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Stem Cells in Urology

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Chapter 1
Introduction

Seyed Mohammad Kazem Aghamir

Abstract Mesenchymal stromal cells (MSCs) are the type of cells with self-renewal and multi-lineage differentiation potentical and are placed especially in bone marrow. The ability of MSCs to differentiate into mesoderm, ectodermal, and endodermal cells in vitro, make them as a choice for tissue engineering and several disease treatment including urology disorders. On the occasion of renal tubular injury, MSCs not only prevent fibrosis of renal tissue and prohibit apoptosis of renal cells, but have a virtual role in regeneration of renal tubular cells indirectly. Several role for MSCs in the erectile dysfunction, bladder dysfunction, kidney injuries and regenerative medicine in urology are considered. In this book we represent the exact place of stem cells in urology disorders

Keywords Mesenchymal stromal cells (MSCs) · Self-renewal · Urology disorders

Mesenchymal stromal cells (MSCs) with capacity of self-renewal and multi-lineage differentiation can be detected especially in bone marrow and also, it can be harvested through various tissues throughout the adult body including bone marrow, umbilical cord blood, adipose tissue, and peripheral blood [1]. The ability of MSCs to differentiate into mesoderm, ectodermal, and endodermal cells in vitro, make them as a choice for tissue engineering [2]. After finding MSCs in inflammation states [3], tissue injury [4], and tumors [5], the role postulated for MSCs in these conditions. Additionally, when renal tubular injury occurs, MSCs not only prevent fibrosis of renal tissue and prohibit apoptosis of renal cells, but have a virtual role in regeneration of renal tubular cells indirectly as well [6].

Cancer stem cells (CSCs) are known as subdivided of tumor cells that have ability to renewal themselves and differentiate heterogeneity [7] and moreover, are responsible for creation and development of different type of tumors [8]. Among various cells of a tumor, the merely cells that have potential to originate tumors are

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CSCs owing to limitless replications and capable of self-renewal [9]. The recent studies elucidate that CSCs can be used as pharmaceutical targets to enhance the chance of treatment and quality of life of patients with cancers [10]. The implication of CSCs in urological cancers as well as numerous other sites of cancers has been proved especially, prostate cancer; however, research has not reach any specific CSCs for bladder cancer and renal cancer yet [11]. Hence, larger studies are warranted to understanding CSC of bladder and renal cancer in order to advance new treatments.

Organoids, which are multi cell structures that can virtually mirror the entire aspects of an organ in vitro, has become one of the hottest topics in current tissue engineering research [12]. The spectrum of usage of organoids are limitless and there have been lots of attempts made to apply organoids in urogenital diseases particularly, urological cancers that require radical surgeries due to more suitable function and shape in comparison to usual treatments [13, 14]. Organoids mainly can be utilized in urological- congenital malformations [15], bladder disorders [16], urethral stricture [17], kidney failure [18], ureteral stricture [19]. Albeit there have been evolutions regarding biomaterials applied for producing organoids and new approaches are going to substitute old approaches, each approach and biomaterial include both benefits and complications. With respect to organoids in urology, the majority of studies and research is belonged to animal studies and there is scarcity of clinical studies.

Regenerative medicine is a newfound field that dedicated to reconstruction and repair of tissues and organs [20]. Any situation that causes tissues or organs damage, for example, congenital disorders, tumor, and trauma may necessitate considering regenerative medicine [21]. The most important difference between regenerative medicine and traditional treatments is regenerative medicine's focus is on maintaining the function of organ or tissue rather than controlling the process of illness [22]. Tissue engineering for keeping the normal function of organ or tissue employs three principles: (1) cell transplantation (2) material science (3) biomaterial engineering [23]. Also, there are different options for source of creation of tissue or organ such as autologous cells, stem cells, and therapeutic cloning [24]. Satisfactory outcomes prompted investigations on regenerative medicine in urology. However, there are limitations in this regard that must be aware of including restriction in growing genitourinary-associated cells in large number which nowadays, this difficulty resolves partially with new protocols [25, 26].

Erectile dysfunction (ED) is a prevailing problem that badly affect the quality of life of the men and their partners [27]. The prevalence of it is increasing notably that can attribute to remarkable rise in diabetes mellitus [28] which is one of the leading cause of ED [29]. Those with ED mostly response to oral type-5 phosphodiesterase inhibitor which is commonly the first line of treatment for ED. If not, the alternative options should be taken into consideration for patients. One of the options that has showed promising results is stem cell therapy [30] which most studies are restricted to animal studies and clinical studies are limited [31]. Following JY Bahk's study, seven patients with diabetes mellitus who had suffer from ED in the last 6 months, were treated by stem cells with the source of human umbilical cord blood which improve all

blood glucose, libido, and ED [32]. In spite of satisfactory outcomes regarding stem cell usage in patients with ED, need further human studies to bring it into practice.

A variety of problems such as overactive bladder, urgency, or urinary incontinence may account for Bladder dysfunction (BD), which involves huge quantity of people universally [33]. For viable bladder function appropriate coordination between sympathetic, parasympathetic, and somatic system is necessitates and any interruption in each system can contribute to BD [34]. Returning function of bladder is essential for maintaining the kidney functional and also, increase in quality of life of patients [35].With respect to applying stem cells in BD, MSCs were the first stem cells that had pertained for solving BD and sophisticated results were obtained [36]. Thereafter, neural stem cells, umbilical blood derived cells, induced pluripotent cells, Schwan cells, and olfactory ensheathing cells were used [37–41].

Kidney impairment, which is described as health-threaten condition that last at least for 3 months [42], includes both acute kidney injury and chronic kidney injury [43]. When glomerular filtration rate decreases to 60 and less, it defines Chronic kidney injury (CKD) which is an ongoing and irreversible process [43]. CKD is accompanied with dangerous complications such as cardiovascular diseases, hyperlipidemia, anemia, and bone diseases [44]. While CKD manifests asymptomatic in the early stages, even in early stages it can be diagnosed only with some simple laboratory test. Thereby, the process of emerging of CKD and its complications can be procrastinated [45]. CKD is one of the casual causes of end stage renal diseases and in this stage replacement of kidney, which has three choices including hemodialysis, peritoneal dialysis, and kidney transplantation (KT), is obviously plausible. Supportive evidences stated KT produce lower mortality rate in comparison two other options and considered it as a best choice for patients with end stage renal diseases [46].

Induction therapy has great advantage in the normal process of organ transplantation by prohibiting initial immune response [47, 48]. In patients with KT, induction with antithymocyte globulin despite encouraging outcomes that has been revealed, may result in several serious complications [49, 50]. Currently, whereas there is an increasing tendency to use MSCs as an original method for graft rejection treatment as a result of decrease in the odds of rejection and the dosage of immunosuppressive drugs [51, 52], treatment-related complications such as tumor formation obstacle us to bring MSCs into clinical practice [53].

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Chapter 2
Overview of Mesenchymal Stem Cells

Fateme Guitynavard and Seyed Mohammad Kazem Aghamir

Abstract Over the past two decades, clinical use of stem cells (SCs) for treatment of various medical conditions has become an interesting issue and attracts many investigators' attention. To date, the understanding of tissue regeneration processes mediated by SCs has become significantly developed. So far, many studies have been conducted to achieve a better concept of SCs physiology and their several interesting characteristics as well as their immune function ant their interaction with the immune system. Beside the growing studies for developing basic sciences about SCs, there is a marked increase in the clinical use of SCs.

In this chapter, the SCs characteristics and their clinical use are briefly discussed.

2.1 Background

Mesenchymal stem cells (MSCs) are multipotent, thin, fibroblast-like stem cells derived from mesoderms found in almost every tissues and organs, including bone marrow, fat tissue, periosteum and synovium, placenta, amniotic fluid, umbilical cord, and fetal tissue. MSCs have capability for self-renewal and differentiation into many different cell lines. These MSCs also have immunomodulatory functions, and they can repair damaged or inflamed tissues. MSCs can be found in many tissues in large quantities and can be cultured in vitro. It is so interesting that under certain conditions they can differentiate into different cell lines and form various tissues such as fat, cartilage, bone, muscle, nerves [1–3].

In view of give a comprehensive definition of MSCs, the International Cellular Therapy Society identified MSCs in 2006 with the following three minimum criteria: [1] under standard culture conditions, MSCs should be plastic adherent; [2] MSCs should express proper cell markers such as CD73, CD90 and CD105 without the expression of CD45, CD34, CD14, CD11b, CD79α, CD19 and human

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leukocyte antigens (HLA)-DR; [3] In vitro, MSCs should maintain their ability of trilineage differentiation into adipocytes, osteoblasts and chondrocytes [1].

However, it may now be unnecessary to have a global description of MSCs. Descriptions of different MSCs' subsets may be appropriate and sufficient [4].

2.2 Stem Cell Characteristics

2.2.1 Differentiation

MSCs have the capacity for multi-linear differentiation. Many studies have shown that in vitro, they are able differentiate into mesodermal (fat, cartilage, bone, etc.), ectodermal (neuron, epithelium, etc.) and endodermal (liver, muscle, etc.) cells [2, 3]. Thus, MSCs are so favorable for tissue engineering.

MSCs also have ability to migrate to inflamed or injured tissues and tumoral tissues after systemic infusion. This is called 'homing capacity 'and can be used for targeted treatment of diseases or tumors when MSCs used as a vehicle of a specific drug or gene [5]. In addition, MSCs inhibit fibrosis and apoptosis in the damaged tissues by their immunomodulatory ability and controlling the inflammatory responses through paracrine and endocrine secretion of various cytokines, and encourage angiogenesis to stimulate damaged tissue regeneration rather than direct differentiation into tissues specific cells [6].

2.2.2 Anti-Inflammation Ability

By flow cytometry, it has been shown that MSCs express major class I histocompatibility complex (MHC) molecules, but do not express MHC category II and co-stimulatory molecules, like B7-1 (CD80) and B7-2 (CD86) as well as CD40 [7], resulting in lack of these cells' immunogenicity the fact that makes these cells so qualified for transplantation.

In fact, a theoretical basis for their use in extensive transplantation is this unique characteristic.

By secreting bioactive molecules such as chemokines and prostaglandins and also by between cells contacts, MSCs exert their immunosuppressive effects which are summarized in Fig. 2.1.

Inhibiting the macrophage activation, suppressing monocyte differentiation and interfering with dendritic cells' growth, differentiation and maturation, are other MSCs' immunologic effect. In addition, MSCs can minimize natural killer cells' IFN- γ secretion, inhibit their proliferation, alter their cytokine secretion, and ultimately impair their cell killing function [8]. MSCs secret Indoleamine 2,3-dioxygenase (IDO), an enzyme that can suppress proliferation processes of T-cells. The enzyme degrades tryptophan which is an essential amino acid for initiation T-cell cycle, resulting an arrest in the G0/G1 stage of the cell cycle.

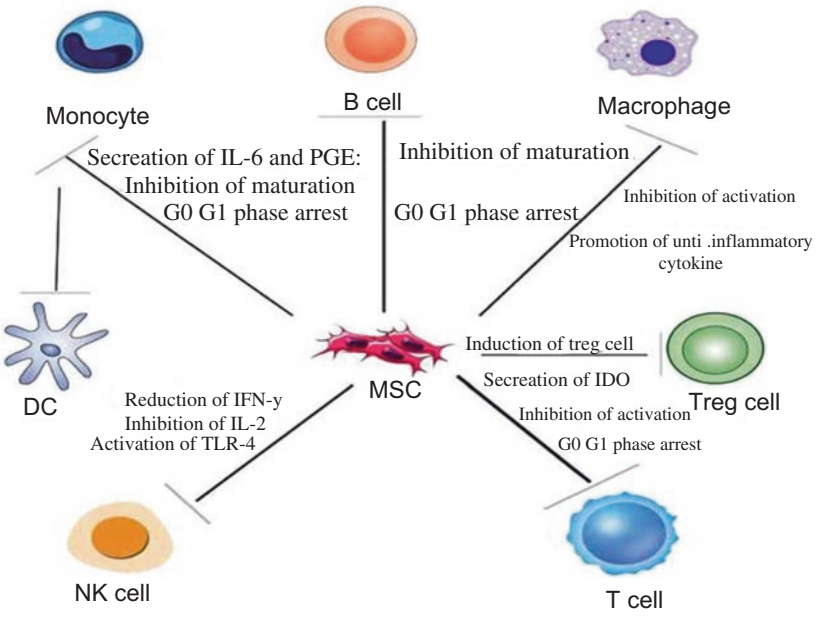

Fig. 2.1 Proposed Immunomodulatory Mechanisms of mesenchymal stem cells (MSCs). IDO indoleamine 2, 3-dioxygenase, IFN interferon, IL interleukin, PGE2 prostaglandin E2, TLR Toll-like receptor

MSCs increase the regulatory cell function of $CD4^+$ $CD25^+FoxP3^+$ cells and may cause an arrest in phase G0/G1of B lymphocytes cell cycle [9]. Via T-cells, MSCs can inhibit B-cell maturation and proliferation, impair their migration, and alter their antibody production [10].

The inflammation is essential for MSC to develop their immunosuppressive functions [11] and the inflammatory status induces the role of MSCs in immunoregulation. High inflammation allows the immune response to be suppressed by MSCs, whereas poor inflammation contributes to increased MSCs immune reaction. MSC1 and MSC2 are, MSC's phenotypes one with proinflammatory functions and the other with anti-inflammatory properties respectively [12]. TLR activation can have an effect on MSC's inflammatory functions [13]. When the proinflammatory cytokines are absent, TLR4 activation results in MSCs differentiation into MSC1 phenotype. In comparison, differentiation into MSC2 phenotype is induced by delivering anti-inflammatory signals via TLR3 to MSCs [12–14].

It has been demonstrated that cultured MSCs induce a shift in the macrophage phenotype from inflammatory (M1) to reparative (M2), by their anti-inflammatory properties [15]. MSCs also inhibit proliferation of lymphocytes by interleukin (IL)-10 and F secretion as ligand [16].

Finally, MSCs suppress co-stimulatory molecules including CD40, CD80, and CD86, evoking an allogeneic immune response of T-cells [17].

2.2.3 Homing

Stem cell's ability to migrate preferentially to inflammatory sites is believed to play a crucial role in the success of organ injury cell therapy [18]. Intravenous or intra-arterial MSC infusion often initially results in the entrapment of administered cells in different organs capillary beds, particularly in lung and liver [19]. In uninjured states, intravenous MSCs tend to migrate to the bone marrow [20, 21]. After the injury, however, MSCs preferably reside at the inflammation site where they migrate across the inflamed endothelium and enter the injured tissue bed [22–27] It has been shown that MSC migration is guided by various interactions between chemokines released from the damaged tissue and chemokine receptors expressed by MSCs. For example, stromal cell-derived factor-1/CXCR4 pathway, which is upregulated under ischemic or hypoxic conditions, can mediate the position of injected MSCs. When the extra-cellular matrix is exposed, another major pathway is the interaction between CD44 expressed in the damaged tissue by MSC and hyaluronic acid [28, 29].

In summary, homing is MSCs' another unique ability which is controlled by inflammation states via the expression of different chemokines and ligands in both injured/inflamed tissue and MSCs.

2.3 Tissue Regeneration

Over the past two decades, understanding of the concept of the processes underlying MSC tissue regeneration has grown considerably. The definition of the mesenchymal "stem" cell and propose them for cell "replacement" therapy may have delayed development to some degree. For example, it was difficult to reject the concept of trans-differentiation of hematopoietic progenitors into cardiac cells, despite rigorous studies that did not support the theory [30].

Nonetheless, the possibility that cell re-programming and acquisition of certain characteristics of the desired lineage maybe play a key role in the tissue regeneration mediated by MSCs, remains to be investigated [4].

2.4 Clinical Application

Although, the basis for the clinical use of MSCs has lagged behind laboratory findings, particularly in regenerative medicine, to maximize their scientific rigor, it is important to optimize the MSCs' studies design based on the most recent preclinical results.

Another issue is the trouble of systematically reviewing conducted clinical trials that their findings in international peer-reviewed publications have not been yet officially published.

As an example of their clinical application it is interesting that MSCs have been used assuming their ability to support kidney transplantation. In a prospective study, 159 patients who had received a live donor kidney transplant were randomized whether to receive induction therapy using (IL-2 receptor antibody) or autologous BM-MSCs to determine the risk of rejection [31]. While patient survival and graft survival were not different among the groups, patients receiving MSCs had a lower incidence of acute rejection, a lower risk of opportunistic infections, and ultimately, they had more improved kidney function.

Another example of MSCs clinical use is the preclinical data that suggest MSCs may have a role in controlling acute myocardial infarction [32].

Also, another interesting issue is the relationship between MSCs and cancer [33].

Future human studies are needed to be conducted that also include in vivo patient monitoring and resolve some of the inherent limitations of xenogeneic animal models.

2.5 Conclusions

In summary, the MSC studies in the past two decades have been surprisingly successful. It is important to conduct many studies to further hasten the process of improving our knowledge about MSC biophysiology and expanding available well-designed clinical trials. Due to the various kinds of tissues as the sources for stem cells harvest and the identification of sub-populations with particular characteristics, definitions of this cell population should be revisited. It is also a priority for researchers in this area to uniformly use the definitions supposed by International Cellular Therapy Society. What makes MSCs so interesting for using in cell therapy is their ability of multi-linear differentiation, immunomodulatory functions as well as migration and homing. To achieve a better identification and investigate the biophysiology of the MSCs, it is necessary to define more animal models. Close cooperation between laboratory and clinical researchers is so crucial for designing successful clinical trials.

Future studies should focus on in vivo patient monitoring to resolve some of the inherent limitations of animal models.

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Chapter 3
Cancer Stem Cells

Fatemeh Khatami, Maryam Aghaii, and Fatemeh Dadkhah Tehrani

Abstract Cancer Stem Cells (CSCs) are a minor group of cells in tumors that have self-renewal, differentiation, and tumorigenicity properties. Some known markers such as CD44, CD24, and CD133 on the cell membrane are often used to identify and enrich CSCs in several tumors, including prostate tumors, testis tumors, kidney cancers, and bladder tumors. Actually, CSCs can pass through vessels and shed into the blood and circulate freely to form circulating tumor cells (CTCs). CTCs are suggested as the real-time representers of the tumor. They are the main component of liquid biopsy together with cell free DNA (cfDNA) and exosomes, which are important diagnostic and prognostic markers in uro-oncology. Currently, numerous studies are about the isolation of CSCs from cancers of genito-urinary tracts, especially in prostate, urothelial and kidney cancer origin. Tumor's progress reveals new ways of finding more effective treatment strategies. In this book chapter, we summarize the current understanding of CSCs and explain the current achievements in cancer stem cell research in urological malignancies.

Keywords Cancer Stem Cells (CSCs) · Circulating Tumor Cells (CTCs) · cell free DNA (cfDNA)

3.1 Introduction

Tissues like the intestinal epithelium and the hematopoietic system can continuously be renewed by tissue-specific stem cells [1]. Stem cells are long-lived cells that generally make progenitor cells to restore the several specific, short-lived, and differentiated cells that eventually complete tissue-specific functions. A stem cell is an undifferentiated cell with a high potential to proliferate or differentiate to other

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specific types of cells. Cancer stem cells (CSCs) are a cluster of tumor cells within tumors that have the same features of a typical stem cell, particularly the power of the self-renewal and conversion to differentiated cells. In a tumor, CSCs are tumor-forming cells, because contrary to the non-tumorigenic cancer cells, they are potentially able to make cancer cells [2, 3]. CSCs have a vital role in urological malignancies. CSCs are considered as individual cells mainly responsible for relapse and metastasis of the tumor. Thus, the success of cancer therapies by targeting CSCs can increase the survival and quality of life of cancer patients, mainly for patients with metastatic diseases [3, 4].

In oncology, the point that one round of chemotherapy does not kill all tumor cells is known as the fractional kill, or fractional cell kill [5–7]. That means that chemotherapy, which has been used for a limited period, can kill a fraction of the cells, free from the absolute number of cells. Consequently, repeated doses of chemotherapy need to be administered to shrink the tumor size continuously. Currently, cyclic drug treatments are applied during chemotherapy regimens, with the regularity and treatment period restricted by its toxicity [8]. The fractional kill regularly estimates the effectiveness of cancer treatments in the initial phase of cancer. As CSCs form a small portion of the tumor cells, they can escape from the drugs that act precisely on the stem cells. So, conventional chemotherapies, unfortunately, cannot kill CSCs with self-renewal ability that remains untouched and can cause relapse.

