of the antimuscarinic group at 4 weeks; this effect occurred without engraftment of human MSCs	19
ED				
Albersen et al., 2010	ADSCs and ADSC lysate	Intracavernosal injection of ADSCs or ADSC lysate in a rat bilateral cavernosal nerve injury ED model; erectile function was measured 4 weeks later by assessing ICP; immunohistological analysis included measurement of nNOS, smooth muscle and collagen content and apoptotic cells in the penile tissue	Animals in both treatment groups had markedly improved erectile function, preservation of nNOS and smooth muscle fibres and reduced fibrosis compared with PBS-treated controls; in the ADSC group, only a small fraction of cells were observed in the cavernosal tissue after 1 month, suggesting a paracrine effect	109
Sun et al., 2012	MSCs and MSC-CCM	Intracavernosal injections of MSCs or MSC-CCM in rats with diabetes-induced ED; erectile function was measured 4 weeks later by assessing ICP immunohistological analysis included measurement of nNOS and neurofilament content in the penile tissue	Both treatment groups experienced partial restoration of erectile function compared with untreated controls, although the effect was smaller in the MSC-CCM group; immunohistochemistry demonstrated increased staining of nNOS and neurofilament fibres	110

ADSC, adipose-derived stem cell; AKI, acute kidney injury; CKD, chronic kidney disease; DBD, diabetic bladder dysfunction; ED, erectile dysfunction; EUS, external urethral sphincter; GFR, glomerular filtration rate; ICP, intracavernosal pressure; LPP, leak point pressure; MSC, mesenchymal stem cell; MSC-CCM, MSC-conditioned culture medium; NCM, non-conditioned culture medium; nNOS, neuronal nitric oxide synthase; OAB, overactive bladder; SUI, stress urinary incontinence.

Box 3 | **Clinical trials of MSC therapy for stress urinary incontinence**

In the past decade, several clinical trials have demonstrated the safety and efficacy of mesenchymal stem cell (MSC)-based therapy for stress urinary incontinence (SUI). In the first trial of stem cell therapy for SUI, eight women received sphincteric injections of autologous muscle-derived stem cells (MDSCs)[32]. After a follow-up period of 1 year, five of the women showed marked improvements in their symptoms as measured by pad weights, bladder diaries and quality of life measures. Notably, one of these women achieved total continence. In a follow-up study[140], the investigators randomized 38 women to low-dose (1–16 million cells) or high-dose (32–128 million cells) injections and found that high-dose treatment was more efficacious — 89% of women had a 50% reduction in pad weight and 58% of women had a 50% reduction in leaks.

A similar study conducted in Poland enrolled 16 women with SUI to receive sphincteric injections of MDSCs[33]. Two years after a single treatment of low-dose (~6 million) MDSCs, the investigators reported a 75% success rate, including 8 out of 16 patients who achieved full continence. Sèbe and colleagues[34] investigated the use of autologous MDSCs in the treatment of 12 women with scarred, fixed urethras who had failed previous surgical intervention. Even in this inoperable cohort, 3 of 12 patients were dry after 3 months (0–3 leaks per week and a 5 g decrease on pad test), and more than half of patients demonstrated objective improvement in symptoms.

In each of these studies, adverse effects were minimal and limited to bruising and discomfort at the injection site. Collectively, these trials demonstrate the efficacy of MSC therapy for the treatment of SUI in some women, with a minority achieving a durable response. Larger randomized, placebo-controlled trials are required to further investigate this promising therapy.

paracrine mechanism of action. For example, in a bilateral renal pedicle clamp model of AKI in rats, Tögel and colleagues[98] showed that intracarotid administration of MSCs substantially improved renal function 48 hours after renal ischaemia compared with vehicle-treated animals, but administered MSCs were undetectable in the kidney after 24 hours of administration. Nonetheless, 24 hours after injury, the kidneys of MSC-treated animals had decreased expression of the pro-inflammatory cytokines IL-1β, TNF and IFNγ and increased expression of the anti-inflammatory cytokine IL-10, with concurrent upregulation of renotrophic growth factors and anti-apoptotic proteins. The brief time interval between MSC treatment and renal functional recovery, and the absence of MSCs at the injury site after 24 hours, suggests that the injected cells did not differentiate and replace injured host tissue.