For the first time, the theory of CSCs was suggested by Furth and Kahn in their landmark article mentioned that leukemia cells, when vaccinated into inbred mice over the sequence of tests, presented effective transplantation of about 5% of leukemia cells in 1937 [9]. Then John Dick indicated to the CSCs in acute myeloid leukemia in the late 1990s. Dick evidenced the central role of CSCs and he points to the CSC hypothesis over their seminal research on hematopoietic cells characterization and the acute myeloid leukemia (AML)-initiating cell with the capacity of leukemia initiation in non-obese diabetic mice with severe combined immunodeficiency disease (NOD/SCID). In addition, they verified that the self-renewal potential of these cells holds ultimately $CD34^{+}CD38^{-}$ cell markers and can distinguish leukemic blasts [10]. Over the last two decades, the cancer research has mainly concentrated over considerate the CSCs' characteristics and mechanism of their formation, due to their aptitude to start tumor growth, self-renewal characteristics, and medication resistance.

The presence of CSC was shown in numerous tumors like urological cancers, as well. In prostate cancer (PC), the CSCs were effectively separated and characterized. So, it was possible to develop new therapeutic strategies that may revolutionize PC treatment. However, the origin of bladder cancer and renal cancer from CSC is under the debate due to the hard isolation of CSCs from a tumor. Extensively assumed participation of CSCs in urological malignancies origination and progression offers the new insight into the tumor biology, medical course and carries excessive chance for future less empirical management. The main feature of CSCs is their well-evaluated resistance to radiation and chemotherapeutic agents. The statement that tumors have a population of continually growing stem cells capable of surveying systemic treatment and then starting tumor regrowth can be the main idea behind the designing of new treatment attitudes and starting different aspects of cancer management.

3.2 Cancer Stem Cells Specific Properties

CSCs are a group of cancer cells which has specific cell surface markers and the potential to auto-regenerate, proliferate, and differentiate into multiple cancer cell lines over symmetric and asymmetric cell division [11–13]. Despite numerous studies of CSCs, some limited basic properties of CSCs are highlighted. For instance, at least a minor number of CSCs should be present at the site to start a new tumor, and their self-renewal capacity outperform their differentiation, their identification and isolation is possible by identifying their exact and individual surface markers, they play the leading role in metastasis, they are the origin of CTCs, they have the capacity to be transplanted generation to generation, and they are resistant to chemotherapy and radiotherapy [14, 15]. So, it is critical to abolish CSCs in order to stop cancer and avoid future relapse. Recent studies are mainly focused on the identification and precisely targeting CSCs.

CSCs have been the subject of debate for more than a century, after which scientists believe that cells with the ability to regenerate themselves produce cancer [16]. There is, of course, substantial evidence from the "cancer stem cell hypothesis" as well. Self-renewal, a key feature of stem cells, relates to their ability to divide continuously. During proliferation, other cells are transformed into two daughter cells that resemble the parental cell, but one stem cell can be transformed into a new stem cell and an ancestral cell. The progenitor cell loses the ability to self-regenerate, but gains the ability to alter or differentiate into the cell types of the tissues maintained by the stem cell. After such division, some stem cells remain unchanged.

Because a stem cell is destroyed and a cell is created, so the stem cell population rebuilds itself as it creates new cells for the tissues. In fact, stem cells were first identified in a particular type of leukemia in year 2 by John Dick et al., at the University of Toronto. They were more challenging to identify in tumors (such as liver and kidney masses, etc.) because biologists had no means of identifying markers or molecular markers located in a stem cell. However, in the year 2000, Dr. Michael Clarke found stem cells in breast tumors. Dr. Clark showed that many of these cells are unable to grow and proliferate in human breast tumors.

The small populations of cells are capable of producing new cancers, and these cells resemble stem cells in their ability to proliferate and produce mature cells. In 2012, Dr. Peter Duke of the University of Toronto diagnosed stem cell-like cells in brain tumors, and Dr. C Parker Gibbs of the University of Florida identified them in bone cancers. Biologists are still not sure how CSCs are created. Stem cells may undergo mutations or changes in their DNA structure, disrupting their control over self-renewal capacity, resulting in more tendency for regeneration rather than differentiation. CSCs also maintain the ability to differentiate into non-regenerating cells that form a significant part of the tumor. CSCs are similar to normal stem cells in terms of self-renewal and metabolic properties. CSCs regulate tumor invasion and metastasis (Fig. 3.1).

Only recently have biologists devised methods to identify stem cells and their presence in tumors. To preserve a tissue or organ, stem cells are likely to regulate their numbers by receiving messages through chemical exchanges when they reach

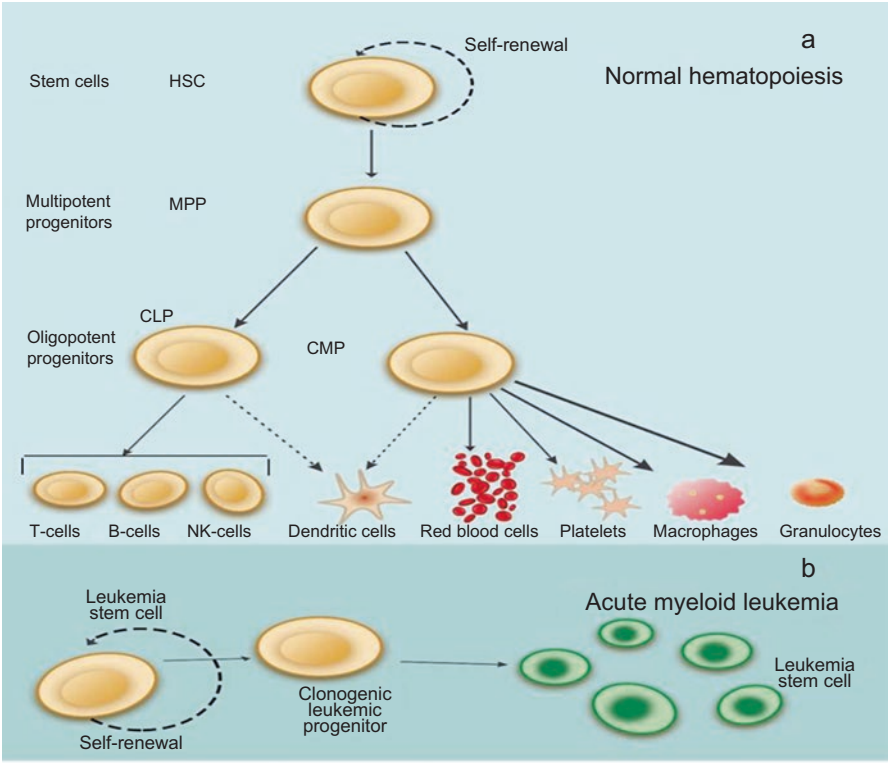

Fig. 3.1 Diagram showing the development of different blood cells from haematopoietic stem cell to mature cells

a quota. Cancer cells are different as they lose control of their population size. Many body tissues are prone to cancer, such as blood, skin, intestinal lining, which are short-lived cells that are constantly shedding and regenerating. The cells become malignant only after a series of mutations that disrupt their genetic control system.

Pathologists have long known that tumors have different types of cells. Including some cells that are specific to the tissue from which cancer originates, but not all of these cells are cancerous. If the cells of one tumor are injected into another part of the patient's body, as in Experiment 1, which is considered immoral nowadays, more than one million cells must be inserted into the new site before a tumor can be formed. This experiment confirms the idea that only a small number of cells in cancer are able to maintain the tumor.

Dr. Vogelstein believed that the appeal of the cancer stem cell hypothesis is that if 1% of the cells that survive after successful chemotherapy are cancer stem cells, there are different pathways for the different forms of the treatment that target these remaining cells," says Dr. Wegelstein." Dr. Gillian, a proponent of the theory, confirmed that 5% of the cells examined in solid tumors such as those of the liver, lung, etc., had stem cell-like properties. However, with better markers, it is clear that far

fewer of them are actual cancer stem cells. “If the growth of solid cancers is derived from cancer stem cells, there will be many problems for the treatment,” says Dr. Irving Weisman. Treatments specifically for stem cells may lead to more extended treatment responses and even cure spread tumors.

The development of diagnostic and therapeutic approaches in basic and clinical cancer research is dependant on a better understanding of how cancer stem cells are formed and identifying the control pathways of these cells. Some researchers believe that at the center of any tumor, there is a small number of abnormal stem cells that continue to grow malignant and abnormal tissue. If this is true, it can explain why tumors often rebuild even after anti-cancer drugs almost destroy them.

The finding also provides a different approach to the discovery of anticancer drugs, suggesting that these drugs should be selected for killing cancer stem cells rather than for their ability to destroy any cells and shrink tumors. “I think this is one of the most exciting advances in cancer research in the last five years and more people are embracing it, and there is a lot of evidence gathering that stem cells are there,” says Robert Weinberg, a cancer geneticist at Cambridge University. “Cancer is a large group of tumors.”

The idea that cancer cells have characteristics similar to stem cells has been around for years (Fig. 3.2). Various molecular markers have been proposed for the isolation and identification of cancer stem cells (Table 2.1), including CD44, CD24, CD133, CD166, ALDH1, Cassette Transporters Binding-ATP (ABCG2, ABCB5), EPCAM, CXCR4 [17]. Interestingly, many of these markers, including CD44, CD24, CD133, and aldehyde dehydrogenase (ALDH) are also expressed in normal adult stem cells [18, 19]. CD44 is a specific receptor for hyaluronic acid, a class I membrane glycoprotein (Fig. 3.3).

There are some suggestions that a group of these cells that enter the bloodstream turn into cells called circulating tumor cells (CTCs), which are the primary mediators of metastasis and include fluid biopsy in addition to cfDNAs and exosomes [20–22]. To have a more in-depth view of CTCs, we need a brief explanation of epithelial to mesenchymal transition (EMT) and metastasis. In the following part, we discuss the relationship of CSCs in urological cancers.

3.3 Testicular Germ Cell Tumors (TGSTs)

The origin of TGCTs can explain the relation between normal stem cells and CSCs. Factually, teratomas have given the primary evidence that tumor cells can differentiate into the tissues made up of three main embryonic layers. Currently, it is clear that pluripotent stem cells resulting in normal stem cells are accountable for this occurrence. The fact that germ stem cells can lastly start the tumor in testis is the most significant evidence of the idea that stem cells can transform into CSCs [23]. The pattern of gene expression, such as OCT3/4, SOX2 and Lin28, can indicate the pluripotency and self-renewal ability of primordial germ cells (PGCs), was shown in TGCTs [24]. Isolated cells from TGCTs display an

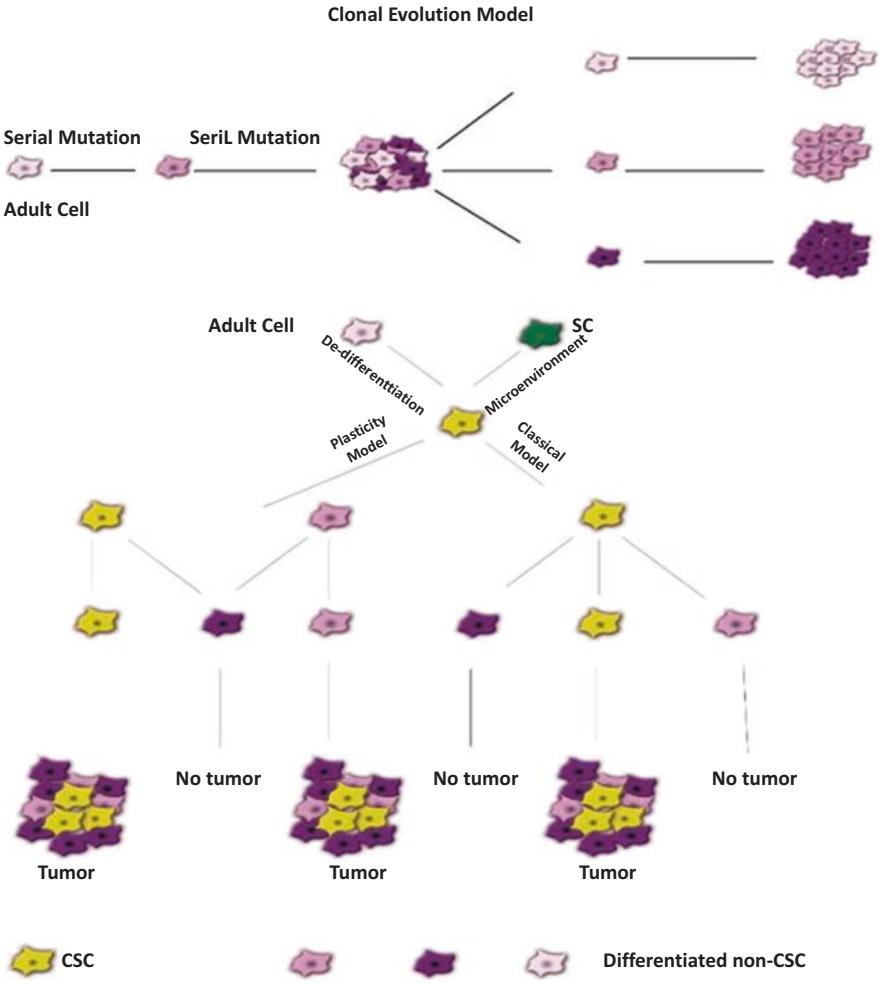

Fig. 3.2 The clonal evolution theory describes a way in which cancer cells with diverse phenotypes could arise within a tumor and distinct cancer cell populations evolve progressively during multistep tumorigenesis due to heritable genetic and epigenetic changes

excessive dependency to embryonic cells from which they arise [25]. Oncogenic transformation occurs during the migration of germ stem cells to genital ridge or through initial steps of gonadal organogenesis. Throughout fetal life, some genetic alterations make the connect for additional stages of oncogenic transformation and includes postnatal environmental factors [26–28]. Consequently, CIS (*Carcinoma in situ*) cells originating from embryonic cells in the first prenatal development steps previously than gonadal tissue mature (Fig. 3.4).

Table 2.1 Various molecular markers have been proposed for the isolation and identification of cancer stem cells

Tumor (references)	CSCs markers
Pancreas [18–20]	CD133, CD44, CD24, CXCR4, c-Met, ALDH1, ABCG2
Breast [22–27]	CD44, ANTXR1, ALDH1, CXCR4, ALDH1
Colorectal [28–30]	CD133, CD44, CD44v6, CXCR4, CD26
Gastric [31]	CD44
Glioblastoma [32, 33]	CD133, MMP-13
Lung [34, 35]	CXCR4, ABCG2, CD133, ALDH1
Osteosarcoma [36, 37]	CD133
Retinoblastoma [38]	ABCG2
Head and neck cancer [39]	c-Met
The ovary [40]	CD133

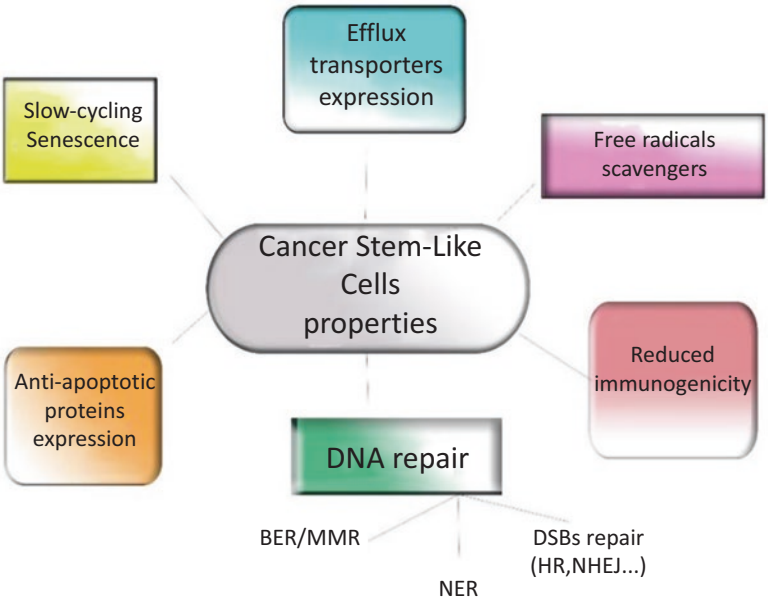

Fig. 3.3 Several Cancer Stem like properties that give the capacity of self-renewal and for causing the heterogeneous lineages of cancer cells that comprise the tumor

3.4 Prostate Cancer Stem Cells (PCSC)

Prostate Cancer Stem Cells (PCSC) indicated to the minor percentage of the tumor. Collins et al. estimated that just 0.1% of the tumor has specific properties of the stem cells [29]. The central policy of CSC identification exploited the theory that normal prostate stem cells have PCSC markers as well. In many studies, CD44,

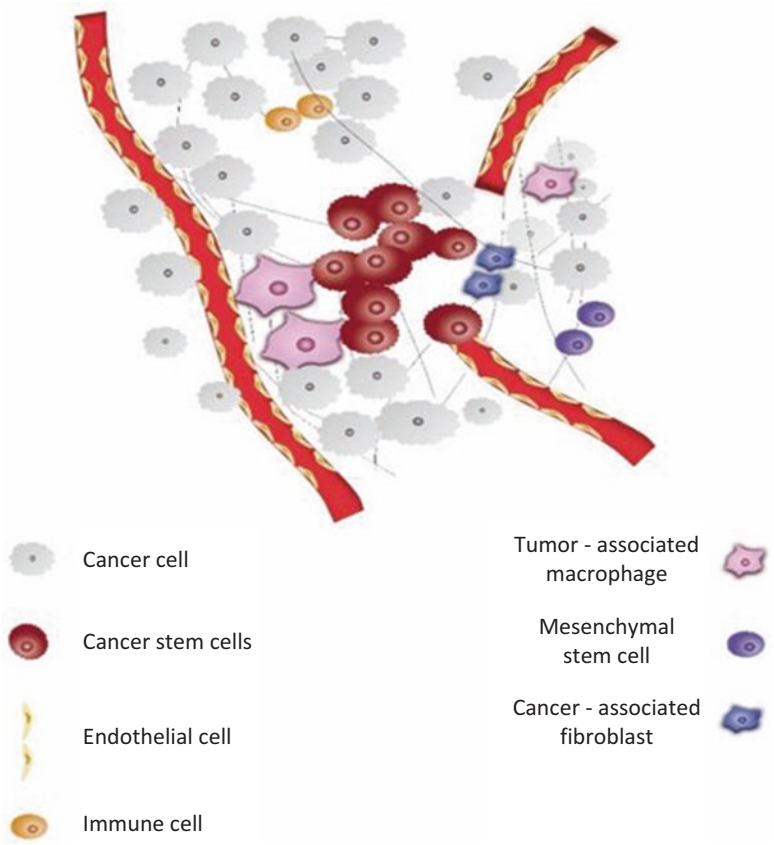

Fig. 3.4 Schematic presentation of cancer stem cells shedding into the blood vessel and converting to circulating tumor cells

CD133, α2β1hi integrin, FAM65B, MFI2, and LEF1 are the main markers that were considered as a way of PCSCs detection [30]. The response of several studies to the query of the cellular origin of PCSCs is unconvincing. Although the present state of the investigation, which they all agree upon, can be: Transient Proliferating/Amplifying (TP/A) intermediate stem cells, prostate stem cells, and basal cells or luminal cells [31]. On the other hand, the same amount of research indicates that PCSC is developed either from the basal layer or the luminal layer of prostatic epithelium.

The CSC model states that CSCs are the driving force of cancer evolution and the resistance to cancer therapies [32]. Prostate cancer grows from high-grade prostatic intraepithelial neoplasia (HGPIN) and develops to invasive carcinoma following by metastatic cancer. Prostate cancer mostly metastasizes to the bone [33]. At first, it was shown that surgical castration and estrogen injection caused significant tumor

regression in 15 of 21 patients with metastatic prostate cancer (mPC) [34, 35]; this finding directed to androgen deprivation therapy (ADT) as the standard treatment in patients with mPC.

Mechanisms of prostate cancer initiation and metastasis are widely under consideration. While some phenomenon remains uncomprehensible, this enormous research effort exposed complex mechanisms to play a role in prostate cancer initiation and metastasis that can be joined to regulating PCSC.