As MSC incorporation or differentiation is rare at the site of injury, the hypothesis that the therapeutic benefit of MSCs stems from the immediate secretion of regenerative factors that might activate innate repair mechanisms is plausible. In a cisplatin-induced model of AKI in mice, intraperitoneal and intravenous injections of MSCs improved renal function, animal survival and histological parameters compared with NCM-treated mice[99] (TABLE 1). Similar to the Tögel et al. study[98], the authors demonstrated that no transplanted MSCs resided in kidney tubules after 24 hours of cisplatin-induced AKI, but the therapeutic benefits lasted for up to 6 days. These effects were also observed when MSC-CCM was administered intraperitoneally, supporting the concept that renal recovery after AKI did not depend on the physical presence of MSCs in this model. Blood urea nitrogen was markedly improved after treatment with MSC-CCM, but the authors did not report the changes in creatinine observed with MSC-based therapy; thus, direct comparison between the MSC-CCM and MSC treatment groups is difficult. However, the observations that paracrine factors secreted by MSCs initiated rapid-onset renal protection in two distinct (ischaemic[98] versus cytotoxic[99]) models of AKI is noteworthy. Thus, we posit that the regenerative effects of the stem cell secretome involve divergent pathways (that is, anti-inflammatory[76,77], pro-angiogenic[82–84] and anti-apoptotic[84,90] pathways) working in concert to promote renal protection following injury.

The close relationship between AKI and CKD is well established — AKI accelerates progression to CKD, whereas CKD predisposes patients to AKI[100]. Accordingly, van Koppen et al.[4] hypothesized that MSC-CCM could also influence renal recovery in a rat subtotal nephrectomy model of CKD (TABLE 1). After establishment of CKD, rats were intravenously injected with MSC-CCM or NCM twice daily for 4 days. MSC-CCM-treated rats had markedly higher glomerular filtration rates (GFRs) than animals injected with NCM 6 weeks after treatment. Histological analysis showed that kidneys treated with MSC-CCM had developed less glomerulosclerosis and tubular damage than NCM-treated kidneys. These results[4] are congruent with another study by Da Silva et al.[5] showing that MSC-CCM attenuated renal fibrosis in a rat model of CKD caused by unilateral ureteral obstruction (TABLE 1). In this experiment, animals underwent unilateral ureteral ligation or sham injury followed by intravenous injection of MSCs or MSC-CCM. Although the study was limited by the absence of renal functional assessment, the authors demonstrated that both MSCs and MSC-CCM decreased pro-inflammatory cytokine expression (FIG. 2), collagen formation, fibrosis and apoptosis in the kidney at 14 days after treatment compared with untreated controls.

Together, these studies indicate that the MSC secretome can not only prevent AKI but also reverse CKD. The specific mechanisms responsible for this renoprotective effect have yet to be elucidated but are probably multifactorial. From in vitro studies[64,77], we speculate that anti-inflammatory cytokines present in the secretome, such as PGE_2, might contribute to suppression of the acute inflammatory phase of renal injury. Furthermore, MSC-derived cytokines responsible for modulating fibrosis and apoptosis could limit progression to CKD[76,90]. However, whether renal protection is a result of the direct action of MSC-secreted factors or an indirect outcome (through their stimulation of endogenous progenitor cells) requires further investigation. Clinical trials are currently underway to investigate the use of MSCs for the treatment of AKI and CKD[101–104]. Encouraging results from early-phase, single-arm trials have demonstrated the safety[102] and efficacy[104] of autologous stem cell treatments.

Bladder dysfunction. Bladder dysfunction is a term encompassing a wide range of pathologies that affect micturition, including urinary retention, overactive bladder (OAB), neurogenic bladder and interstitial cystitis, among others. Many pharmacological and surgical treatments exist for these disorders, but none offers the regenerative and reparative potential of MSCs and their secretome.