The isolation of androgen-dependent and androgen-independent PCSC provided innovative vision into the mechanisms accountable for androgen resistance advanced prostate cancer [36]. The hormone dependency of PCSC is mysterious, and there are some suggestions that at least a subgroup of prostate cancer stem cells express androgen receptors [37–39]. Vinagolu et al. recognized stem-like human prostate tumor-initiating cells with no androgen receptors and Prostate Specific Antigen (PSA). So it is possible that these cells are resistant to treatment and diagnostic processes and have a role in disease recurrence and exhibit increased NF-κB signaling [40]. Direct targeting of PCDC can bring revolution to prostate cancer therapy due to the ability to remove cells in charge of metastasis and recurrence. Liu et al. have just presented that such attitude is potential and presents new repressing CD44 expression [41]. Vis et al. also show the loss of CD44 on the surface of a potential therapeutical agent like microRNA miR-34a. The miR-34a inhibits PCSC and metastasis by directly of prostate PCSC is self-determining prognostic predictor of clinical recurrence [42].

3.5 Bladder Cancer Stem Cells (BCSC)

Urinary bladder tumor is heterogeneous in genetics and molecular aspects and several genetic alterations were recognized to take part in urothelial cell carcinoma (UCC) development and progression [43]. The origin of CSCs is under debate. It is extensively expected that CSCs is originated from normal stem cells with some genetic mutation and the group of CSCs from usual stem cells is intricated [38, 44–46]. It is shown by Yang et al. that bladder cancer stem cells (BCSCs) initiated from bladder cancer stem cells (BCSCs) or bladder cancer non-stem cells (BCNSCs) by clonal homogeneity among BCSCs and BESCs or BCSCs and BCNSCs. The single-cell sequencing discloses modifications in ARID1A, GPRC5A and MLL2 driving self-renewal of HBCSC [47].

BCSCs have been isolated and defined hardly to some extent because CSCs identification in urinary bladder carcinoma arise have no established process agreeing to isolated CSCs from other cancer cells [48]. In fact, tumor progress is shown by several genetic alterations in charge of self-renewal capacity, migration and metastasis, treatment resistance, and additional malignancy features [49].

Urothelium layer consists of three main cell types: basal cells, intermediate cells, and umbrella cells. While urothelial cells usually form clonal units as a collection of cells

resulting from stem cell confined in the basal layer [50]. All clonal units dynamically replace the urothelium through aging. Castillo-Martin et al. have recently suggested that two different progenitor cells make intermediate cells and "umbrella" cells [50, 51].

Some cytokeratins are identified within the urothelium inner surface of the urinary bladder [50]. However, CD44, pancytokeratin, p63, and cytokeratins CK5, CK10, CK17 are completely over expressed in the basal and intermediate cellular layers [52]. Expression of CK18 and CK20 is restricted to umbrella cells and oncogenic transformation can be documented by checking alterations in biomarkers expression patterns. Malfroe et al. noted that the upregulation of CK20 can be linked to the overexpression of p53 and Ki-67 which can be recognized as the main molecular markers of malignant alterations within urothelial mucosa [53].

The OCT 3/4 overexpression was established in human bladder cancer cells and is directly connected to the higher migratory and invasive properties of bladder cancer cells [53]. The presumed CSCs are supposed to have their niches on the basal layer of the urothelium. Some researches shed light on the putative bladder cancer stem cells based on basal cells surface markers. Nonetheless, it is still undecided whether these cells are CSCs or they are sub-population of cancer cells with higher tumor formation potential as the tumor-initiating cells.

BCSC was firstly identified in 2009 *through* the isolation of the specific markers of the normal stem cells [54]. The biological properties and phenotypes of tumor cell lines can unwantedly alter during long-term *in vitro* culturing and several passages, so the first passage of the cells are the best candidates for BCSCs isolation and identification. Chan et al. established that about 40% of the samples collected from transitional cell carcinoma of the bladder patients contained $CD44^+$ cells. So, the CD44 splice isomer (CD44v6) was a candidate for $CD44v6^+$ epithelial membrane antigen-negative (EMA^-) stem cell subtype isolated from whole bladder tumors [55]. Besides, the $67LR^+CEACAM^-$ BCSCs were identified with two other cell markers; the 67 kDa basal layer laminin receptor (67LR) and carcinoembryonic antigen-related cell adhesion factor 6 (CEACAM6). The 67LR is presented in the junction of tumor stroma in about two/third of high-grade invasive bladder cancer, and CEACAM6 is the non-specific poor reaction antigen [55]. ALDH1A1 is the other BCSC marker that gives the cells better colony formation and tumorigenicity characteristics [56]. Furthermore, the colony formation and tumorigenicity of the BCSCs were considerably decreased by shRNA knockdown of the *ALDH1A1* gene. The $ALDH1A1^+$ cells are subtypes of $CD44^+$ cells and can have more primitive BCSCs [56].

3.6 Renal Cancer Stem Cell (RCSC)

Renal tubular cells are described as the cellular origin of renal cell carcinomas. Several studies have tried to separate and describe a population of CSCs between tubular cells by stem cell markers or functional assays [57–59]. In clear cell

RCC (ccRCC), tumor recurrence and metastasis are the main origins of poor survival, and CSCs are hypothetical as the accountable one for tumor propagation, and metastasis formation like renal cancer [60–62]. Based on the CSC hypothesis, usual treatment like radiation and chemotherapy can eliminate the mainstream of cells in the tumor bulk but sparing the CSC pool [63]. Nevertheless, several studies on these biomarkers show that the CSCs markers are not distinguishing tumor types diagonally; consequently, discriminative factors for CSC types cannot be useful in renal cancer. Some recent studies propose that different CSC subgroups can coexist in the single tumor, and new CSC (sub-) clones can be created, chosen, and fight with each other in the same way with the stochastic model through tumor progression and treatment [64]. So, several biomarkers can be applicable in some steps through tumor development and progression, while they progress into the obsolete in others. The main concerns raised about the stem cell hypothesis and CSCs are measured as an occasional slow cycling subgroup of cells questioned the option of their involvement in treatment resistancy, in support of mechanisms of acquired or intrinsic resistance.

CSC traits are continued by interaction with the tumor microenvironment (TME) niche [65]. The CSC niche is the functionally distinct TME existing within a tumor that maintenances and sustains CSC characteristics [66]. It is made up of the extracellular matrix ECM, cancer-associated fibroblasts, mesenchymal stem cells, endothelial, and immune cells [67]. Stem cell niches are regularly placed in hypoxic areas where low O_2 altitudes impose a slower proliferation rate and reduce DNA damage due to reactive oxygen species reactive (ROS). Inflammation, hypoxia, angiogenesis and EMT happen usually within the TME and have a role in establishing CSC fate through acting critical regulatory pathways of CSCs: Wnt, SHH, Notch, TGFβ, and growth factor- receptor tyrosine kinase (RTK) [68, 69]. Remarkably, tumor cells in the non-CSC parts can instinctively submit to EMT and obtain a CSC-like phenotype and surface marker expression [70].

The exact mechanisms and properties of CSCs can be suggested by novel genomic and functional assays and can improve CSC studies for the better. The addition of therapies that accurately target CSCs through their surface markers can prevent CSC-related signaling pathways. CSC-specific therapeutics, in addition to focusing on the CSC niche with conventional chemotherapy and radiotherapy, can result in RCC patient survival [67, 71].

3.7 The Process of Epithelial to Mesenchymal Transformation (EMT)

The process of epithelial to mesenchymal transformation (EMT) is a complicated process that can lead to loss of epithelial tissue and gaining of mesenchymal traits over opposite differentiation way and then enlarged motility by rearrangement of cellular junctions and finally cell adhesion deletion. During EMT cells are partially or entirely transformed from epithelial phenotype to mesenchymal [72, 73].

EMT occurs naturally in organogenesis and wound healing, but in the case of cancer, it plays a vital role in tumor cell proliferation. This transfer enables the tumor cells to acquire the ability to spread through the body, facilitating escape from the primary tumor site, penetrating through the vessels, and exiting from them.

EMT produces tumor cells that have stem cell characteristics with a phenotype similar to CSCs. Evidence suggests that CSCs are in an intermediate state of EMT with decreased levels of E-cadherin expression and exhibit mesenchymal features, including metastasis-related invasion. These findings illustrate the mechanisms underlying EMT and are highly dependent on their fundamentality. Recent reports from several laboratories have identified new mechanisms of EMT regulation and fundamentality, including epigenetics, microenvironment, and early differentiation.

CTCs have also been shown to exhibit EMT properties, although it is unclear what part of CSCs they possess. The EMT features of both CSCs and CTCs are associated with resistance to current clinical therapies. They indicate that targeting CSC in addition to more differentiated tumor cells is required for long-term responses. Therefore, the EMT properties of CTCs may prove to be useful biomarkers for effective treatments for many cancers. From a molecular perspective, the EMT process is stimulated by several transcription factors such as SNAIL, TWIST, ZEB1, ZEB2, SLUG, BMI-1, etc. [74]. With impaired epithelial adhesion and loss of cellular polarity, carcinoma cells in the tumor have become invasive, allowing them to circulate through the bloodstream [75].

It seems that EMT is not only for the presentation of neoplastic epithelial cells with only mesenchymal and invasive phenotype but may also enhance embryonic features. In fact, cells undergoing EMT process acquire stem cell characteristics, which are detected by overexpression of CD44 marker and low expression of CD24 marker, as well as increased expression of other stem cell markers in differentiated epithelial cells [76, 77].

In this way, EMT may propagate or even produce newly formed cells with tumor and metastatic properties. Mani et al. for the first time, demonstrated that EMT is sufficient to induce a cell population with high migratory stem cell characteristics. However, EMT is often a transient and reversible process and re-establishment of micrometastases in distant sites to the reversible process, called mesenchymal transition to epithelial (MET), requires cells to regain the epithelial properties necessary for colony formation. Therefore, the EMT-MET transfer process is considered to be the driving force of metastasis that can occur in most cancer cells. EMT is a dynamic process that occurs in both cancer stem cells and non-cancer stem cells, and only cancer stem cells are capable of enhancing metastatic cancer stem cells through EMT.

In this context, it is essential to note that by definition, non-cancerous stem cells cannot induce tumors *in vivo*, indicating that the potential for a new generation of cancer stem cells via EMT (or other mechanisms) is minimal [78]. Conditions such as hypoxia or TGFβ, which increase EMT in human breast cancer, also increase the proportion of CSC cells with the CD44 + / CD24- phenotype.

CSCs are able to alternate between EMT and MET modes [79]. As determined by immunohistochemistry, EMT-stage CSCs were primarily observed in the invasive tumor, whereas MET-stage CSCs were more frequent in the central regions of the tumor. The role of EMT in the spread and progression of bloodstream disease in a study of CTCs in human breast cancer is described. This study showed that epithelial and mesenchymal markers were simultaneously expressed in primary tumor cells but only enriched mesenchymal cells were found in patients' CTCs. It has also been reported that mesenchymal CTCs were found both in single cells and in multicellular clusters [80, 81].

In addition, fucosylated is also involved in the metastasis process and is one of the most common glycosylation changes, involving oligosaccharides in glycoproteins or glycolipids. Fucosylated is also one of the most important types of glycosylation in cancer, the importance of which was first reported in 1979 by the tumorigenic liver tumor cells compared to normal hepatocytes. Studies on CSCs have shown that inhibition of focalization affects the ability of cancer stem cells to invade [82–84]. Therefore, fucosylated is a novel mechanism by cancer stem cells to acquire features for invasive and metastatic to produce metastatic cancer stem cells and seems to be an important therapeutic intervention.

3.8 Metastasis in Urothelial Cancer

In some malignant tumors, cancer cells lose contact with tumor tissue and pass to other organs through the bloodstream or the lymphatic system. These cells start to grow in second place and produce a new cancer cell in other areas. The process of metastasis causes the tumor to spread to various tissues and organs of the body. Metastasis occurs in advanced stages of cancers and when a cancer tumor reaches the metastatic stage, treatment of the patient becomes difficult. In some tumor cells, a small number of primary cell properties change and the tumor cell is still more or less like the primary cell from which it originated.

The tumors that result from these cells are called benign tumors. Benign tumors have limited proliferation, and the tumor can be seen only at the same location as the primary cell. These cells express proteins that are not normally expressed in healthy cells. They also prevent the expression of some proteins in these cells. These changes in the expression of cellular proteins cause the cancer cell to lose contact with adjacent cells migrate from its original site to the cell site by decomposing the intercellular matrix, where it also causes tumor formation [45, 85].

Some cancer cells may not have the ability to invade and metastasize, while others can mediate to a large extent and increase the ability to develop a malignancy. Invasion and metastasis are biological features of malignant tumors and are the leading cause of physical and morbidity due to cancer. The act of migration of cancer cells from one site to another is called metastasis, and these types of tumors are called metastatic tumors. Tumors can spread to distant organs in three different ways:

through the circulatory system, the lymphatic system, the wall trunk into the abdominal cavity, and the chest. The metastasis process involves several stages:

– Separation of the tumor cells from each other
– Destruction of the extracellular substrate
– Migration of the tumor cell
– Propagation of the vein and implantation of tumor cells in a new location

Based on recent data from whole-genome sequencing, next-generation sequencing, and transcriptome profiling, bladder cancers have been clustered into basal and luminal molecular subtypes that hold different biological and clinical features [86, 87]. Basal cells have specific biomarkers of CSC and EMT that are considered in BC metastasis. Patients with the basal/CSC BC subtype tend to be more high stage metastasis tumors. Also, basal/CSC human BC orthotopic xenografts in mice can be more metastatic than luminal/epithelial cells [88]. So, CSCs are essential in metastasis of cancers including bladder cancer.

Prostate tumor cells usually migrate to the bone and CSCs possibly will differentiate into tumor cells. It was shown that the CD133 overexpression can be important in keeping the stability of CSCs in the human PC cell line, LnCaP, produced bone metastasis in a mouse model [89]. The cytokine arrays indicated that cytokines have role in bone metastasis. Also, EMT characteristics, like reduced E-cadherin and vimentin over-expression, wound gap distance, and cell migration improved.

3.9 Liquid Biopsy in Urological Malignancies

CTCs, or circulating tumor cells, are the cells that flow from the tumor into the vessels or lymph and enter the circulatory system. CTCs have established the sequential growth of tumors (metastases) in body tissues that are the leading cause of cancer death. In the mid-1990s, the importance of CTCs in cancer research was highlighted by Dallas, Trestapan, and Liberty, and investigations into the presence of circulating tumor cells began in the earliest stages. It is shown that CTCs have a potential role as a prognostic marker for risk stratification in patients with non–muscle-invasive bladder cancer (NMIBC), to predict both recurrence and progression [90].

CTCs, which together with exosomes and cfDNAs are referred to as fluid biopsies, actually show metastasis and provide explicit information about the individual's disease status [91, 92]. As we know, blood sampling is a safe and straightforward method and sampling can be done at different times. Typically, tumor biopsy involves an invasive process that may be associated with patient disagreement. Monitoring the progress of the disease at different time points improves the treatment, symptoms, and quality of life of the patient. Various techniques with high sensitivity and no need for surgery and repeatability to identify CTCs have been introduced in cancer patients, especially metastatic patients.

These circulating molecules are trackable in different body fluids (blood, urine, saliva and seminal plasma). Liquid biopsies is the gifted source for personalized medicine. Molecular profiling of CTCs and cfDNA can represent the multi-marker tests into the clinic noninvasively. Here we discuss the importance of liquid biopsy in kidney, prostate and bladder cancer diagnosis and prognosis. We will also argue the pros and cons of this non-invasive cancer biomarker test [93, 94].

As the name suggests, CTCs are free and circulating cancer cells in the bloodstream. By definition, it can be expected that the number of these cells in the blood may vary depending on the extent of the tumor's spread in the patient's body. CTCs are detectable in the blood both in metastatic and in dormant conditions, so even in dormant conditions, the release of these cells can cause metastasis in the individual. Although one tumor releases millions of CTCs into the bloodstream on average per day, not all of these cells can be considered as threatening and underlying metastases, as few CTCs are able to exit the vasculature and form a secondary tumor. Some information propose that cfDNA is a very truthful for the presence of prostate cancer cells on needle biopsy [95].

Given the above, it can be argued that by exploring the potential of CTCs to detect cancer and improve the lives of those with the disease over the next 10 years, screening these cells will become one of the most common and reliable strategies for cancer detection and metastasis prevention. In recent years, many researchers and companies have been studying the physical and biological properties of CTCs and have devised methods to detect and estimate their numbers in peripheral blood. In fact, CTCs are cancer cells that break away from their original site and circulate in the blood. CTCs are considered part of the long process of cancer metastasis. Molecular evaluation of CTC using liquid biopsy and examination of isolated cancer cells has provided an excellent opportunity to understand cancer biology and the metastasis process.

It is shown that higher *FGFR3* and *PIK3CA* mutated DNA in urine and plasma can be the sign of bladder tumor metastasis. So the bladder tumor DNA mutations can be monitored in urine and plasma for both diagnosis of progression of the tumor [96–98].

3.10 Conclusion

The CSC characterization and cultivation can change the cancer treatment strategies to develop a new one that is precisely targeting responsible cells for disastrous features like tumor recurrence, metastasis and treatment failure in urogenital malignancies. It is considered that kidney, prostate, bladder, and testicular cancers are originated mainly from CSC. Their effective documentation between populations of cancer cells could offer new objects for effective therapies.

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Chapter 4
Organoids

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Abstract Nowadays, quite amount of attentions address to organoids which are well-accepted structures originated usually from stem cells through cascade of events that mimic both structure and function of intact organs. The great vast number of urogenital diseases necessitate considering organoids as treatment choice rather than limited routine treatments owing to more appropriate function and shape. The usage of organoid is not restricted to any specific organ of urogenital system and additionally, it can be used in a wide range of diseases. Studies with regards to organoids in urogenital diseases showed a substantial improvement in this field in both experimental and clinical scenarios. So far, lots of various biomaterials have been used to produce organoids and advantages and disadvantages of them have been assessed. Also, there are different approaches in this regard and recently, most of studies have focused on cell-based approach that uses autologous cells and synthetic materials.

Herein, we discussed studies related to development and usage of organoids in urogenital diseases. It is expected that organoids have potential to overcome current treatments. Nonetheless, this goal has not been reachable yet and in most of diseases the usage of organoids is limited to animal studies, not fully translated to clinical studies and practice. New studies with larger population and different animals are essential to prove the efficiency of organoids in urology.

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4.1 Introduction

Organoids are organized cells derived from *in vitro* cells that can indicate structure and physiology of intact organs. Tissue engineering has been challenged in the past two decades and most progression in this field is due to building tissues form thin sheets of cells including skin, bladder, and arteries [1]. It is well-known that tissues can be alive, and have sufficient function if they receive nutrients and oxygen from sources not farther than 3 mm away [2]. Although there have been lots of efforts to construct thicker tissues, some barriers prevented us from achieving this goal. Distance from source of oxygen and nutrition plays a pivotal role in not being successful with constructing organoids for thick tissues such as liver and muscle [3].

In recent years, the spectrum of organoids usage has been abundant because of multiple structural and functional problems of the urogenital system (UGS) which occur in various organs that cannot be fixed unless organoids are used. In UGS, there are multiple congenital or acquired diseases leading us to utilizing organoids.

First, congenital UGS malformations are divided into upper UGS and lower UGS, including bladder exstrophy, epispadias, neurogenic bladder and severe hypospadias. Choices for treating patients with these malformations are restricted to two options, which are tissue reconstruction and replacement. In many cases, the tissue cannot be preserved and the mere option is replacement [4].

Hypospadias are one of the frequent UGS malformations that has various numbers of manifestations and severity depending on the location of malformation and the accompanying defects [5, 6]. Hundreds of surgical modalities have been tested over the past years and albeit new studies offer two-stage procedures, the rate of postoperative complications are still high [7, 8]. In some cases, grafts are essential for urethral reconstruction. Grafts, commonly taken from buccal mucosa (BM) may not only make a few difficulties for donor, but also are not quantitatively enough. Moreover, using adjacent tissues as a graft has an association with severe morbidity in long-term follow-up. As a result, scientists were led to considering TE as a better option [9].

Bladder dysfunction is attributed to vast diseases that leave no choice except bladder reconstruction. A wide range of diseases from malignancies to congenital malformations contribute to bladder failure that may have a detrimental effect on patients' health condition [10]. Bladder can be spared if no muscle invasion is seen in malignancy; if bladder muscle is involved in malignancy, the treatment is radical cystectomy. The most important are the complications after surgery, which occur as a result of urinary diversion postoperatively. These complications reinforce the importance of TE. Although great deal of attempts have been made to reconstruct bladder with tissue engineering (TE), significant issues preclude scientists from achieving this aim. The first challenge is the complexity of bladder anatomy that comprises of heterogonous tissues [11]. The second belongs to bladder function, with the necessity of appropriate coordinated working between neurological system and bladder itself [12]. So, it is reasonable to address attentions to regenerate bladder, considering both structure and function.

Urethral stricture (US) affects a significant number of patients worldwide accounting for 0.1% of men older than 65 years of age. For elder patients, infection, trauma, pelvic fractures, and even spontaneously US can be emerged [13]. Urethral reconstruction using BM as a graft is the best way to treat patients with complex urethral strictures longer than 1–2 cm [14, 15]. Urologists are looking for new methods instead of buccal grafting (BG) due to bad effects including pain and numbness of the donors mouth [16]. A significant piece of research on using TE in urethral construction has been published with hope to replace BG in the future [17].

Kidney impairment (KI) can be deleterious by commencing a cascade of events, imposing a great burden on individuals and society [18, 19], so it is logical to find a way to create kidney substitute by searching solutions for healing diseases individually. Besides, generating human kidney (HK) could help us find about the process of HK growing and how it develops and matures. The other benefit we can earn from creating HK in the laboratory would be the understanding of how HK may be affected in chronic kidney diseases in order to understand its mechanisms and find new treatments to procrastinate the process of decreasing the HK function. Millions of people suffer from chronic kidney diseases and die prior to or from complications of dialysis and kidney transplant [20]. Creating HK in laboratory has been studied for years but there is still a long way to achieve this goal completely.

Engineering autologous vagina was implanted in four patients with vaginal aplasia suffering from Mayer-Rokitansky-Küster-Hauser syndrome, showing no complications within 8 years of follow-up and the implantation worked as good as the nature organ. This method can open up new methods for treating puberty problems that affects many people in the world [21].

Ureteral reconstruction (UR) has been one of the greatest difficulties that urologists deal with mainly as a result of ureter damage during colorectal, gynaecologic, and vascular pelvic surgeries in the elderly [22]. Ureter has the potential to anastomosis to bladder by the psoas hitch, and the boari flap, which consist of quite a few of complications [23]. Besides, the chance of primary anastomosis seems extremely low in patients who suffer from long ureteral damage with length >3–5 cm. Hence, intestinal ureter might be the best option in this situation [24]. TE in UR has not been studied as much as bladder and urethral regeneration. Given this fact, more studies are to be performed to assess advantages and disadvantages of TE in UR.

The spectrum of organoids usage has been abundant in recent years because of multiple structural and functional congenital or acquired problems of urogenital system (UGS) that cannot be fixed, leading us to consider utilizing organoids.

4.2 History of Organoids

The initial introduction of cell culture traces back to twentieth century that was used for studying nerve extension of frog embryo and thereafter, much of interest were attracted to utilizing cell culture [1]. Tremendous advances in cell culture lead to

organ culture, which can mirror the selected tissues' the function as well as the structure. Of note, in compare to the other methods of tissue culture, several limitations may arise following organ culture from cell culture including technical obstacles to gain enough cultured samples, trouble through measuring cells and tissues, and challenges regarding replication of cells. With the purpose of solving these issues, organoids, which is defined as three-dimension structure and somehow, makes connection between in vivo and in vitro systems. It has various sources, but not limit to, such as pluripotent stem cells, embryonic stem cells, induced pluripotent stem cells, and adult stem cells [2, 3].

In vitro, Embryonic stem cells contain kind of specific feature that can produced themselves repetitively without any limitation, which is called "self-renewal" if the well suitable situation provide for them and this self-renewal property will be continued without any harm to differentiation potential. Ectoderm, mesoderm, and endoderm which all are the originations of all body tissues and can be produced by embryonic stem cells [4]. Induced pluripotent stem cells, which can be produce from any source of mature cells, along with embryonic stem cells, due to impressing improvements in methods has revolutionized the field of organoids, that prompted numerous investigations to fulfill lack of information in this regard and besides, assist to induce a great progression in regenerative medicine [5].

In 1987, Y Barrandon and his associate used adult stem cells in order to making organoids and they showed that epidermal stem cells have a high degree of reproducibility and also, can give rise to a number of epithelium cells in vitro [6]. One of the leading source of organoids are adult stem cells with the unique ability, that is differentiation into cells of adult tissues and their genome will be intact. Adult stem cells have been pertained to generate mammary gland, bone, stomach, small intestine, colon, and liver, which were successful and promising results have been reached [7].

4.3 What Is an Organoid?

An organoid is defined as a three-dimensional structure grown and developed *in vitro* and derived from cluster of cells including primary tissue, stem cell, ESCs, and IPSCs, which has the potential to resemble the function, and architecture of the tissue considered as origin. The technology of organoids is similar to that used in cell-mixing experiments. Other types of organoids are generated based on artificial extracellular matrices with lack of mesenchymal, stromal, immune, and neural cells that has superiority over basic organoids in self-organization into similar structure of native organ [25, 26].

4.4 Organoids in Cystoplasty

There are numerous studies on organoids in urological problems. Gastrointestinal segments have been used as donor tissue for urological problems, however, the results were not satisfactory due to complications including metabolic disorders, urolithiasis, increased mucous production, and malignancy, which may even worsen the situation and limit its use [27].

These complications of gastrointestinal segments forced scientists to focus on other methods and new tissues as organoids. The first attempt belonged to Neuhof in 1917. He tried to use fascia augmentation for reconstructing the bladder instead of gastrointestinal segments in dogs but he failed. After that, several other materials have been used for free grafts experimentally and clinically [28]. Cheng et al. investigated the effects of reversed seromuscular flaps of ileum in 16 dogs in need of cystoplasty and uretral replacement. They revealed that using reversed seromuscular flaps produces the excellent re-epithelialization of the serosal surface with transitional cells; also, the existence of contraction was little, which was the result of trauma occurring during the mucosal stripping [29]. Porbst et al. used bladder acellular matrix (BAM) as a graft instead of bowel segments to show the benefits that may be provided by ABM in comparison to bowel segments. They performed bladder augmentation in 34 rats after partial cystectomy. They realized that the grafts were surrounded by vessels and smooth muscles of the hosts. After 8 weeks, the grafts resembled normal bladder histologically and most parts of the component of bladder wall, except the neural system were produced. So, BAM can be considered as a reliable graft causing no rejection, and in addition to no risk of rejection, it has the ability to work as a framework to augment the bladder [30]. In 2018, Davis et al. mentioned the benefits of tissue engineered extracellular matrix (ECM) and progressions that have been made in this field. Diseases of lower urinary tract can be solved with ECM scaffolds and for diseases of upper urinary tract, we need to seed ECMs with various cell types before ECM implantation. They believed that a wide range of studies are needed to bring bladder engineering into urological practices [31].

Additionally, synthetic materials have been studied in experimental settings. Polyvinyl sponge is used for replacement of surgically created bladder defects in dogs by Kudish et al. The sponge failed to incorporate with normal bladder tissue by firm fibrous union. Lack of adequate collagen infiltration was evident microscopically. Grossly, the implants were partially extruded into the bladder lumina [32]. Collagen/vicryl (Polyglactin) composite membrane was used to repair full-thickness defects in the urinary bladder of rabbits by Mansour et al. The material has been shown to be biodegradable, prevent leakage of urine, and is readily replaced by collagenous scar tissue lined with urothelium. They suggested that this material can be used in contracted bladders and in the repair of fistulae in human subjects

[33]. Rohrmann et al. investigated silicone rubber prosthesis for alloplastic replacement of the urinary bladder. All tests including blood chemistry, ultrasound, and histopathology showed positive results in animals. The positive outcome of this animal experiment suggested that this material can be implanted in human as well [34], however, the synthetic materials may not be capable of defeating mechanical abnormalities and formation of stones of urinary system. Moreover, the capacity of bladder will be reduced as a result of scarring, and fibroblast remains after the insertion of synthetic materials into the bladder. In summary, evidences so far implied that no better option than gastrointestinal segments could be found, so they are considered as the best option for tissue engineering in bladder reconstruction.

The next step was building a new bladder based on cell-base approaches. Cell-base approaches are techniques using autologous cells as a base cell. They obtain autologous cells from host bladder by biopsy and then, separate and expand the cells, attach to matrix and re-implant them in the same host.

Atala et al. used biodegradable polymers as a delivery vehicle for building new urothelial structure from dissociated cell in New Zealand White rabbits. Cells were seeded onto nonwoven meshes of polyglycolic acid and after 1–4 days, they were implanted in omentum, and mesentery of mice. After 30 days, anticytokeratin western blot showed the evidence of existence of urothelial cells. They showed that urothelial cells can be produced using autologous urothelial cells after some engineering procedures [35]. They also showed that human cell-polymer xenografts can be recovered from host animals at extended times after implantation and when human urothelial cells and bladder muscle cells were implanted in polyglycolic acid fibers, they shape a new structure, which constitutes both types of cells [36]. Atala et al. used human urothelial cells to assess the biocompatibility of biomaterials, used in urinary reconstructive surgery. They concluded that human urothelial cells can be considered as a good choice for finding out the interactions among biomaterials and if any of biomaterials can functionally support the bioactive cells [37]. Southgate et al. elucidated that some studies proved the benefit of bladder engineering though longer follow-up and the understanding of the exact interactions among engineering, material science, biology, and medicine are necessary before gastrointestinal segment can be replaced as the best choice for urinary reconstructive surgery [38]. They performed a study on seven patients with myelomeningocele and poor bladder functions. They biopsied bladders of patients and grew them in culture, and seeded them on a biodegradable bladder-shape scaffold with collagen, and polyglycolic acid. They followed-up the patients for approximately 4 years and the results showed that the mean bladder leak point pressure decrease, and the volume and compliance increase were the greatest in the composite engineered bladders. They suggested that patients who need cystoplasty may profit from TE [38].

Recent studies have focused on joining various biomaterials instead of using special biomaterials. Horst et al. designed a study on 16 rats based on using BAM with polylactide-co-glycolide (PLGA), the combination of which enhances the porosity of electrospun of scaffolds and presents normal bladder wall *in vivo* and *in vitro*. The common restriction of PLGA is shrinkage, which was resolved by mixing PLGA with BAM, and the mechanical resistance of TE bladder was increased by

using both PLGA and BAM. They concluded that the porosity of electrospun hybrid scaffolds enhance the chance of tissue ingrowth *in vitro* and *in vivo* [39]. Adamowicz et al. investigated the mixture of amniotic membrane (AM), which has the capacity to re-epithelization regardless of remaining scars, and electrospun nanofibers that may contribute to the enhancement of mechanical resistance of AM. The result was satisfactory and this combination was capable of bladder augmentation without a significant decrease in the bioactivity of AM [39].

Recently, two approaches, namely acellular and cellular scaffolds are considered as the best choice to encourage bladder regeneration [16]. The acellular scaffolds can be made by either natural or synthetic biomaterials, which can lead to activating the process of regeneration of bladder working as a framework to be surrounded by vessels and tissues of the host bladder [40].

Clinically, bladder augmentation depends on large scaffolds that may complicate the situation with scars, and fibrosis. Roelofs et al. demonstrated the implantation of multilayer collagen scaffolds which are supplemented with heparin and mixed with vascular endothelial growth factor (VEGF), fibroblast growth factor 2, and heparin-binding epidermal growth factor is capable of regenerating great parts of a bladder in animals. Urodynamic studies showed that bladders which were produced with this method have virtually the same capacity, and compliance of the native bladder [41]. The studies showed that using acellular scaffolds with enriching synthetic biomaterials result in new bladders that are difficult to distinguish from native bladder. For better outcomes, it is necessary to figure out the regeneration pathways that may lead to understanding of the mechanisms of the synthetic biomaterials participation in the bladder regeneration process and control the process as a result.

One of the new techniques for bladder augmentation in TE is electrospinning in which a variety of biomaterials can be generated based on the compliance and mechanical resistance. The electrospinning capability of being the source of new scaffolds is the result of flexibility and constructing fibers with various diameters [42]. Poly (ε-caprolactone)/poly (l-lactic acid) was produced by Shakhassalim et al. using electrospinning technique for replacement of bladder wall. They concluded that when new-scaffolds fabricated by electrospinning are accompanied by cell transplantation, they can firmly act as a supportive agent for regeneration of the bladder wall [43].

TE in clinical practice is just available for partial bladder replacement, which the indications are limited compared to the need for total bladder replacement. Therefore, new studies should be done in order to find solutions with ability to augment the whole bladder [44].

4.5 Organoids in Pelvic Floor

Pelvic organ prolapse (POP) is a prevailing problem affecting 25% of all women and the burden of it cannot be overlooked [45]. The extreme stretching and tearing of peripelvic organ tissues which is common during vaginal birth are among factors

responsible for POP occurrence [46, 47]. The possibility of vaginal delivery without hurting any structure helping the pelvic to be stable is low [47]. Reconstructive surgery either with or without mesh to enhance the stability of pelvic floor can be the origin of complications in many women [48].

Using TE in pelvic floor problems has been brought up in the last 30 years and can be used as an alternative treatment also in stress urinary incontinency (SUI). The first approach in TE for POP repair was done in 2010. Authors seeded human vaginal fibroblast on a polylactic glycolic acid-knitted mesh and then implanted them into mice and followed them for 12 weeks and the results showed well-shape fascia with a high collagen I/III ratio. They also illustrated a better function of different biomaterials with adding adipose-derived mesenchymal stem cells [49]. In Li et al. study, a higher rate of neovascularization and a lower rate of inflammatory cell infiltration for POP repair was reported by designing a gelatine-coated polyamide knit mesh, which was seeded with endometrial mesenchymal stem cell [50]. Roman et al. showed promising outcomes concerning capacitation of attachment of both human oral fibroblast and human adipose-derived stem cells to scaffolds [51]. In contrast to developments that have happened regarding using TE for treating SUI and POP, the rate of complications is still high, and it is the main reason of forbiddance of using TE for pelvic floor diseases in human. Before TE can be translated into clinical practice the efficiency of TE must be proven in animals [52].

Nowadays, SUI which is a type of incontinency happening during strain, sneezing, and coughing [53] affects more than 200 million people. SUI constitutes various types and there are several well-known causes that account for emerging SUI [54]. The treatment options are divided into non-surgical, and surgical [55]; however, in many cases, the first option is the non-surgical approach regardless of the severity. Albeit, injection of urethral bulking agents is the harmless surgical approach, its efficiency is under question and furthermore, it has an association with dysuria, abscess formation, and pulmonary embolism [56]. The more invasive method is the implantation of artificial sphincter, which also results in some complications that limit its usage [57]. The surgical methods do not seem to be convincing so far, thus TE may be the solution for relieving patients who suffer from SUI.

The first step regarding TE in SUI was done by Chancellor et al. in 2000. They injected myoblast in only eight rats with no control groups in terms of treating SUI. The results showed that surprisingly, myoblast could mix with the local tissue of sphincter [58]. Cell therapy was first utilized in Yokoyama et al. study. They gained muscle cells from gastrocnemius muscle in 6 rats. They found out that 88% of transgene expression lasted for at least 30 days and the muscle cells which were injected were capable of being survived [59].

The functional studies started from 2003, and the studies have shown good improvements in SUI using cell injection. The studies were performed on different animals and used various methods in sciatic and pudendal nerve transection, each of which has its own strengths and limitations [60, 61, 62, 63]. Mitterbereger et al. demonstrated that as the number of injections increase, the incontinency improvement increases as well and the episodes of urinary retention would be seen if the cells have the concentration ratio of 7.8×10^7 or above [63].

Adipose-derived stem cells (ADSC) have been studied as a source of cell transplantation in some studies. First, Lin et al. gained ADSC from periovari fat and in 12 rats, ADSC was injected in urethra and in six rats were injected intravenously and 10 rats were the control group. After 4 weeks of follow-up, they realized that rats receiving ADSC have a higher rate of elastin content even compared to rats with normal voiding function. They concluded that ADSC can be used not only in treating SUI but also, in prevention of SUI [64]. The other studies have shown similar results [65, 66] and they illuminated that ADSC has the capacity to preserve the urethral sphincter function [66].

Bone marrow mesenchymal stromal cells have been investigated in only four studies and the studies were restricted to animals. Moreover, one model of SUI was used in these studies. One of the limitations of studies is the bone marrow procedure, which is aggressive and harmful [67, 68, 69, 70].

The efficiency of Amniotic Fluid Stem Cells (AFSC) has been limited to few studies owing to the ethical issues of administering AFSC. In 2012, Chun et al. illustrated that AFSC has the potential to encourage the process of differentiation, and proliferation of myoblast and that is why it is wise to consider the combination of AFSC and ADSC [71, 72].

Human umbilical cord blood mononuclear stem cells (HUCBMSC) can be named as a new source for stem cells. HUCBMSCs included the capacity for translation into muscle when they were injected intravenously. More studies are necessary to support HUCBMSCs into practice [73, 74].

4.6 Organoids in Urethra

Obstacles encountered in harvesting tissue for urethral reconstructive surgery forced us to look for better options. TE has priority over donating graft tissue from BM due to predictable side effects and limited number and size of grafts [17, 75].

A few types of biomaterials have been used in different preclinical studies. The first one was small intestine submucosa (SIS), which was only used in partial reconstructive urethral surgery. SIS has potential in complete reconstructive surgery if it is accompanied by stem cell seeding. Studies on the effect of combining BAM gained from porcine or leporine bladder with cells such as keratinocyte, ADSC and urethral cells have shown that these combinations can be relied on as promising biomaterials [76, 77, 78]. Chun et al. made a homogenous biomaterial from a cellular bladder submucosa matrix and autologous urethral tissue. They clarified that the rate of rejection decreased as a result of using autologous urethral tissue that can also make the infection rarer [79].

A wide number of synthetic biomaterials has been studied but the most effective one is polylactide-co-glycolide that was used in Raya-Rivera et al. study that showed the feasibility of it for urethral reconstruction.

In addition to preclinical studies, some clinical trials have been performed in TE for reconstructive urethral surgery. A clinical study on 50 patients with ure-

thral stricture carried out by Fiala et al. reported that 40 of the patients undergoing urethroplasty using SIS as a biomaterial of TE showed excellent results with 80% success rate; additionally, the rate of wound infection, fistula, urinary tract infection, and rejection was zero compared to another method of surgery which uses BG and flap skin [80]. The efficiency of BAM obtained from cadaveric donors was studied by El Kassaby et al. The result showed the feasibility of using BAM rather than using BG in patients who had less than two operations [81]. Considering urethral acellular matrix as a biomaterial, it has been investigated in several studies and they showed that benefits might be obtained from this biomaterial in various ranges [82].

Although TE in urethroplasty has shown promising results, the tough and costly process of providing the biomaterials in TE is one of the greatest restricts. A systematic review carried out by Versteegden et al. revealed that time for replacing TE in urethroplasty instead of usual treatment has not come yet and studies in both preclinical and clinical studies are needed once the translation of preclinical studies into practice has not occurred [83].

4.7 Organoids in Ureter

The field of TE in UR has not been studied as much as bladder and urethra. So, this field has lots of capacities to be investigated, and there is a long way to gain possibilities to translate TE in UR into clinical practice. First, Davis in 1943 showed that the ureteral lumen and wall can be appropriately preserved in patients with ureteral stricture by clinically inserting a stent through ureter. Hinman et al. emphasized the importance of smooth muscle integrity preservation aiming to avoid scar formation preserving peristaltic waves [84].

The studies discovered that arteries and ureters are structurally similar [85, 86]. Hence, they made significant efforts in order to use arteries as synthetic scaffolds for UR. However, some studies showed only little progress in regrowth [87] and most of studies have concluded that this procedure cannot be relied on. The complications of implanting autologous venous graft after 6 months were reported by Engel et al. They revealed that after 3 months post implantation, an increasing rate of hydronephrosis appeared. Thus, more investigations were suggested to assess the long-term results of this procedure [88].

Functionally, ureter organ is predisposed to ischemia due to weak plexus of blood supply that makes the ureter susceptible to extensive fibrosis, which forces scientists to explore a way to overpass the defects [89]. When it comes to regenerating ureter, local ischemia of ureter directed urologists to perform a two-step surgery technique that provides the chance for progressing vascular plexus rather than a one-step technique. The excellent outcome was achieved by Kloskowski et al. by implanting poly (L-lactide-*co*-caprolactone) into the omentum to gain a rich vascular plexus that enclosed the scaffold [87].

In cystectomy candidates, a urinary conduct which works as a ureter must be formed. Hence, the material of ureteral scaffolds and urinary diversion are the same [10]. Using TE in urinary diversion has not been studied enough to provide the potential to translate into clinical; furthermore, the results of studies were disappointing. The incorporation between ureter and the synthetic conduits was not accomplished, and most of conduits did not work appropriately. Also, administering decellularized materials cause serious complications such as shrinkage of graft, hydronephrosis, and blocking of the site of anastomosis [87].