At present, no studies have directly examined the effects of the MSC secretome on bladder dysfunction, but stem cell studies can provide insight into the mechanism of action. Zhang et al.[105] investigated the use of ADSCs in a rat model of diabetic bladder dysfunction (DBD) induced with a high-fat diet and streptozocin (TABLE 1). Animals received intradetrusor or intravenous (tail vein) injections of labelled ADSCs or PBS. Results showed that 100% of rats receiving PBS had voiding dysfunction as measured by conscious cystometry 1 month after treatment, whereas only 60% and 40% of rats receiving tail vein and bladder ADSC injections, respectively, showed bladder dysfunction. However, only a small fraction of injected ADSCs were observed in the bladder mucosa after 1 month of treatment, suggesting that the functional effects of the ADSCs were primarily paracrine in nature. As the pathogenesis of DBD is probably related to detrusor decompensation and neuronal damage[106], the benefit of stem cells for DBD could be a result of upregulation of smooth muscle and neuronal growth factors. This study would have benefited from a gene expression analysis of bladder tissues from ADSC-treated and control rats to investigate the expression of such genes.

In another study, OAB was induced with urethral ligation in female rats[19] (TABLE 1). Four weeks after injury, either labelled human MSCs or PBS (negative control) were injected into the detrusor muscle of the bladder, and these groups were compared with a positive control group receiving the antimuscarinic solifenacin intravenously. At 2 and 4 weeks after treatment, both MSC-treated and solifenacin-treated groups had marked decreases in their detrusor contraction frequencies compared with the PBS group; however, the therapeutic effect was superior in the MSC-treated group at 4 weeks. Analysis of the bladders of MSC-treated rats revealed upregulation of native pluripotent stem cell markers (such as *Oct4* (also known as *Pou5f1*), *Sox2* and *Stella* (also known as *Dppa3*)) without any engraftment of the transplanted human MSCs. Lastly, consistent with other studies[40,77,84], MSC treatment resulted in overexpression of trophic cytokines such as SDF1, HGF, PDGF and VEGF (FIG. 2). This study supports findings from other organ systems and demonstrates that, without engraftment, transplanted MSCs release soluble factors that might have therapeutic benefit in OAB. Moreover, the MSC-induced upregulation of native stem cell markers suggests that the mechanism of action might occur via mobilization of the endogenous stem cells of the bladder. Notably, stem cell treatment was more effective than the antimuscarinic solifenacin, a competitive cholinergic receptor antagonist, after 4 weeks. Anticholinergics, although effective for the management of OAB in humans, are a symptomatic treatment and are associated with adverse effects such as dry mouth, constipation, urinary retention and cognitive impairment. Thus, MSCs and their secretome might be a safe and effective treatment option for bladder dysfunction that targets the pathophysiology rather than symptoms. These animal studies show promise, but the role and therapeutic potential of the MSC secretome in human bladder dysfunction require further investigation.

Erectile dysfunction. ED, or the inability to attain penile erection satisfactory for intercourse, affects up to 30 million men in the United States[107]. The aetiology of ED is related to vascular compromise from chronic hypertension or diabetes, pelvic trauma or nerve damage (for example, as a result of prostatectomy). Current therapies for ED aim to enhance the blood flow to the penis using pharmacological agents (phosphodiesterase inhibitors), intraurethral suppositories or intracavernosal injections[107]. In addition, penile prosthesis implantation is a viable surgical option for patients in which conservative measures have failed[108]. The MSC secretome, with its vasculogenic and neuroregenerative properties, has shown preclinical potential in various animal models of ED and could be a treatment option for impotent men in the future.

Albersen et al.[109] developed a bilateral cavernosal nerve injury model in rats that was designed to simulate ED after radical prostatectomy (TABLE 1). Immediately after injury, rats received intracavernosal injection of labelled ADSCs, ADSC cell lysate or PBS. Erectile function was assessed by measuring intracavernous pressure (ICP) after electrostimulation of the distal cavernosal nerve 4 weeks later. Both ASDC-treated and ADSC-lysate-treated animals had major increases in the ICP to mean arterial pressure (MAP) ratio compared with injured rats treated with control. Moreover, rats treated with ADSCs or ADSC lysate had markedly higher numbers of nNOS-positive nerve fibres, greater preservation of smooth muscle content and less fibrosis than control-treated rats. In animals treated with ADSCs, very few labelled stem cells were observed in the cavernosal tissue after 28 days, although the number of remaining cells was not quantified. Thus, the authors concluded that the benefit of ADSCs does not result from incorporation or transdifferentiation into the host tissue but rather from soluble neurotrophins released by the cells.