Results in most studies have shown that TE can play a substantial role in UR, which is one of the most complicated problems that urologists face. At the same time, a great number of studies in this field belong to animals and clinical studies are not sufficient enough, and the population of studies should increase. Besides, longer follow-up is needed to prove the benefits and safety of TE in UR in the long run.

4.8 Kidney and Organoids

The technology of generating a human kidney (HK) took lots of efforts to accomplish in the past six decades, and each related study contributes to upgrading the methods of one another. The first challenge was to find out how a normal HK develops anatomically. HK has three classes in mammals including pronephros, mesonephros, and metanephros, which a mature organ originated from. The morphogenesis of HK was completely described by Potter and Osathanondh. The number of kidney cells among people is different, and some factors may influence the number of glomerular, varying the number of them between about 0.25 and 2.0 million individually.

The second challenge was discovering the biology of HK development. First, Grobstein et al. [90] described the biology of HK development and some studies completed that [91, 92]. The studies showed that ureteric bud branching and mesenchymal to epithelial transition cannot be alive without each other in vivo, and each of them contains a factor that stimulates the other [93]. New studies hypothesized that the pattern of developing of kidney in humans and mice is similar, and differences were seen in only some aspects [94, 95].

The third challenge was how to generate a HK *in vivo*. Different strategies have been employed to generate HK from PSCs (Fig. 4.1). The first line of study was producing MSCs from human embryos (flash1), recently produced also from skin, blood cells, and mature kidney cells [96] (flash2). The second line was waiting until MSCs self-renewed, (flash3) and after that, make them to differentiate into intermediate mesoderm-like cells [97, 98] (flash4). Here, there are three choices: (1) After a week, may branching tubule-like and primitive kidney be produced form MSCS (flash5). (2) Differentiated cells were selected and implanted in three-dimensional masses (flash6), and after that it is possible to implant the cells derived from PSC into immunosuppressed mice

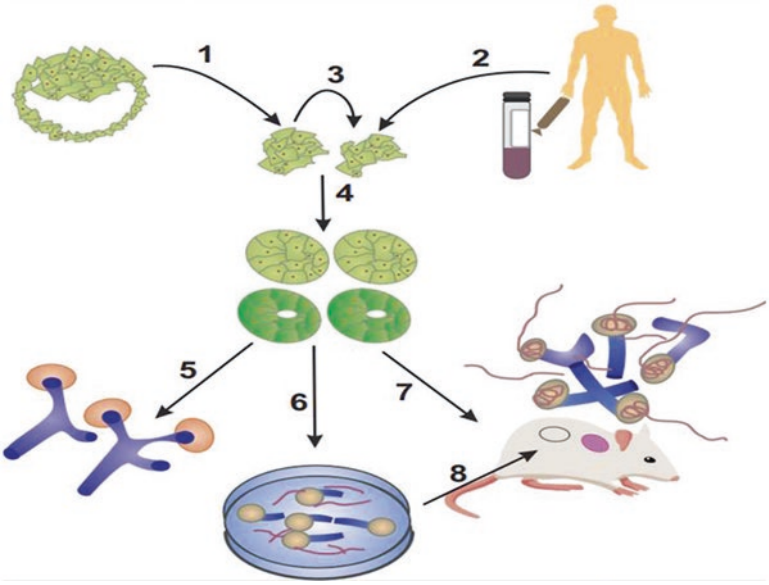

Fig. 4.1 Generation of a human kidney in vivo

(flash8). (3) Implanting differentiated cells into immune-deficient mice and vascularized glomeruli will be observed after several months (flash7).

4.9 MSC in Renal Disorders

Mesenchymal stromal cell (MSC) has been identified as a suitable source for cell therapy in TE owing to the potential ability to differentiate into both mesenchymal and non-mesenchymal cells, and self-renewal. The usage of MSCs in kidney disorders has been reported. MSCs can have a decreasing effect on proteinuria, slow down the process of constant decrease in kidney function, and may lead to better proliferate glomerular cells [99, 100, 101]. The role of MSCs in patients with acute renal failure (ARF) has been demonstrated. They showed the capacity of MSCs in differentiating tubular epithelial cells, and enhancing the process of healing. Impressively, increasing the proliferation of tubular was observed [102]. The promising results were published in cases, who suffered from ARF as a result of cisplatin and glycerol toxicity that underwent bone marrow MSCs. Exogenous MSCs had the ability to differentiate normal renal epithelial cells, and bring back the function of disabled kidney [103]. Togel et al. noted that VEGF which origins from MSCs can play an important role in renoprotection. Furthermore, animals showed no signs of fibrotic lesions, prevalent in the process of acute kidney injury in long-term follow-up, and decreasing rate of renal function reduced significantly as well [104].

The number of studies related to effect of MSCs in chronic kidney diseases is limited. Ninichuk et al. presented that although the ability of MSCs differentiating in chronic renal failure (CRF) is poor, MSCs can still produce VEGF and bone morphogenetic protein-7, and regeneration of kidney cell will increase. Semedo et al. illustrated that the level of pro-inflammatory cytokines will decrease while MSCs is administered for patients with CRF. On the contrary, the level of anti-inflammatory cytokines will increase as MSCs is administered for patients with CRF [99].

Overall, the outcome of studies demonstrated that MSC can be used as an alternative therapy in animals with both CRF and especially, ARF. Most of the studies are small animal experiments and it is necessary to make the population of animal studies larger, and also, perform studies on animals with some other kidney diseases. Then, it would be possible to study the effect of MSCs on humans with kidney diseases [105].

4.10 Conclusions

Recently, lots of attention in urology belongs to organoids, which can produce better tissue survival and better outcomes in comparison to common treatments. While studies have showed satisfactory results especially in animals, demonstrating evidences for using organoids instead of routine treatments, they are not enough to bring organoids and TE into clinical practice yet. Most of them suggested that new studies with larger population and different animals are essential to prove the efficiency of organoids in urology and it is expected that someday the routine treatments are replaced by organoids completely.

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Chapter 5
Regenerative Medicine in Urology

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Abstract There has been always challenge regarding repair and reconstruction of damaged tissues and organs and this issue causes health care systems face so many troubles. Traditionally, for handling this problem, surgeons had limited options which consist of resection of the lesions and repair the site of defect with autologous tissue, or allografts. Using autologous tissue as tissue for transplantation has some obstacles that can limit wide-spread use of that, for example, appropriate of site of donor and additional damage to donor site and allogenic transplantation has the rejection chance. Due to these reasons, scientists put their force on to overcome these difficulties by regenerating tissues and organs within new method and technology. Regenerative medicine (RM) is an emerging field that focuses on how to regenerate tissues and organs to substitute tissues and organs that were damaged in order to maintain their routine and normal function.

Keywords Regenerative medicine · Urology · Stem cells · Wound healing

5.1 Introduction

First time the term of RM was used in 1999 by William Haseltine who defined RM as a new branch of medical science that discuss about usage of tissue engineering with the purpose of improving in biological substitutes [1]. In tissue engineering (TE) or RM, after cells that were harvested from a patient, integrated with a biodegradable structure that denominates "scaffold" and now new structure is ready for implantation in the patient. The scaffold is considered as a basic structure that

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should have some features to make it suitable for TE and RM. For instance, scaffold ought to have capacity to significantly biodegrade, adherent properly to cells, and support physically a new structure. Some metabolite indicators (oncometabolites), genetic mutations, proteomics and epigenomics biomarker can take in the account of both cancer diagnosis and prognosis [2]. The merely difference between TE and RM is related to the citation of where cells are grown and in other aspects no major differences are existed Materials that have potential to apply as scaffolds differentiate into two main groups: (1) natural materials which include collagen and fibrin (2) synthetic materials which includes polyglycolide, polylactide coglycolide. The process of generation of a new tissue or organ through RM can be explained briefly in four phases: phase1. growing a new tissue or organ around a scaffold that were integrated with cells. phase2. the new growing tissue must be close to tissue-separating structure. phase3. lamination of 100 μ thick tissue layers grown on special bioreactor. phase4. providing capillaries inside the regenerated tissue. Although there have been great attempts to improve all the phases, most efforts are belonging to phase 1 and the main improvement in this phase have been seen. There are several problems that scientists must dealt with concerning RM: (1) lack of essential means for cells growing except oxygen that has capacity to reach cells. (2) as mentioned above, several factors are warranted to create applicable scaffold. (3) as stated above, is challenging to choose suitable biomaterial among different biomaterials that are available. (4) remaining the new tissue or organ stable structurally [3]. As increasing tendency in RM field has been reported, our study aimed at the investigated the feasibility of RM in urology base on previous studies.

5.2 Stem Cells

Scarcity of organ donors for RM, lead scientists to research on viable substitution that will be able to be pertained in RM and the fittest substitution for that are stem cells (SCs) and that is as a result of increase in knowledge of biology of SCs and moreover, their potential to differentiate into progeny, which has shown promising results in regenerating damage tissues in human studies. The viability of using SCs in TE and RM has been proved and therefore, they have been employed in two fields widely [4–9]. The various types of progenitor cells exist during adulthood, which are the reasons of revitalization of tissues [10, 11] and considered as sources to be applied in wound healing [12]. Four main types of stem cells platform with specific properties are currently presented for cell-based therapy [13].

5.3 Embryonic Stem Cells

Embryonic stem cells (ESCs) which derived from the inner cells of blastocysts and also, can derived from a variety of species including human, mouse, etc. [14, 15]. While traditionally, believed that human ESCs, which were described by Shapiro

SS et al. in 1998 [14], were the only sources of multipotent cells, recently, the other sources for multipotent cells have been recognized [16]. Nowadays, according to prior studies, the generation of non-ESCs that are stem cells without any source from embryonic cells [17] have been successfully used in different medical fields for example, ophthalmologic field [18], hematological field [19], and neurology field [20]. There was a revolution in production of pluripotent stem cells that was achieved by K Takahashi et al. They eventually succeed at induction of pluripotent stem cells via introducing four transcription factors from mouse ESCs or adult fibroblasts [21].

5.4 Perinatal Stem Cells

Perinatal stem cells (PSCs) derived from amniotic fluid, fetal membranous, placenta, and mostly derived from umbilical cord blood (UCB) is one of the leading platforms for RM [22]. UCB cells may be beneficial at stem cell transplantation owing to similarity with adult bone-marrow progenitors and furthermore, less maturity in compare to adult stem cells lead to lesser odds of graft-versus-host disease [13, 23].

5.5 Adult Stem Cells

Adult stem cells (ASCs) can be derived from a board spectrum of tissues such as bone marrow and adipose tissue and also contains a broad spectrum of progenitors. Among various stem cells that have been used, the most prevalent used stem cell is belong to ASCs owing to easy access to them, not difficulty in gaining them in vitro, and desirable potential to proliferation [24]. The primarily source of ASCs is bone marrow, which comprises of two main progenitors including hematopoietic stem cells and mesenchymal stem cells (MSCs) [25, 26].

5.6 Wound Healing in Urology Using Mesenchymal Stromal Cells

When an injury occurs, MSCs migrate to the site of wound in order to repair the site of injury and before its happen, they provide the suitable microenvironment with the purpose of releasing growth factors that are necessitate for wound healing, modulating the immune responses and promote the process of wound healing by decrease in the level of pro-inflammatory cytokines and increase in the level of anti-inflammatory cytokines and before modulating immune system reactions [27]. Nakamura et al. speculated that the power of wound healing of MSCs can be increased by engineering them to express stromal cell-derived factor1 (SDF-1). After they transfected

MSCs with SDF1 plasmid DNA, SDF-1 was secreted from MSCs for 7 days. It was shown that the combination of them brings about rise the migration of MSCs and enhanced dermal fibroblast in comparison to use MSCs individually. They significantly released the endothelial growth factor, hepatocyte growth factor, and interleukin 6 and not only decreased the size of wound, but also increased the newly blood vessels in quantity [28]. Most studies pertained autologous cells to reconstruct a bladder; On the other hand, in patient who suffer from bladder cancer gaining autologous cells from patient bladder is not possible and hence, it was hypothesized that MSCs can be used as substitution for autologous cells in patient with bladder cancer. Tian et al. showed that if myogenic-differentiated MSCs seeded on the porous Poly-L-lactic- acid, the structure can be applied for bladder constructing in patients with bladder cancer [29]. The potential of differentiating of bone marrow-derived MSCs and amniotic fluid into smooth muscle cells in rats, who had cryoinjured bladder, has been investigated. This feature of MSCs was researched in De Coppi et al's study and they demonstrated that while MSCs have not great effect on regeneration of smooth muscle of bladder, they hamper inappropriate hypertrophy of smooth muscle cells that were existed after injury possibly through a paracrine mechanism [30]. The feasibility of using adipose-derived MSCs on tissue-engineered preuce scaffold has been demonstrated in Kajbafzadeh et al'study. Wall regeneration of rats' bladders was assessed by measuring CD31, CD34, and SMC α-actin and it was revealed that in group with tissue-engineered scaffolds that were seeded with adipose-derived MSCs higher portion of CD34 and CD31 was detected versus in group with electrospun nonofibrous matrix [31]. The potential of MSCs in kidney injury has been studied in experimental studies. H Asanuma et al. declared that exogenous MSCs can implicated in recovery of acute or chronic kidney injury [32]. The mechanisms of MSCs that participate in recovery of kidney injury have studied by M Morigi et al. They expressed the majority of MSCs' ability to healing kidney injury is attribute to paracrine mechanism. Although lots of preclinical studies showed the capacity of MSCs in healing kidney injuries, the effectiveness of them in clinical applications remain to be elucidate [33].

The aim of cell therapy is to renew, substitute or reestablish biological function of an injured tissues or organs [34]. Between several stem cells, adult stem cells the most common type in tissue engineering because their handling is easy and their culturing has not ethical issues like embryonic stem cells. In fact, bone marrow is the main source of adult stem cells which is made of mesenchymal stem cells (MSCs), haematopoietic stem cells (HSCs), cellular precursor and endothelial progenitors.

HSCs growth to the monocytes, erythrocytes, white blood cells (WBC), dendritic cell (DCs), [25]. MSCs are presented in the mesodermal germinal layer in trace, and can differentiate into chondrocytes, osteoblasts and adipocytes. More than bone marrow, MSC are seen in the umbilical cord, skin and adipose layer as well [34]. MSCs can migrate from bone marrow to the wound site on the injury occasion, through the inflammatory stage of wound healing, MSCs control cell cycle and proliferation. MSCs can act as immune response modulators and support the transplanted cells [27]. Nakamura et al. considered the aptitude of genetically

engineered cells with stromal cell derived factor-1 (SCDF) to restore injuries and showed that gene transfection, MSCs secreted SCDF within 1 week. Studies over the fluorescently labelled cells indicated that percent-age survival of SCDF–MSCs was much greater than MSCs [28]. Tian et al. produced a very porous poly L-lactic acid (PLLA) scaffold via the paraffin sphere leaching method. Growth factors cause bone marrow MSCs nourished and then embedded subcutaneously into the nude mice. It is shown that PLLA scaffolds permitted cell matrix diffusion, myogenic differentiation, and tissue remolding with gorgeous duct formation (Fig. 5.1). The consequences confirmed that the PLLA scaffold is able to be possibly used for cell based tissue engineering in bladder damaged patients [29]. De Coppi et al. have proved that amniotic fluid and bone marrow derived MSCs can be changed to smooth muscle cells in the cryo-injured rat bladder model and possibly will stop compensatory hypertrophy of surviving smooth muscle cells. Thus, they stated that stem cell transplantation could control post-injury bladder remodeling [30]. Asanuma et al. have considered the therapeutic potential of MSCs to healing renal damage [32, 35]. Kajbafzadeh et al. confirmed the possibility of adipose derived MSCs seeded over the engineered bladder [31].

Adipose derived stem cells (ADSCs) are a group of cells that separated by liposuction techniques from adipose and are suitable to make muscle cells and blood vessels. Dissimilar to the bone marrow stem cells, ADSCs are extremely plentiful and simply available. Zhang et al. revealed that ADCSs could be differentiated into the urothelium cell phenotype when they were co-implanted with cells of the mature urothelium cell line, and the ratio of differentiated cells raise from 2 to 4 weeks [36–38]. Fu and co-workers stated that typical or artificial polymers do not suggest a suitable curing solution for lengthy urethral faults. They effectively used ADSCs alongside with oral mucosal epithelial cells for making tissue engineered urethras. Poly glycolic acid was chosen as a scaffold materialto overcome pathogen infections [39].

Embryonic stem cells (ESCs) are pluripotent stem cells originated from the primary embryo (blastocyst). The major restrictions of ESCs are the ethical issues [40]. Lee et al. considered the benefit of ESCs on wound healing in110 diabetic rats. ESCs were injected into full-thickness skin wounds. The mRNA levels of EGF, VEGF and fibronectin were noticeably elevated and the injury sizes were seriously healed. It is shown that mouse ESCs are able to be differentiated to the bladder cells. Some specific endodermal transcription factors including Foxa1 and Foxa2 are important in this way. The embryonic bladder mesenchymal cells can turn ESCs endodermal derived urothelium [41].

In order to solve the problem of graft rejection, induced pluripotent stem (iPS) cells can be reprogrammed to the stem-cell like condition. Nevertheless, enormous concern are about cancerous potential of iPS [42]. Li et al. used iPS for the treatment of diabetic wounds. After reprogramming somatic cells into iPS cells, adopted them directly to the lineage reprogramming with the more enriched population power [43]. Franck et al. have invented silk based biomaterials in grouping with extracellular matrix coatings for bladder tissue engineering with primary and pluripotent cells. These scaffolds were equipped by the gel spinning procedure and

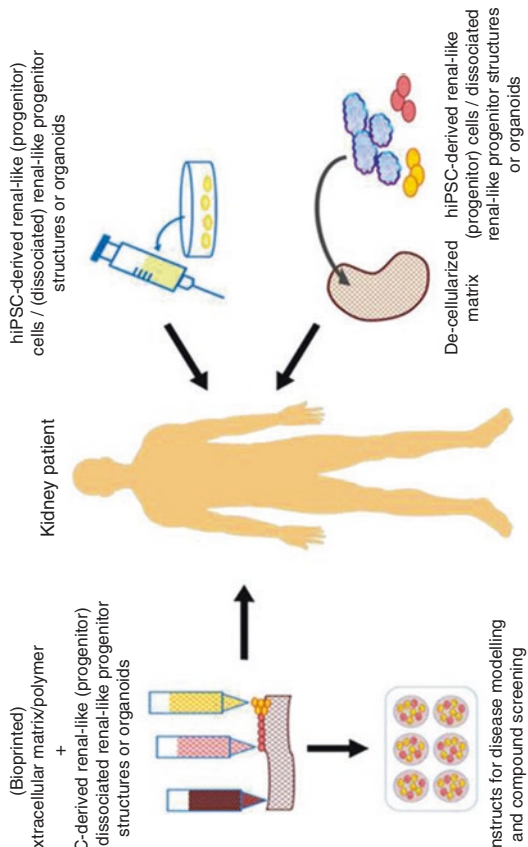

Fig. 5.1 Potential application in regenerative research

were covered with collagen I, collagen IV and fibronectin. The RT-PCR outcomes presented that fibronectin coated scaffolds eased ESC and induced pluripotent stem cell differentiation to urothelial and smooth muscle lineages simultaneously [44].

5.7 Regenerative Medicine in Bladder

So far, a great number of pathological conditions have been recognized that can account for bladder dysfunction (BD) or bladder damage and these conditions lead surgeons to consider cystoplasty as the choice of treatment. Previously, for performing cystoplasty, the intestine tissues were used as the substitutional. Nevertheless, treatment-related complications including stone formation, infection, mucous production, perforation, and tumor formation limited the utilization of intestine tissues. Consequently, researches on the efficacy of using stem cells in cystoplasty instead of using intestinal tissues have been performed and promising results have been showed [45–49].

5.8 Regenerative Medicine for Bladder Using Cell Transplantation

The promising results have been reported concerning the capacity of regenerative medicine in constructing of a viable bladder segments with cell transplantation and rise hopes for using this method clinically in the near future. There are factors decrease the chance of failure of cell transplantation strategies for bladder reconstruction that should be focused on. For instance, the possibility of using donor tissue at the first must be considered, or assess if there are proper situations for cell growth and differentiation. So far, although various types of cells have been pertained for bladder reconstruction, present evidences are persisting on using native cells on account of extreme ability in reproduction and the facility of expansion in large amount [50]. It has postulated that basal part of neck and trigone of a bladder contain more progenitor cells in compare to other bladder regions [51, 52].