Sun et al.[110] studied the effect of MSCs and their secretome in a rat model of diabetes-induced ED (TABLE 1). In this study, at 4 weeks after rats received intracavernosal injections of MSCs or MSC-CCM, partial restoration of erectile function (as measured by ICP to MAP ratio) was observed in both MSC and MSC-CCM groups compared with untreated controls, although the effect was smaller in the MSC-CCM group. This improvement was accompanied by an increase in positive immunostaining for nNOS and neurofilament in nerve fibres of the cavernosal tissue. These therapeutic effects were probably a result of MSC-derived neurotrophins such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), which were found to be highly expressed in MSC-CCM (FIG. 2). Studies in the neurological literature have established that MSCs, through secretion of neurotrophins such as BDNF, glial cell-derived neurotrophic factor (GDNF) and neurotrophin 3 (NT3), improve central nervous system recovery in animal models of neurodegenerative disease[111–113]. Similar to the neuroprotective effects of MSC-CCM after pudendal nerve injury in a model of SUI[3], the recovery of erectile function after stem cell injection might be in part caused by the neuroregenerative effects of the MSC secretome.

In the past 10 years, several phase I–II trials investigating the use of stem cell therapy for organic[114], diabetic[115] and post-prostatectomy[116,117] ED have been published, with varying results. However, cell-based therapy raises concerns about malignant transformation, which is particularly important in the setting of post-prostatectomy ED, during which prostate cancer recurrence is a possibility. Future human studies should investigate the utility of the MSC secretome in recovery of erectile function.

Characterizing the secretome

The emerging paradigm that stem cells function, in part, through secretion of bioactive molecules has led the stem cell research community to characterize the MSC secretome using a variety of proteomic methods and approaches to examine EVs. However, considerable work remains to fully characterize the MSC secretome.

Proteomics. Proteomics methods can include targeted detection using antibodies or shotgun-based, antibody-free methods (FIG. 3). Detection using antibodies, such as the enzyme-linked immunosorbent assay (ELISA), is sensitive, reliable and reproducible; however, its main limitation is the need for preselection of the antibodies[118]. Thus, investigators must know what they are looking for. For example, using an antibody array of 120 common cytokines and chemokines, Park and colleagues[119] assessed the secretion profile of human bone marrow-derived MSCs. They demonstrated that IL-6, metalloproteinase inhibitor 2 (TIMP2), MCP1, VEGF and osteoprotegerin (OPG) were constitutively secreted by the MSCs in culture, independent of donor characteristics. In another study, western blot analysis, another antibody-based detection method, revealed that MSC-CCM contained high levels of the angiogenic cytokines VEGF and angiopoietin 1 (REF.[120]). This approach is also limited by the availability and ability of antibodies to detect certain proteins. Moreover, given the need for antibody preselection, targeted protein detection fails to detect novel bioactive molecules that have not yet been defined.

Untargeted proteomics techniques have enabled investigators to study the secretome more broadly than targeted methods (FIG. 3). A commonly used approach is liquid chromatography with tandem mass spectrometry (LC–MS/MS)[118,121,122]. This analytical tool is a powerful one, in which a sample can be separated into components that can be subsequently analysed in detail using the detection of charged ions[123]. Researchers have used this technique to discover novel proteins in the MSC secretome. Indeed, investigators have used LC–MS/MS to detect pigment epithelium-derived factor (PEDF), a major chemoattractant of fibroblasts, and protein CYR61, a pro-angiogenic cytokine, in MSC-CCM[124,125]. Untargeted proteomics approaches enable the identification of novel bioactive molecules that are important for stem cell therapy, but these methods are limited in their ability to detect small quantities of secreted cytokines. Accordingly, Sze et al.[126] used a combination of LC–MS/MS and antibody arrays to identify 201 unique proteins in a line of human ESC-derived MSCs, a unique subtype of MSCs derived from embryonic tissue with greater proliferative ability and differentiation potential than adult MSCs. The investigators used computational analysis to confirm that these gene products were involved in important biological pathways such as metabolism, immune response and differentiation. Their results demonstrate that a combined approach using targeted (antibody arrays) and untargeted (LC-MS/MS) methods can improve the robustness of MSC secretome profiling; however, more research is required to compile the vast array of biological factors secreted by MSCs in vivo.