To obtain autologous cells, a variety of sources have been investigated. Amniotic fluid, bone marrow-derived stem cells, and ESCs are the quintessential of autologous sources with high potential for differentiating into bladder tissues and urothelial cells [16, 41, 53, 54]. Nonetheless, in many counties ethical and politics issues hamper scientist to routinely use ESCs as a source of autologous cells and hence, unfortunately, these cells are excluded in clinical studies [55]. In addition to ethical dilemmas, not identifying the best condition for culturing ESCs and the reports of tumor formation confront us to apply them in regenerative medicine [56]. As mentioned, adult stem cells are the most prevalent cells that have been studied in stem cell therapy [25, 57]. This is as a result of tremendous capacity to be applied in a

wide range of diseases and also, no immune system reactions were observed when they applied. These reasons make them desirable sources for regenerative medicine [56, 58]. The variety of tissue types that MSCs can be differentiate into such as smooth muscle, urothelium, and endothelial cells is limitless [59]. Chung et al. performed a study that investigated on the efficacy of MSCs in reconstructing a bladder. They seeded porcine small intestine submucosa with MSCs in case group and in control group, no seeded with MSCs was done. The results showed that rats who were in the case group, exhibited urinary bladder with an excellent function and structure in compare to the control group [60]. Interestingly, recent studies have reached to novel method for collecting progenitor cells and that involves voided urine, which oddly, can extract progenitor cells from it and differentiate it into urothelial and smooth muscle cells in suitable conditions [61, 62].

Accumulative evidences have shown that both urothelial and muscle cells have ability to expansion in vivo, seeded on scaffolds, and shape sheets of cells irrespective of source [63] and also, there is a fundamental difference between urothelium and muscle tissue and that is regenerative power of urothelium is higher than muscle tissue and that is justifiable by consideration greater reparative capacity [64]. In the light of this, an interesting hypothesis was formed which said that in vivo constructing a 3-D bladder would bring about not only the better terminal cells differentiation after in vivo implantation but modulate the immune reactions as well. This hypothesis was in consistent with WI de Boer et al's study that showed the capacity of bladders in dogs who their bladders were augmented with the allogenic bladder submucosa with cells (that were generated by tissue engineering) were superior in comparison to dogs who their bladders were augmented with cell-free allogenic bladder submucosa [65]. Although encouraging results were achieved, one main question left necessitates to revert and that is enhancing the function of bladders was as a result of tissue engineered tissues or the remaining native bladder tissues. F Oberpenning et al. performed a study to investigated that and they showed that improvement in function parameters of bladders is because of tissue-engineered bladders that were seeded with cells and besides, bladders that were seeded with cells showed a normal cellular arrangement histologically and all the layers of a normal bladder were existed that was confirmed by immune cytochemical analyzes; however, histology of non-seeded bladders was not as normal as cell-seeded bladders [66]. Thereafter, plenty of studies have proved these results in animal population and their results were in line with F Oberpenning et al's study [67, 68]. So, it can be concluded that virtually no evidence warranted to show the efficacy and advantages of cell-seeded scaffolds and no serious complications can be expected as well [69, 70].

On the other hand, some limitations were found in applying several scaffolds if the volume of the bladder that should be replaced was high in quantity, namely according to Y Zhang et al's study that was investigated on 22 dogs who had a 90% partial cystectomy and divided into bladder augmentation with cell-seeded group, bladder augmentation unseeded group, and the group without bladder augmentation and they used SIS as graft. They were assessed at 1 month after bladder augmentation and it was shown that strangely, both cell-seeded and unseeded groups showed

graft shrinkage, adhesion and regeneration of bladders was not as much as expected. After 9 months, all three layers were formed and bone formation was detected. In the group without bladder augmentation, existence of acute inflammation, fibroblast, and hypertrophy of muscles were detected. This study provided challenges with respect to bladder augmentation with using SIS in bladders that badly damaged [71].

In addition to type of scaffolds, whether using bioreactors can influence the outcome of a bladder regeneration. Bioreactors provide an excellent environment for cells to attach and proliferate well on scaffolds and also play pivotal roles in the process of maturation of cells and secretion of various molecules especially, extracellular matrix [72]. In vitro, bioreactors support muscle development, are require in endothelial layer of blood cells, and for bladder, help to tight control of urinary filling and emptying, rise the interactions between cells and scaffold, and decrease the time that is needed for gaining normal function [73].

Over the time, new methods have been experienced and promising results that correlating with better function, enhancing the compliance, and improving in the dynamic parameters have been achieved. One of the best example of these methods is using omentum as coverage for bladder regeneration. First, Atala et al. performed a study on 7 children who suffer from myelomeningocele and had neurogenic bladder. They seeded merely collagen or composite collagen, polygglycolic acid bladder acellular matrix with autologous cells and implanted them with or with omental wrap. They followed their patients for between 22–61 months and they highlighted the importance of both autologous cells and omental wrap base on the better results in the group with omental wrap [74]. Joseph et al. designed a study on 11 children with neurogenic bladder as a result of spina bifida base on the study of Atala et al. Similar to Atala et al., they seeded composite collagen with polyglycolide/polyactide mesh with autologous cells with omental wrap. Against all the odds, after 36 months follow up, no significant improvement in neither compliance nor capacity of bladders occurred and more importantly, notable complications faced most patients [75]. Although the method and technique of two above studies are virtually similar, there are not any reasonable explanations for discrepancy between two above studies yet.

Tissue-engineered Neo-Bladder-augment (NBA) was assessed in two clinical studies. Joseph et al. in 2009 investigated on the possibility of using NBA in children with neurogenic bladder secondary to spina bifida at Children's Hospital Boston. The indications for augmentation cystoplasty were bladder pressure over 40cmH_2O and/or emerging upper tract complications and all the patients were eligible for augmentation. Subsequent open biopsy of bladder from each patient, autologous cells were obtained from the biopsies and seeded on scaffolds which were made of NBA. Then, after implantation of the constructs, and final results showed that 6 of 10 patients experienced improvement in clinical status and urodynamic parameters [76]. The next study belonged to Shenot et al. that were performed on 6 (age over 18 years) patients who suffer from refractory neurogenic bladder due to spinal cord injury. The procedure was alike to Joseph et al's procedure, which used NBA for bladder augmentation. After 2 years' follow-up, 2 of 6

patients responded to bladder augmentation completely, 2 of 6 patients responded partially, and no responded were detected in 2 of them. The point that both of above studies insist on was the important role of normal bladder cycling on final outcome of bladder augmentation and those patients who did not have normal bladder cycles the results were unmet and some uncertain explanations support that.

M Sloff et al. summarized all 28 preclinical studies with respect to the possibility of using tissue engineering to construct a bladder until 2014. Despite the design, characteristics, populations of study, and model of studies are different from each other, mots studies showed promising results. However, there are some challenges, such as clinical studies did not reach the similar results as animal studies reach, that hamper scientist to translate these preclinical studies into clinical studies [77].

5.9 Detrusor Dysfunction

BD is a prevailing problem that involves people regardless of age, race, and culture, and impose a great burden on society by impairing both physical and psychological aspects of life [78]. The most common bladder functional problems include Overactive bladder (OAB), that often manifests with urinary frequency and nocturia [79], may has association with detrusor overactivity (DO) [80], and underactive bladder (UAB), which is characterized by hesitancy, straining, and incomplete bladder emptying [81].

Although, alternative options for treatment of OAB are available, no promising treatment for UAB is available. However, definite treatment that takes it into consideration as curative treatment or the treatment that its object is preventing the ongoing process of pathology despite a number of studies, is yet unreachable. In spite of disappointing studies with regard to treatment of OAB and UAB, awareness towards stem cell therapy and regenerative medicine in this regard is sustainably increasing. In this section, most studies that were revealed the benefits and disadvantages of stem cell therapy in these diseases, will be reviewed.

Treatment of uncomplicated OAB should be initiated with conservatively such as bladder retraining, specifics pelvic floor excise, and anticholinergic drugs. Bladder retraining helps to improve in increase the capacity of bladder and decrease in bladder spasms. The pelvic floor exercise can be utilized in both OAB and stress incontinency. Nowadays, the best choice for treatment of OAB is drug therapy, which are antagonist of anticholinergic muscarinic receptors [82].

For treatment of UAB there are not validate options: (1) physiotherapy including straining to void, intermittent catheterization, and double void. (2) drug which includes α-adrenoceptor antagonists, Muscarinic receptor agonists, and Acethylcholinesterase [83, 84]. (3) stem cell therapy that is one the progress and promising outcomes have been demonstrated and it was illustrated that stem cells helps detrusor muscle to be stronger and thereby, the contractility will be improve and gene therapy can enhance the contractility as well as stem cell therapy [85, 86]. Harada et al. showed that distigmine, which binds to muscarinic receptors directly,

apart from its adverse effects, may result in downregulation of muscarinic receptors that can worsen the situation more than before [87].

Following recent studies, chronic ischemia, spinal cord injury, diabetes, and bladder outlet obstruction (BOO) can lead to OAB in animal models. YC Huang et al. induced OAB in rats by diet with high fat and it was shown that hyperlipedimic situation has association with higher urine frequency and lower nerve intensity and blood vessels. Also, it was showed these adverse events surpassed when they injected adipose derived stem cells (ADSCs) were injected through veins of rat tails [88]. HJ Lee et al. induced BOO in rats with collagen deposit and investigated on the effect of MSCs on BOO. The level of expression of both collagen and TGF –β suppress and also, residual urine volume and voiding pressure ameliorate after injection of human MSCs. Finally, they explained that human MSCs could considered as a novel therapy for patients who were diagnosed with BOO [89], which was in concordance with YS Song et al's result [90]. OAB can be emerged by inducing occlusion in middle cerebral artery in rats according to Liang et al. They transplanted amniotic fluid stem cells in female rats' bladders and that bring about better function of OABs with increasing in nerve growth factor, M_2, M_3 muscarinic and P2X1 purinergic receptors [91]. Another method for inducing OAB in rats were provoking injury to common iliac artery that was performed by Liang and colleges. They showed when amniotic fluid stem cells inject into the tails of rats, the histological features and voiding parameters will be improved significantly by suppressing the chain of events that lead to oxidative stress [92].

There is scarcity of studies regarding employing stem cells in patients with UAB and the investigations are in early-stage. S Nishijima et al. made efforts to reinstated the contraction capacity of UAB by bone marrow transplantation in 12 female rats. In transplanted group, contraction of bladder increased and the layers of smooth muscle were seen in the wall of bladder. They concluded that stem cells can differentiate into cells that are alike to smooth muscle and thus, the power of contraction can be increased. Ischemia can be the cause of BD and capable of inducing BD that pathologically and functionally is alike to BD that aging induce. They injected bone marrow MSC into the common iliac artery of the rats and after that, bilateral iliac arteries were ligated and doxazosin was admitted orally. The result was satisfactory which demonstrated that with using this procedure the quantity of smooth muscle cells and nerve cells in the bladder wall would be rise and therefore, the better contraction of bladder can be expected.

A variety of models for inducing UAB are existed; but the model that is related to injecting streptozotocin has the most prevalence among them [93]. CC Liang et al. designed a study to assess the effect of human amniotic fluid stem cells on UAB due to induced-streptozotocin in female rats. They concluded the plausibility of amniotic fluid stem cells in diabetics rats with UAB that can be explained by recovery of nerve growth factor and muscarinic receptors [94].

In 2015, the first clinical study was done on a 79 years old man, who underwent transurethral resection of the prostate twice and while used alpha blocker drug, suffer from repetitive urine retention. After, FDA approval, autologous muscle-derived stem cells (MDSCs) were cultured with density of 250 million for 6 weeks.

Thereafter, 30 intradetrusor injections were performed without any complications. The result was extremely fascinating and urinary symptoms of patient improve versus before the surgery. Although the outcome was satisfactory, larger studies are warranted to used stem cells in patients with UAB widely [95, 96].

5.10 Stress Urinary Incontinence

The term urinary incontinency consists of different types of disorders, such as stress urinary incontinency, urgency urinary incontinency, and mix of both. Stress urinary incontinence (SUI) defined as unintentional leakage of urine during sneezing, coughing, or exertion and urgency urinary incontinence defined as sudden desire to urine which leads to unintentional loos of urine. SUI involves female more than male, and has various prevalence universally, which is between 29% and 75%. Hypermobility of urethral and impairment in intrinsic sphincter of urethra are the prominent cause of SUI in females [97, 98]. The bulk of treatments ranging from weight loos to surgical treatments are available for SUI. Surgical treatments encompass various approaches including mid-urethral and pubovaginal sling, and recently, while research pay attention to injection therapy and in the cases with sphincter dysfunction, limitations obstacle us to use both routinely [99]. In male, SUI occurs due to nerve injury following radical prostatectomy, or external sphincter damage during transurethral resection. Although different types of treatments have potential to be applied in male with SUI, selecting the treatment choice for patients depends on the severity of the disease. As above mentioned, using artificial sphincter in female with SUI necessitates more evidences. On the contrary, artificial sphincter in male with sever SUI provides satisfactory results despite the limitations that are existed. Inadequate options for treatment of SUI in both male and female addressed focuses on using stem cell therapy to provide a better function for urethra sphincter [100].

Two main types of SUI can be induced in animal models: (1) Temporary SUI generated by inflating a balloon with 2 ml of saline solution in the vaginas of female rats. This result in vaginal dilation, attenuating the muscles that support urethra, and damaging the nerve plexus of pelvic [101] (2) Persistent incontinence can be induced by injury to pudendal nerve, which regulates the function of external sphincter of urethra [102].

Reviewing the previous literatures that investigated on the feasibility of using stem cells in the patients with SUI revealed that different types of stem cells have been studies in this regard [103], and among various types of disorders that lead to BD, SUI has been studied **more than the others.**

First, MB Chancellor and colleges showed MDSCs that were injected into urethra and bladder of 8 rats with SUI, have potential to differentiate to myoblasts, which was confirmed by desmin test [104]. Longer time of survival (higher than 28 days) reach in Yokoyama et al's study, which was designed base on MB Chancellor et al's study [105]. Carr et al. chose 8 female with SUI who had not

responded to conservative treatments and injected them MDSCs. After 12 months of follow-up, 6 rats showed improvement in pad tests and quality of life and interestingly, in one rat incontinency was resolved completely [105]. The effect of different dosage of MDSCs in 38 female patients was assessed in 2013 clinical trial. It was shown that patients who had received doses $\geq 32 \times 10^6$ showed the better outcome versus who had received the dosage lower than that [106]. Two hundred and twenty two male patients with SUI due to postprostatectomy were treated with intraurethral injections of MDSCs. Approximately 50% of patients responded to treatment of whom 12% responded completely and 42% responded partially after 4.7 months of follow-up [107].

The benefits and efficiency of ADSCs in patients with SUI have been studied and the results were encouraging. G Lin et al. obtained ADSCs from ovary fat and injected them into 12 female rats via urethra and 6 female rats via tail vein and 10 rats received saline as a control group. After four weeks, 8 of control group, 4 of the urethra-ADSCs group, and 2 of tail vein-ADSCs group had abnormal voiding. They concluded that intra-urethra and intravenous injection of ADSCs may be beneficial in patients with SUI [108]. S Silwal et al. tried to improve recovery of cryoinjured rabbit urethras by ADSCs. They described that ADSCs through some mechanisms including increase in regeneration of neurons and differentiate into myogenic cells have potential to promoted the function of cryoinjured rabbit urethras and this results can be translated into clinical practices for the patients who suffer from SUI as a consequence of radical prostatectomy surgery [109]. In 2014, 5 female patients with SUI undergone injection therapy by ADSCs that were combined with bovine collagen and saline. They tested patients with cough test after 1 years from injection and cough test was negative for 3 patients and besides, according to questionnaires that were asked from patients, improvement in several subjects were seen [110]. Although satisfactory results were obtained regarding using stem cells in urethral disorders, lack of both experimental and clinical studies hindered us to bring them to clinical routines.

5.11 Penile Reconstruction

Several well-established acquired and congenital diseases of penis including micropenis, hypospadias, epispadias, aphallia, impotence, etc. may leave patients with no choice other than reconstruction of penis. This surgery due to spate difficulties despite numerous investigations that have been performed, remains challenging and the main challenge is lack of normal autologous tissue for repairing [111–113]. It was displayed that corpus cavernosum muscle can be generated as human corporal smooth muscle and endothelial cells seeded on polyglycolic acid scaffolds with two dosages (20×10^6 and 10×10^6 cells per cm^3), [114]. G Falke et al. designed a new method for generating corporal tissue, which containing cavernous smooth muscle, endothelial cells, and 3D acellular collagen matrices and was greatly similar to native tissue. After 4 weeks, engineered tissue implanted into mice and after

8 weeks, all tests such immunocytochemical showed present of both corporal muscle and endothelial cells [115]. In rabbits with penile defection, engineered corporal tissue was assessed structurally and functionally that both of them were satisfactory [116]. Although the engineering corporal tissue that had been formed was capable of erection, penetration, and ejaculation properly, it could not act as well as native tissue acts. With adding bioreactors to previous method the better function and structure of corporal tissue had been gained and corporal tissue that was generated with using bioreactors was much similar to native tissue versus corporal tissue that was generated without using bioreactors [117].

5.12 Penile Transplantation

While abundant improvement in reconstruction of penis has been occurred, for some patients this surgery is not applicable, especially whom with profound injuries that cause penile loos or had extremity injuries that cannot be reconstructed with autologous cells. As the incidence of these injuries has substantially increased, a more viable solution for penis reconstruction is needed. It has postulated that penile transplantation (PT) is desirable substitutional for penile reconstruction in patients who lack in flap donor sites or rejected penis reconstruction [118]. To date, PT has been performed 3 times. First, in 2006, a 44-year-old man that had lost his penis through an awful accident underwent PT. For that, dorsal arteries, superficial dorsal vein, and deep dorsal vein were anastomosed successfully and patient was treated with immunosuppressive drugs postoperatively. Although the final result of surgery was acceptable, after 2-weeks, patient requested to remove the transplanted organ due to psychological issues that he and his wife faced. Second, was done in South Africa on 21-year-old-male. In spite of thrombus formation after 4 days of surgery, after 3.5 months from surgery patient had normal sexual and urinary function [119]. The third was performed in US in patient with penile cancer [120]. The leading limitation of this procedure is necessitating long immunosuppressive therapy postoperatively, which can cause some serious complications itself [121].

5.13 Spermatogonial Stem Cell (SSC) Autotransplantation

Infertility has become the great concern worldwide with an incidence of over 15% in couples, who are at the age of productivity [122], and its prevalence mostly as a result of side of chemotherapy drugs are remarkably increasing [123]. A variety of treatments are presented to couples who produce functional gametes including hormonal therapy, IVF, ICSI, and intrauterine insemination and for those who are not able to produce functional gametes the merely clinical option that is available is donor gametes. There are situations that may account for disability of gametes, such as genetic syndromes, chemotherapy, radiotherapy, and immunosuppressive drugs

[124]. Hence, the incidence rate of population with disable gametes is rising and consequently, these conditions addressed attentions to explore the suitable treatment for those with disable gametes.

Recent researches provide hopes to use germline progenitors that are located in the gonads in patients with disable gametes in the future. The main germline progenitors are spermatogonial stem cells (SSCs) in men and ovarian stem cells in women. Two separate functions can be attribute to SSCs which are generating spermatogonia (initiate the spermatogenesis process) (figure) and self-production [125]. Brinster et al. described the novel method for transplanting SSCs of a donor into recipient and they also showed that if so, donor cell spermatogenesis is similar morphologically to native cell and also is capable of generating mature spermatozoa [126]. In 2001, transgenic animals by infecting SSCs with retoviral vector were produce [127]; however the lack of technique for culturing SSCs hampered to manipulated gene efficiently and this problem resolved when glial cell line-derived neurotrophic factor was introduced [128]. Another approach was explored by M Kanatsu et al. in 2006 that was based on transfection of SSC with a vector that contained a gene which was resistant to drug [128].

The transplantation approach gradually has been evolved. The first experience belongs to Brainster et al. that showed the feasibility of SCCs transplantation to restoring fertility in three rats that lasted for 8 months. Ogawa et al. showed SCCs transplantation from steel mice can bring about to restore fertility for 5 months. Most of studies using SSCs from sexually immature animals and the effectivity of SSCs from mature animals had not been experienced. The first SSCs transplantation in primates was belong to BP Herman et al. that assessed the feasibility of autologous SSCs transplantation in 18 adults and 5 prepubertal macaques, who were infertile as a consequence of using alkylating chemotherapy. The sperm of one recipient was intracytoplasmic injected into 85 rhesus oocytes. Eighty one of oocytes were fertilized successfully and producing embryos with different cell content [129].

So far, no clinical trials on human have not been experienced and there are some issues that must be handle before doing that. First, if patients who suffer from malignancy, especially hematopoietic malignancies volunteer for donating SSCs, no signs of malignancy should not be existed in transplanted cells [130]. Second, it is very important to maintain the stability and integrity of DNA of SSCs through whole the procedure and if that happen, the next generation of offspring can be affected seriously [131].

5.14 Conclusion

With respect to repairing damaged tissue or organ, due to various complications arise following use of autologous tissue or allograft, lots of attempts have addressed to find the better substitution rather than using them. Investigations explored new methods and techniques that can be pertained to remaining the normal function and structure of a tissue or organ that has been injured by using stem cells. Also, the

application of stem cells has been studied in numerous studies related to urological diseases. While, a vast number of studied showed encouraging outcomes concerning urological diseases, the majority of studies were performed on animals and clinical studies were not as much as experimental studies. Additionally, there are limitations that obstacle us to translate stem cells into clinical applications. Therefore, studies in this regard necessitate further verification and expansion to have potential to bring them into clinical practices.

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Chapter 6
Erectile Dysfunctions

Seyed Mohammad Kazem Aghamir and Fateme Guitynavard

Abstract So far, many basic studies addressing the concept of stem cell therapy for ED. The stem cells mechanism of action is to induce angiogenesis and so increase the cavernosal smooth muscle cells within corporal bodies. Generally, erectile dysfunction treatment focuses on the symptomatic reprieve and, thus, aims to provide a temporary relief rather than cure or reverse the cause. The large number of difficult-to-treat patients has motivated the researchers to look for new treatment approaches that instead of offering ad hoc symptomatic care focus on the cure and restoration of the underlying cause. Regenerative medicine has evolved widely over the past few decades and the effects of growth factor therapy, gene transfer, stem cells, and tissue engineering to restore erectile function have been demonstrated in preclinical trials.

With the subject of administration of stem cells in animal models of erectile dysfunction due to aging, type 1 and type 2 diabetes, cavernous nerve injury, Peyronie disease, and even penile trauma, a number of preclinical studies have been published. Based on these studies, there is a general consensus among researchers that using mesenchymal stem cells -mainly from the bone marrow and adipose tissue- would be a promising approach for treatment of ED.

Human umbilical cord blood stem cells have recently demonstrated beneficial effects on erectile function when administered into the penises of men with severe type 2 diabetes. This influence, however, has been short-lived and not lasting. In an open dose-escalation study, another Phase I trial investigated the intracavenous administration of bone marrow cells following radical prostatectomy and reported no serious adverse effects.

Conclusions on the efficacy of these trials should be drawn with the caution as these trials are designed to test safety (no control group); however, preliminary efficacy results were encouraging, with improvement in erectile function and penile vascularization measurements in a small set of patients. Although these preliminary safety data are promising, there is an eager anticipation for larger Phase I – III studies and practical tests.

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This chapter discusses the current status and future outlook of stem cell therapy for erectile dysfunction treatment.

Keywords Erectile dysfunction · Treatment · Stem cell

Erectile dysfunction morbidity is around 50% in adult men, which means that more than 300 million males worldwide are troubled by this condition. Oral inhibitors of type 5 phosphodiesterase (PDE5-Is) are commonly used as first-line ED treatment. PDE5-Is' clinical efficacy exceeds 70% with mild side effects; however, PDE5-Is' clinical efficacy for serious ED caused by diabetes, surgery, and severe cardiovascular disease is limited [1]. While recent data have revealed that daily intake of tadalafil (5 mg) helps improve endothelial cell function in ED patients, this is only a preliminary research and its mechanism is still unknown [2]. Second line ED treatments include vacuum devices and intracavernosal injection therapy. Generally, penile erection is induced after 5–10 min after the injection of the drug at an effectiveness of about 70%, but may followed by common complications such as pain, priapism and fibrosis [3]. Prosthesis implantation is typically the last option for patients who do not respond to first-line and second-line treatments. Although the effective rate of implantation of prosthesis is about 90 percent, it implies significant limitations including high cost, risk of infection, erosion, and failure of equipment [4]. All of these existing ED approaches are primarily used, as on demanded treatments, to temporarily improve erectile function just to relieve symptoms and not to reverse pathological changes in erectile tissues [6, 7], and most patients require an optimal approach to restore their normal erectile function, creating an important scientific question for future ED studies. Stem cells (SCs) are considered to be unspecialized precursor cells capable of differentiation as well as self-renewal. SCs including adipose-derived SC (ADSC) [5], bone marrow SC (BMSC) [6], embryonic SC (ESC) [7], endothelial progenitor cell (EPC) [8], and skeletal muscle SC11 have recently attracted great attention in reversing pathological changes in animal models of erectile ED tissues. Increasing evidence has shown that SCs primarily secrete certain cytokines to boost improvements of pathological changes in ED [5, 9, 10]. Because of ethical problems and immunogenicity, the use of ESC imposes certain limitations. New technologies have recently emerged in order to reprogram adult somatic cells into a pluripotent state [11]. Such cells, usually called "pluripotent stem induced (iPS) cells," facilitate transplantation without immunosuppression. But we should also note that iPS cells are at risk of tumorigenicity and may induce an immunological reaction in some circumstances. On the other hand, most postnatal tissues contain SCs usually referred to as "adult SCs (ASCs)" or "tissue-specific SCs." ASCs have recently developed as a basis for regenerative therapy because they are readily accessible and used after ex vivo purification and expansion for therapy. However, time/cost-intensive and invasive methods and the low retention rate at the injected site restricted the clinical use of ASCs [12]. Stem/progenitor cells exist throughout any adult organ and tissue that may play a significant role in homeostasis of tissue and healing injury. While increasing evidence indicates the

existence of penile endogenous SCs, the regenerative capacity of endogenous SCs/progenitor cells provides new insights into ED therapy [13–15] while the underlying mobilization and recruitment mechanisms about the mobilization and selection processes are far from being fully known.

6.1 Stem Cell Niches

A stem cell niche is a highly organized microenvironment, consisting of signaling molecules, intercellular communication, and interaction between SCs and their in vivo environment. SCs are in contact with the supporting cells within a niche, which provide short-range signals by soluble factors as well as transmembrane proteins. SCs also maintain close contact with the extracellular matrix, a dynamic network that provides biochemical and mechanical signals chemically and physically cross-linked. It is known that blood vessels represent niches, bear long-range signals, and recruit circulating cells into bone marrow (BM) or tissue-specific niches [16, 17]. In addition, metabolic signals such as reactive oxygen species can also affect the niche function [18]. The SC niche's key role is to maintain the quiescent and activated SCs 'fluid balance. Physiologically residing SCs in tissue-specific niches will support/replace the SC pool and differentiate into multiple matching cell lines [19]. It is now well-documented that SC niches are present in many adult organs and tissues including BM, brain, skin, skeletal muscle, heart, gut, and ovarian epithelium [20–22]. The BM is many forms of SCs' main reservoir. A small number of SCs are constantly leaving the BM under a steady state, joining the blood or tissues, and traveling back to the BM or peripheral tissue-specific niches. Specific cellular components within the BM niche, including vascular/perivascular cells [23], nestin positive MSCs [24], osteoblasts [25], macrophages [26, 27], and sympathetic nervous system neurons [28] were identified as important regulators for maintaining and functioning SCs. The interstitial cells produce adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) and the SC factor (SCF) binding to the HSC receptor α4 β1and c-kit, respectively, as shown in the hematopoietic SC (HSC) niche [29]. However, the binding of stromal derived factor-1 (SDF-1, also referred to as CXCL-12) to its receptor (cxc chemokine receptor 4, CXCR4) on the HSC surface also plays a key role in the preservation of HSC within the BM [30]. It is believed that the SC microenvironment (niche) influences/controls the "stemness" of SCs, i.e., self-maintainment or differentiation [31]. The value of niche as a SC regulator for deciding cell fate is exemplified by the fact that when separated from their microenvironment and cultivated in vitro, SCs appear to differentiate quickly. An interesting theory is that depending on the correct epigenetic signals in mature tissues, SCs may undergo different patterns of differentiation. For example, after direct exposure to myoblasts and transplantation into the skeletal muscle, Galli et al. [32] demonstrated that human neural SCs could produce skeletal muscle cells in vitro and in vivo, respectively. Zhao and others [33] Human BMSCs implanted in the brain are found to be capable of differentiating into neural cells expressed as astrocyte markers (GFAP(+)), oligodendroglia (GalC(+)) and neurons

(beta III(+), NF160(+), NF200(+), hNSE(+) and hNF70(+)). These findings support the ability of SCs to produce terminal differentiated cells specific to their host niche.

However, further investigation still involves the mechanism of SC differentiation governed by the particular niche.

6.2 Endogenous Stem Cells

For physiological tissue turnover and regeneration, tissue-specific ASCs are essential. Mammalian ASCs are typically in a mainly quiescent state (out of cell cycle and lower metabolic level) [25], but are capable of exiting quiescence and growing and differentiating rapidly in response to stress.

In fact, SCs have different destinies in their niches: (i) SCs I remain in a relatively quiet (non-dividing) state, (ii) undergo apoptosis or death, and (iii) cause self-renewal divisions resulting in two daughter SCs (determined symmetric divisions), either one daughter SC and one dedicated progenitor (determined asymmetric divisions), or two differentiated cells [34]. In general, in rapidly growing tissues, two states of activation and quiescent SCs coexist, which can convert to each other. Improved understanding of regulatory mechanisms that direct the determination of SC fate has great significance in the modulation and activation of existing SCs in the tissues.

It appears that the quiescent state is necessary to preserve SCs' long-term reconstitutive capacity. This is best illustrated by HSCs: it remains quiet in a stable state and only a small collection of HSCs undergo massive expansion to create mature blood cells, ensuring a lifetime supply of blood without draining the HSC reservoir [35].

But, in response to stress, HSC will escape quiescence and proliferate rapidly to restore homeostatic conditions [36]. Deficiencies in regulating the quiescent state resulting in premature HSC pool saturation. Mechanisms and extrinsic signals that work together can regulate the quescence and activation of SCs.

Recent research has shown that p53 is essential to self-renewal and quiescence of SC. Meletis et al. [37] found that p53 suppresses the spread and self-renewal of neural SC. Liu et al. [38] showed that two target genes of p53, Gfi-1, and Necdin, are important HSC quiescence regulators.

Foxo 3a is a transcription factor forkhead-type which is essential for SC quiescence [39]. Without Foxo 3a, NSCs may lose their ability to re-enter a state of relative quiescence which may lead to progenitor amplification and in vivo exhaustion of NSCs [40]. Hypoxia-inducible factor-1α (HIF-1 α) is a master transcriptional regulator under low oxygen conditions. HSCs from HIF-1α-deficient model reduced quiescence and decreased the number during various stresses including bone marrow transplantation, myelosuppression, or aging [41].

The nuclear factor of an activated T cell c1 (NFATc1) has been reported to be preferentially expressed by hair follicle SCs in their niche where its expression is activated by the upstream signaling bone morphogenic proteins (BMPs) and acts downstream to transcriptionally repress CDK4 and maintain SC quiescence.

Hair follicle SCs are stimulated to proliferate and form a new hair follicle once NFATc1 is pharmacologically inhibited (e.g., cyclosporine A) [42]. Goldstein et al. [43] showed that NFATc1 up-regulates the expression of prolactin receptor which drives the quiescence of hair follicle SCs via JAK/STAT5 signaling. For adult hematopoiesis, interactions between SCs and their in vivo environment are critical.

A broad spectrum of cell functions including cell cycle regulation, neuronal differentiation, and survival are engaged in the transforming growth factor-beta (TGF-β) family.

TGF-β1 neutralization releases early human hematopoietic progenitors from the quiescence of early human hematopoietic progenitors from the quiescence into in vitro cycling [44]. Kandasamy et al. [45] showed that TGF-β1 can promote both SC quiescence and neuronal survival.

BMPs have been participating in hematopoietic production as essential regulators. Further more, the interaction between BMP and the Wnt signaling pathway is involved in the activation of SC hair follicles by inducing β-catenin into the nucleus [46, 47].

Arai et al. [25] expressed that the signaling pathway Tie2/Angiopoietin-1 plays a critical role in preserving HSCs in a quiescent state in the BM niche. Adhesive molecules (e.g., N-cadherin and β1-integrin), thrombopoietin, osteopontin (Opn), and more are other extrinsic micro-environmental regulatory mechanisms.

6.3 Stem Cells Mobilization

Effective SC functions require SC trafficking regardless of whether the cell is administered endogenously or exogenously [48, 49]. SC homing refers to the ability of SCs to make their way to a specific anatomical destination in the field of SC research.

The body's self-healing through the host cells is likely to involve recruiting the endogenous SCs from either the BM through the bloodstream or a tissue-specific niche in response to disease or tissue injury [50–52]. Asahara et al. [51] found that there are EPCs in the peripheral blood of humans and that they can be integrated into active angiogenesis sites. Rochefort et al. reported that when animals are exposed to chronic hypoxia, multipotential mesenchymal SCs may be activated into the peripheral blood.

Furthermore, SCs/progenitor cells found within the healthy tissues of the surrounding area may also be recruited for therapeutic purposes to an injury site. Of example, neural precursors in the subventricular zone (SVZ) play an important role in the repair of focal brain lesion [53]. In this regard, endogenous SC homing therapeutic interventions have a great clinical potential.

The most widely used agent for clinical HSC mobilization is the granulocyte colony-stimulating factor (CSF). It was used to recruit CD34(+) cells into peripheral blood, which for autologous and allogeneic cell transplantation has largely replaced BM as a source of SCs [52]. Possible mechanisms underlying its work include

promoting granulocyte expansion, clearing VCAM-1, also referred to as CD106 [54], and disrupting the SDF-1/CXCR4 axis [55]. SDF-1/CXCR4 axis plays a pivotal role in HSC quiescence and retention within BM. A CXCR4 antagonist, Plerixafor (Mozobil, AMD3100) can inhibit the binding of SDF-1α to its CXCR4 receptor. It enables the rapid mobilization into the peripheral blood of CD34 (+) cells from BM [56]. Cyclophosphamide, granulocyte-macrophage CSF and recombinant human SCF are other therapeutic agents used for mobilization of SCs.

Therefore, there are also some new and experimental mobilization methods for SCs. These methods target mainly molecules of cell adhesion (VCAM/VLA4 inhibitors and proteasome inhibitors), redox signals (HIF stabilization), chemokines (CCL2, CXCL-1, CXCL-7, CXCL-12), and their receptors (CCR2, CXCR3, CXCR4). By incorporating SDF-1α to encourage the recruitment of endogenous SCs for in situ tendon regeneration, Shen et al. developed a knitted silk-collagen sponge scaffold. SDF-1α treatment group had increased tendon repair gene markers expression after 4 weeks and had a better therapeutic effect than the control group. Meanwhile, in combination with BMP or TGF-β1, Chim et al. [57] used SDF-1α to induce cell migration in a rat model. The results showed that SDF-1α promotes cell migration into the scaffolds and may result in differentiation between osteogenes and chondrogenes. No exogenous SCs were used in both experiments prior to scaffold implantation. Rather, to achieve tendon regeneration, osteogenesis, and chondrogenesis, they all relied on endogenous SC recruitment and local tissue responses.

6.4 Stem Cell Localization

Tissue-specific SCs are commonly believed to exist in most postnatal tissues. BM is the largest SC pool with two types of SCs at least: HSC and mesenchymal SCs. The former comprises the whole hematopoietic network, and the latter is a perivascular cell subpopulation [24, 58, 59].

Neural SCs reside primarily in their specialized microenvironments: the lateral ventricle SVZ and the hippocampus subgranular area of the dentate gyrus [60, 61].

The skeletal muscle has a remarkable ability to regenerate as a result of mobilizing its own tissue-specific SCs, known as satellite cells, situated between basal lamina and muscle fiber sarcolemma [62]. Lin et al. [63] first described the position of ADSCs in human adipose tissue in 2008 and found that ADSCs are likely to be vascular SCs at various differentiation stages. A study conducted by Bussolati et al. [64] showed that it was possible to isolate adult renal progenitor cells expressing PAX2 and antigen CD133 (a HSC marker) from human renal cortex samples. In adulthood, homeostasis and repair of tissue are critically dependent on both self-renewal and the capacity to differentiate these SCs.

Different approaches were used to classify these cells, including the use of SC markers. Leucine-rich protein-coupled receptor 5 (Lgr5) is a 7-transmembrane receptor that has recently gained popularity in the hair follicle and intestine population as a marker of ASCs [65–67]. A closely related protein, Lgr6 identifies hair

follicle ASCs that produce all skin cell lines [68]. It is worth noting, however, that CD34 is the classic marker for HSCs. Although it also expresses in some non-SCs such as endothelial cells [69] and c-kit, another HSC marker is also expressed in Cajal and urinary tract interstitial cells [70, 71]. Stro-1 is one of MSC's most well-known markers, but is not universally expressed in all reported MSC types [72].

Due to the lack of specific tissue-specific SC markers, potential SCs have been identified in some studies using the technique of "label-retaining cell (LRC)" [73, 74]. Quiescent SCs have some common characteristics, including low RNA, 81 scarce proliferative cell markers, 82 and preservation of some cell labels [75]. LRCs strategy for identifying quiescent SCs is frequently explored. The underlying mechanism is based on the principle that rapidly proliferating cells will in a short time lose the cell label while the quiescent cells and slow-cycling cells will hold the label for a longer period of time. There are two essential parts in a label-retaining assay: a pulse cycle and a chase period.

For a certain time (the pulse) BrdU, EdU, or radiolabel led DNA analogs can be administered to animals to label all proliferating cells. Before the tissues are examined, the labeling reagents will be removed for a prolonged period (the chase). Fast-cycling cells divide and dilute the lable continuously through each division round. Hence, their original label is diluted after the chase to a degree that it cannot be identified anymore. In comparison, during the chase phase, the slow-cycling cells rarely divide. We therefore hold large amounts of the label and function as LRCs. EdU labeling was first developed in 2008 and is one of the most active and important labeling techniques in endogenous quiescent SC research since it does not interfere with cell replication, differentiation, secretion and mobilization [76].

6.5 Penile Endogenous Stem Cells

Penis consists of several tissue types such as skin, tunica albuginea (TA), corpora cavernosa, corpus spongiosa, blood vessels, nerves, and urethra. To date, two types of foreskin SCs namely skin-derived progenitors (SKPs) and MSCs have been identified. Toma et al. isolated, extended, and characterized SKPs from mammalian dermis [77]. They then observed that in the presence of mitogens fibroblast growth factor 2 human SKPs formed in suspension as spheres and expressed nestiny. The clonal analysis showed that single SKPs are multipotent and could contribute to neural as well as mesodermal progeny [78]. In the meantime; MSCs have also been isolated from postnatal human dermal tissues. The isolated cells could expand clonally and be divided into adipogenic, osteogenic, and myogenic lineages [79]. All of these findings suggested that penile skin could provide an open, autologous origin of SCs.

Vernet et al. [13] examined whether cells from plaques of normal TA and Peyronie's disease (PD) undergo osteogenesis, express SC markers, or give rise to other cell lines via TGF-β1-modulated processes. The osteogenic markers (alkaline phosphatase and Opn) and calcification have been found in the osteogenic medium

in TA and PD cells. All cultures expressed SC marker CD34, but in the adipogenic environment, none of the cultures encountered adipogenesis. TGF-β1 incubation increased differentiation of osteogenesis and myofibroblast and decreased expression of CD34 antigen in both cultures. Furthermore, putative endogenous SCs have been shown in parts of penile shaft tissue from murine by detecting CD34 and a potential variant of Sca1 [14].

Lin et al. [15] recently used the LRC technique to identify potential penis SCs/progenitor cells. In this analysis, they found that EdU had marked multiple cells in the neonatal rat's penis, but within 1 week the number of labeled cells fell sharply. Moreover, the labeled cells were distributed primarily in subtunic and perisinusoidal spaces. EdU-labeled LRCs can form cell clones after in vitro isolation and culture, which is one of the SC properties. They also noted, however, that EdU randomly labeling penis cells and label persistence was not correlated with some of the SC markers such as c-kit or PCNA except A2B5. The results suggest that modulation of penile endogenous SC may be a viable approach to ED therapy.