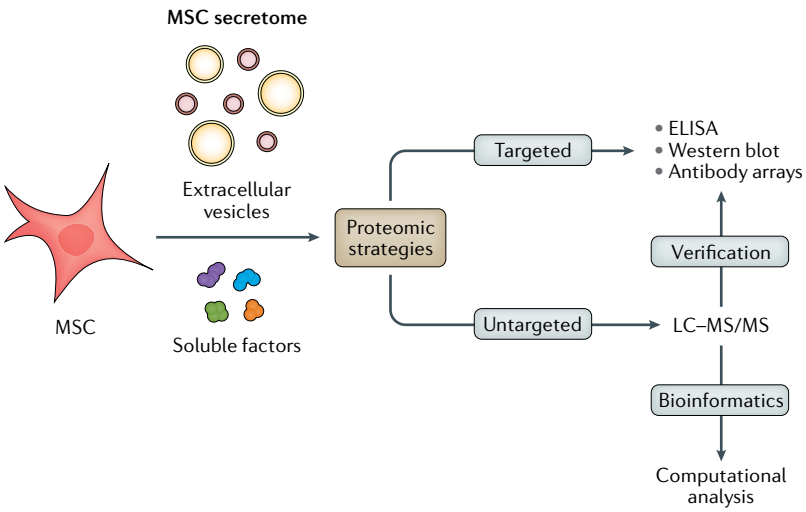

Fig. 3 | **Characterizing the MSC secretome.** To better understand how the individual components of the mesenchymal stem cell (MSC) secretome contribute to its therapeutic effects, researchers have used targeted and untargeted proteomic approaches to characterize the secretome. Targeted approaches, such as enzyme-linked immunosorbent assay (ELISA), antibody arrays or western blotting, rely on the preselection of known bioactive molecules[119,120]. This approach is highly sensitive and reliable but tends to be confirmatory rather than exploratory[118]. Untargeted, or shotgun-based approaches, such as liquid chromatography with tandem mass spectrometry (LC–MS/MS), enable researchers to uncover novel bioactive molecules in the MSC secretome[124,125]. Once identified, they can be verified using targeted approaches and their molecular pathways can be modelled using bioinformatics[126]. In addition, MSC-derived extracellular vesicles, including exosomes and microvesicles, can be isolated, lysed and subjected to these same proteomic techniques in order to investigate their packaged contents[151].

Extracellular vesicles. In the past decade, the therapeutic benefit of MSCs has been proposed to stem not only from individual cytokines working in conjunction but also from cytokines, lipids and nucleic acids packaged in groups of EVs. Exosomes, a class of EV, are membrane-bound nanovesicles ranging in size from 30 nm to 100 nm that are released by MSCs and other cells through exocytosis[127]. Once thought to be the recycling centre for cellular debris, exosomes have now been shown to contain a variety of molecules — including proteins, lipids and even genetic material such as microRNAs (miRNAs) — that are responsible for intracellular signalling[128]. The contents of exosomes reflect those of the parent cell and can be transported to distant targets via ligand–receptor interactions[129]. Microvesicles are a large class of EV (between 100 nm and 1,000 nm in size) with similar contents and functions as exosomes[130,131];

these two terms are often used interchangeably in the literature[132,133]. Both classes of EV are released both constitutively and in response to stimuli by MSCs and other types of stem cells and might influence the behaviour of the target cell by transference of cell surface receptors[134], delivery of proteins[130] or horizontal transmission of mRNAs or miRNAs[135].

A growing body of evidence demonstrates that EVs are partially responsible for the beneficial effects of stem cells in a number of different pathologies[127,136]. A group of researchers from the Netherlands first demonstrated that MSC-CCM reduced infarct size and improved ventricular function in a porcine model of myocardial infarction[137]. Size fractionation of the MSC-CCM demonstrated that the cardioprotection was provided by only the fraction of the secretome containing products >1,000 kDa in molecular mass and between 100 nm and 220 nm in size[137]. Hypothesizing that this fraction consisted of exosomes, the investigators went on to purify the fraction even further using high-performance liquid chromatography (HPLC). They found that a population of phospholipid-bound structures, which had a radius of 55–65 nm and stained positive for the exosome-associated proteins CD81, CD9 and ALG-2-interacting protein X (ALIX; also known as PDCD6-interacting protein), was cardioprotective in their porcine myocardial infarction model[127].

MSC-derived EVs (MSC-EVs) have also been investigated in the field of regenerative urology. In a glycerol-induced model of AKI in mice, MSC-derived microvesicles incorporated into tubular cells reduced apoptosis and protected against acute renal dysfunction[21]. Interestingly, this effect was abolished when the microvesicles were exposed to RNase, suggesting that tubular cell regeneration might be dependent on RNA transfer by EVs. The same group tested MSC-derived microvesicle treatment in a rat model of renal ischaemia, which protected rats from developing AKI and prevented chronic renal dysfunction[22]. Moreover, Zhou and colleagues[23] treated rats with cisplatin-induced AKI with intraparenchymal injections of MSC-derived exosomes and reported that exosome-treated rats demonstrated improved renal function and cell morphology after 5 days. A similar observation was reported by Reis et al.[133] in a gentamicin-induced AKI rat model treated with intravenous injections of MSC-derived exosomes. Additionally, MSC-EVs have been shown to decrease the growth of bladder tumours[24] and promote erectile function in diabetic rats[138].

To date, one human clinical trial investigating the use of MSC-EVs in CKD has been published[139]. Forty patients with CKD were randomized to receive injections of either umbilical cord MSC-EVs or saline control. One year after treatment, there were no adverse events associated with MSC-EV therapy, and patients treated with MSC-EVs exhibited (nonsignificant) improvements in GFR, creatinine levels, blood urea nitrogen levels and urinary albumin:creatinine ratio compared with baseline. The study of MSC-EVs in regenerative urology is still in its infancy, but their great therapeutic potential should motivate researchers to explore cell-free stem cell therapies.

Conclusions

Regenerative medicine offers hope to patients with urological diseases that lack effective treatments. In urology, MSC-based therapy has demonstrated efficacy for the treatment of SUI in several clinical trials[32–34,140]. In preclinical studies, cell therapy has shown therapeutic benefit in animal models of acute[98,99] and chronic renal disease[4,5], bladder dysfunction[19,105] and ED[109,110]. However, the potential of MSC therapy for antifibrosis in urethral strictures, pro-angiogenesis in hypospadias repair or anti-apoptosis in many urological malignancies has yet to be demonstrated. Most investigators now believe that the therapeutic effects of MSCs stem from bioactive factors present in their secretome, given that cell-free treatments have also demonstrated benefit[2–5,99,110]. These factors might be individual cytokines but are more likely to be EVs that function as messengers for the parent stem cell. In fact, a movement in the stem cell research community advocates changing the name of MSCs to 'medicinal signalling cells' to more accurately reflect this theory[141].

Many questions regarding the mechanism of action of the MSC secretome require further investigation. For example, given the short half-life of cytokines, the mechanism by which the therapeutic effects of the MSC secretome persist for days or even weeks after treatment remains unclear. This finding, replicated in several studies[3,5,18,19,84,99,109], implies that secreted factors activate innate regenerative pathways in the host tissue that achieve a durable effect. Moreover, the MSC secretome seems to protect against different mechanisms of injury (for example, ischaemic versus cytotoxic kidney injury), suggesting that the local microenvironment somehow influences the regenerative pathway. Finally, long-term data regarding the effect of MSC secretome administration are still required to translate this therapy into effective clinical treatment.

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D.Z.S. researched data for the article. D.Z.S. and M.S.D. made substantial contributions to discussion of the article contents. D.Z.S., B.A. and P.B. wrote the manuscript. D.Z.S. and M.S.D. reviewed and/or edited the manuscript before submission.

Competing interests
M.S.D. declares an option to license her patent on the mesenchymal stem cell secretome in genitourinary disorders. The other authors declare no competing interests.

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