The translation of physical medical signals into biomedical signals is considered to be the third biomedical revolution. Low-energy extracorporeal shock wave therapy (LESWT) has now been widely used as a novel non-invasive wound healing strategy [80], bone regeneration [81], inflammatory amelioration, osteochondritis dissecans [82], chronic hind limb ischemia [83], plantar fasciitis [84], ED [85, 86] and many others.

Ironically, some of these studies pointed to the possibility of mediating beneficial tissue effects by recruiting mesenchymal SCs. LESWT was found in a study of a rat model of chronic hind limb ischemia to improve the recruitment of circulating EPCs into non-ischemic as well as chronic ischemic tissue [83]. In another study, Chen et al. [87] showed that LESWT care could be improved. More importantly, by promoting the regeneration of erectile components (nNOS-positive nerves, endothelium, and smooth muscle) in the penis, Qiu et al. [88] also found that LESWT can improve diabetes mellitus (DM)-associated ED. We used LRC strategy in the study to classify putative SCs and found that LESWT therapy could significantly increase the penis cell called EdU. Nonetheless, it is still uncertain if endogenous SCs or other pathways are achieving such therapeutic effects of LESWT on ED.

6.6 Endogenous Stem Cell Activation

For the differentiation of different SCs/progenitor cells, p38 pathway is essential. Jones et al. [89] found that somatic SCs could be activated from the quiescent state by activating the p38 pathway and undergoing myogenic differentiation. In addition, adult neural differentiation can also be promoted via the activation of p38 pathway [90]. Herba Epimedii, a traditional Chinese medicine, has long been used in East Asian countries for the treatment of ED. Numerous studies [91, 92] have shown that icariside II (ICA II, C27H32O10, 514.54) has substantial erectogens in ED care. Interestingly, a recent study stated that p38 pathway can be activated by

ICA II [93]. Xu et al. recently conducted a study using LCR strategy to monitor putative endogenous SCs to examine the underlying mechanisms of ICA II in the treatment of ED. The results showed that ICA II in a rat model of bilateral cavernous nerve injury could effectively recover the erectile function and prevent deterioration of penile histopathological changes. However, the results indicated that ICA II's therapeutic effects include enhanced differentiation of endogenous SCs, which can be controlled by p38 pathway. On the other hand, in vascular repair, EPCs play an important role [94]. A study showed a reduction in the number of circulating EPCs in patients with ED [95]. In addition, EPCs residing in the vascular wall are able to differentiate into mature endothelial cells, hematopoietic and local immune cells. These results indicate that EPCs in the penis may also serve as a source for the progenitor cells for postnatal vasculogenesis. Increasing evidence has demonstrated that pathophysiological mechanisms of diabetic ED are associated with oxidative stress [96, 97]. Melatonin, an antioxidant, can reduce the level of oxidative stress induced by diabetes [98]. Qiu et al. [88] found that the effect of melatonin on the prevention of ED in a DM rat model was beneficial. Chronic melatonin administration increased the level of superoxide dismutase in this study and decreased the level of malondialdehyde in BM followed by an increased number of circulating EPCs. Such results indicated that melatonin's beneficial effect on ED could result from the mobilization of EPCs.

6.7 Conclusions

Because the high prevalence of erectile dysfunction in all around the world, different therapeutic approaches have become the focus of many studies, so far. Among them, stem cell therapy is a novel approach with relatively promising results. Another interesting issue in this area which is currently proposed is the use of penile endogenous stem cells for treatment of ED. So, many strong preclinical and clinical trials should be conducted to prove the safety, efficacy and durability of these new approaches.

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Chapter 7
Bladder Dysfunction

Seyed Mohammad Kazem Aghamir and Fateme Guitynavard

Abstract The studies focusing on the use of stem cells in treatment of different medical condition is growing over the time. But yet a few studies conducted to evaluate efficacy and safety of stem cell therapy in different types of bladder dysfunction. In addition, these studies are mainly focuse on experimental models rather than tissue engineering and bladder regeneration. There are some defined models of bladder dysfunction in literature: bladder outlet obstruction, cryoinjured, diabetes, ischemia, and spinal cord injury models. Among the different subgroups of stem cells, adipose derived stem cells (ADSCs), skeletal muscle derived stem cells (SkMSCs) and bone marrow stem cells (BMSCs) are used more commonly in favor of bladder dysfunction treatment. These stem cells with unique characteristics and multiple mechanisms of action (migration, differentiation and their paracrine effect) are so suitable for using in different clinical approaches to treat bladder dysfunction including bladder bioengineering and bioprinting.

This chapter is aimed at providing the current status of using stem cells for bladder dysfunction treatment as well as exploring future prospects on this topic.

Keywords Bladder dysfunction · Treatment · Stem cell

7.1 Introduction

While various therapies have been developed for different types of bladder dysfunction, such as detrusor overactivity or underactivity, but little progress has been made in reduction of voiding dysfunction using stem cells. Recently, growing attractions are toward stem cell therapy in the field of bladder dysfunction and investigators are willing to document promising results in this area.

Stem cells (SCs) or Mesenchymal stem cells (MSCs) have ability of self-renewal and differentiation to create different lines of mature cells [1]. Because of their distinc-

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tive characteristics, unique plasticity of migration, and capacity for tissue repair or regeneration, stem cells are use to perform injury repair in different injured organs. Among bladder dysfunction models, bladder outlet obstruction (BOO) is the well-defined one. The other forms of bladder dysfunction template are yet in an incomplete state. There are current clinical efforts to both prevent and cure BOO. There are studies conducted to provide better understanding of the cellular-level consequences and specific mechanisms responsible for developing BOO. Although abundant reports have demonstrated the MSCs capability to engrave different tissues like brain, heart, liver, and lung, data on bladder dysfunction repair is still scarce [2–4].

7.2 Stem Cells Sources and Their Mechanism of Action in Bladder Dysfunction Recovery

MSCs have self-renewing ability and can differentiate into a range of different cell types, such as chondrocytes, osteoblasts, and adipocytes. While all MSCs including bone marrow stem cells (BM-MSCs), skeletal muscle stem cells (SkMSCs), and adipose tissue stem cells (ADSCs) have similar properties, their availability vary very much based on therapeutic goals [5]. For instance, although SkMSCs need a long expansion with a difficult isolation procedure, it is possible to prepare ADSCs within a few hours. ADSCs are some kinds of mesenchymal cells which are found in the perivascular areas of the adipose tissue [6]. The advantage of ADSCs is that plenty of them are easily accessible in comparison to other types of stem cells. In experimental studies ADSCs showed efficacy on urological diseases [7, 8]. SkMSCs are primarily used in injury models [9, 10]. As a stem cell source for autologous transplantation, SkMSCs have several benefits because the skeletal muscle can be reached quite easy and safe and during surgery SkMSCs can easily be harvested. Cells in the $CD3^{-}/CD45^{-}$ fraction (Sk-DN cells) and $CD34^{+}/CD45^{-}$ fraction (Sk-34 cells) can reconstitute nerve-muscle units of the blood vessel synchronously after transplantation. SkMSC transplantation results in significant functional regeneration of skeletal muscle cells, vascular cells and peripheral nerve cells through cell differentiation [11, 12]. So, different human tissues can be used as the source of stem cells and selection is based on the goal of their therapeutic use.

Stem cell migration, differentiation and their paracrine effect are discussed here for better understanding of these cells mechanism of action for treating bladder dysfunction.

SCs migration into the bladder tends to be associated with improvements in histopathological and functional parameters [13]. MSCs can migrate into the damaged, ischemic or inflamed tissues. This migration is contributed to expression and secretion of specific chemokines by such tissues [14]. There is a wide range of studies on the stem cells migration into many different organs [15–18].

Differentiation is the novel mechanism for stem cell therapy, and bladder regeneration via differentiation has been recurrently shown in models of nonpathogenic bladder. Many studies conducted focusing on non-pathogenic tissue regeneration

models have documented the differentiation of stem cells into detrusor smooth muscles that can finally lead to bladder repair or even replacement [19, 20].

Although differentiation is an important mechanism, it seems rational to assume the effects of paracrine cytokines and growth factors released by transplantated MSCs or adjacent cells. That is called "paracrine effect". SCs secretory factors are shown to induce therapeutic effects by regulating local and systemic immune responses and promoting regeneration of local tissue, as well as recruiting host cells. MSCs replace damaged cells, by secreting growth factor via their paracrine effect [21]. BM-MSCs or ADSCs may secrete multiple growth factors, such as insulin-like growth factor (IGF), hepatic growth factor (HGF) and endothelial vascular growth factor (VEGF) [22]. They play an important role in an antifibrosis pathway in the damaged tissue, which indicates that the reduction of fibrosis is rather contributed to paracrine processes than cell incorporation [15, 23, 24]. HGF as a strong mitogen of hepatocytes has an important role in tissue regeneration [21, 25]. Besides antifibrotic functions, BM-MSCs or ADSCs can also secrete free radical scavengers and antioxidants into ischemic tissues [26].

These three interesting charachteristics of stem cells make them capable for using in treatment of various pathologic conditions and that's why stem cell therapy attracts attentions for treating bladder dysfunction.

7.3 Stem Cell Therapy and Pathogenic Models of Bladder Dysfunction

Kim et al. in a comprehensive review explain deferent models of bladder dysfunction such as bladder outlet model, bladder ischemia model, diabetes model, etc. [27]. The BOO model is the only well-described model of bladder dysfunction and the other pathological models are yet in a challenging condition.

7.3.1 Bladder Outlet Model

Bladder outlet obstruction (BOO) as a result of collagen accumulation is a common condition involving elderly males. Deposition of collagen in the bladder is seen in various pathological processes and ultimately ends in bladder fibrosis and makes the bladder flaccid. The bladder fibrosis impairs function of detrusor smooth muscles and bladder compliance [28]. Bladder dysfunction was observed when the bladder outlet was obstructed [29].

Lee et al. stated that in a rat BOO model, transplantation of human MSCs marked with nanoparticles (superparamagnetic iron oxide) into the bladder, prevented fibrosis and improved bladder dysfunction [16]. Growth factors also have an important role in bladder wall remodeling following an outlet obstruction [30]. This finding

that human MSCs over-expressing HGF inhibite collagen deposition and improved cystometric parameters in rat BOO, was also reported by Song et al. [17].

Fibrosis and hypertrophy are believed to cause vessel compression that lead to reduction of bladder blood flow. So, as a result, severe tissue ischemia can be a possible explanation of bladder dysfunction [31, 32].

Differentiation of MSCs into the detrusor smooth muscles is not only make them suitable to treat detrusor overactivity but also make them useful in underactive detrusors. Nishijima et al. [33] showed that transplanted BMCs would cause an improvement in detrusor muscles contractility after differentiation into smooth muscle-like cells in an underactive BOO bladder.

7.3.2 Bladder Ischemia Model

Using bilateral ligation of the iliac artery [34] or hyperlipidemia [35], The ischemia prototype for the bladder is found. Several research [36] have shown that ischemia can lead to major structural and functional changes in the bladder. The bladder dysfunction mechanism caused by ischemia is complex, and ischemic denervation may be involved. This makes the M-cholinergic receptors hypersensitive to acetylcholine [37] which results in bladder overactivity. Since the ischemia is a high probable process in the elderly, ischemia rat model can be a proper model for investigating detrusor changes caused by aging [34]. Huang et al. [35] indicated that bladder instillation or intravenous administration of ADSCs can improve both tissue and urodynamics parameters in rats with overactive bladder.

7.3.3 Diabetes Model

Diabetic bladder dysfunction (DBD) usually causes gradual and progressive impairment in both storage and voiding phase. In early phase, DBD causes detrusor overactivity. Over the time, detrusor muscle will be decompensated, resulting in an underactive or atonic bladder.

In rats treated with ADSCs, Zhang et al. [38] reported voiding function improvement compared to saline rats treated with phosphate buffer. The DBD trend in their experimental model was hypocontractile bladders. Although some ADSCs have been transformed into detrusor smooth muscles, their paracrine antiapoptotic effects can not be ignored in this process. These data will offer an opportunity for clinical use of stem cell therapy for difficult-treating underactive bladder conditions.

7.3.4 Spinal Cord Injured Model

spinal cord injury (SCI) causes so many lower urinary tract problems such as recurrent infections, impaired bladder compliance and voiding dysfunction [39]. In a study, it was shown that spinal cord injured rats had a higher thickness of bladder wall and a higher collagen to smooth muscle ratio [40].

The main goals of urinary tract care in spinal cord injured patients is to reduce the episodes of urinary infections, maintain function of kidneys, and enhance patients' quality of life. In an animal model study, neural stem cell transplantation into the damaged spinal cord caused an improvement in behavior of the bladder [41].

The functional recovery of the bladder after SCI is limited because new neurons or glial cells are not generated after maturation of central nervous system.

Nonetheless, recent studies have shown that transplanted neural progenitor cells make it easier to restore bladder function by regenerating the damaged tissues [41–44]. Stem cells are directly inserted with a needle into the affected lesion in most of these trials. In an study it was shown that intravenously administered BMSCs resided in L3-4 which cause bladder function improvement in rats following spinal cord injury [45]. So, both intravesical and intravascular administration of the stem cells can be used in treating bladder dysfunction in spinal cord injured patients. Although, more strong studies are required to assess the safety, efficacy and durability of stem cell therapy and studies to make comparison between different rout of stem cell administration.

7.3.5 Cryo-Injured Model

In cyro-injured model, bladder hypertrophy exists but with an inappropriate collagene to smooth muscle ratio just like what happens in BOO models [46]. The main result of stem cell transplantation into cryo-injured model is to decrease surviving smooth muscle cells' size and differentiation of stem cells into the smooth muscle cells. This compensatory smooth muscle cells hypertrophy play a key role in remodeling of the injured bladder.

Huard et al. [47] showed that injected muscle-derived cells (MDCs) could nest in the bladder and enhance the bladder contractility in the cryo-injured model.

Sakuma et al. [48] have shown that fat cells that were dedifferentiated could differentiate into smooth muscle cell lines and contribute to bladder smooth muscle regeneration.

Thus, interestingly not only stem cells but also dedifferentiated cells can be used for treatment of bladder dysfunction.

7.3.6 Other Bladder Dysfunction Models

Based on Nitta et al. [9], transplantation of multipotent stem cells originating from the skeletal muscle in the bladder branch of pelvic plexus (BBBP) causes a drastically higher bladder functional improvement in injured model. Kwon et al. [10] achieved similar results in rats with unilateral transected pelvic plexus.

7.4 Regeneration of the Bladder

As far as bladder tissue engineering is concerned, there are few revolutionary studies which have shown that stem cells or BMSCs derived from embryoid bodies seeded on small intestinal submucosa (SIS) promote regeneration in partially cystectomized model [49–51]. Recently, many other types of stem cells which are seeded on bladder acellular matrix (BAM) demonstrate potential for bladder regeneration like hair stem cells and ADSCs [52, 53]. In studies on the use of synthetic scaffolds instead of using BAM and SIS results showed that BMSCs seeded on thin film of 1,8-octanediol-co-citrate can lead to bladder regeneration [54]. In addition, Tian et al. demonstrated the potential for bladder engineering of BMSCs with myogenic differentiation which are seeded on polylactic acid scaffolds [9, 55]. Similarly, polylactic glycolic acid seeded with human ADSCs with myogenically differentiation preserved both bladder compliance and capacity when transplanted into partially cystectomized rats [19]. In comparison to use of differentiated cells, bladder tissue engineering by the use of MSCs could produce better results. MSCs can differentiate into SMC after migration to the bladder's grafts and [56] such cells will replace the grafts rapidly with a good neural function and also low fibrosis formation [48].

During the past two decades researchers have eagerly waited to see the regenerated bladders full success, while over the last 80 years the intestine was effectively used to replace the bladder. So, one of the organs that can be a target of stem cell researches is the human bladder. Nonetheless, these studies are very limited; there are no systematic reports of dysfunction of the bladder. Only trials focusing on the urethral sphincter and neobladder could be found in literature. Urologists need a suitable replacement for traditional conduits and neobladders due to their adhesion problems, mucus development, emptying difficulties, and metabolic conditions and transformations into malignancies. Autotransplantation was used in innovative work to build artificially engineered bladder tissues [57]. Both urothelial and detrusor smooth muscle cells retrieved by bladder biopsy and cultured for 7 weeks and transplanted into a bladder-shaped biodegradable scaffold mainly consists of polyglycolic acid and collagen.

Many other approaches for reconstructing the bladder [58–60] were investigated in attempt to find safe and usable bowel replacement material and to prevent the complications. Nonetheless, only modest success is yet achieved. Although both

robotic and open route is available for radical cystectomy, open surgery is usually performed in most patients with urinary diversion. Costs of this method vary in different countries. Involvement of an intestinal segment is responsible for the main proportion of the costs.

Hospital readmission rates are high after cystectomy and urinary diversion; thus, the readmission cost is important, too.

Thus, new alternative solutions are looked-for to lessen the significant economic burden of cystectomy and post urinary diversion complications. So, a great deal of the latest research focuses on bioengineering methods for the reconstruction of urinary bladder including tissue engineering, bioreactors and bioprinting.

7.4.1 Tissue Engineering

So far, tissue engineering has focused on the reconstruction of bladder tissue, and significant progress is made. A multidisciplinary approach to bioengineering is mainly based on the human body's potential of natural regeneration and involves the use of a polymers matrix or cell-seeded scaffolds to promote more regeneration [61]. Such complex technologies of regeneration are being studied to create an efficiently designed bladder.

Tissue engineering for bladder reconstruction has significant benefits. It is time-saving in the operating room, helps to prevent digestive problems and increases patient quality of life. Also, this technique is a very promising approach and develops new treatments for other pathologies of the lower urinary tract that do not essentially require a total replacement of the bladder [57]. To date, different animal models were used to ensure the effectiveness of different scaffolds for cell-seeding [62, 63]. The concept of using tissue engineering for urinary bladder regeneration actually goes back to the 1950s.

Type and charachteristics of the scaffolds has a key role to support the complex chemical and mechanical bladder function during both filling and emptying. The matrix microenvironment can influences the stem cells migration, proliferation and differentiation into the regenerating cells [62].

The biomaterials used in bladder tissue engineering should have acceptable mechanical and chemical properties as well as appropriate biocompatibility [64] to provide a good support for structure of several separate layers of cells.

An ideal biomaterial should offer an adequate plane for attachment of urothelial cells at its lumen, and its visceral side should be capable of nesting the muscle cells, which are necessary to form a unidirectional muscle layers and suitable for quick vascularization and innervation [65].

Another main objectives is to prevent the regenerative bladder from rising the host immune response that leads to compromised efficiency and durability of the bladder [66].

As a result, most biomaterials and issues, including acellular tissues, natural or artificial polymers, and composites, were used as substitutes for urinary bladder tissue and matrix scaffolds.

7.4.2 Bioreactors

Bioreactors are advanced modeling biosystems capable for controlling environment by influencing factors such as pH, oxygen concentration and temperature. Simulating the normal physiological functions (both filling and emptying) by bioreactor in vitro can improve the functional results after implantation [67, 68] and can strengthen the stability of the matures tissues. Another promising approach in the field of bladder regeneration is in vivo bioreactors which are used in target scaffold before the main implantation. This preconditioning can further enhance the bioengineered tissue growth, improve tissue vascularization and inhibit fibrosis and consequently prevent contractility loss [65]. Although discovery and use of different types of bioreactors and preconditioning before stem cell implantation in aim of enhancing the outcomes are so interesting, but to date few studies have been conducted focusing on this specific field and more studies are yet required.

7.4.3 Bioprinting

Bioprinting technology is a powerful computer-controlled method for generating cell-based living functional tissues and organs [69]. It needs stem cells for seeding into a biodegradable scaffolds as primary structure and different bioreactors such as growth factors for inducing tissue formation [70]. The great clinical benefit of transplanting such tissues is that they will not raise the host immune response, an issue that cause so many complications in other types of transplantation including allograft tissue transplant.

In this technique a bio-printer first produce a three dimensional (3 D) structure which will be then use as a scaffold for stem cell seeding. Different material can be used as the scaffolds. The most known material is hydrogels. Hydrogels are both biocompatible and biodegradable. In addition, they have specific sites that help cell adhesion that is needed for further cell growth and differentiation [71].

Bioprinting techniques were tested in many kinds of tissues, but some more specific human organs like trachea, bronchi [72], blood vessels [73], and bladder [74] have achieved clinical success in this area of bioengineering, so far. Therefore, we are hopeful that bioprinting will potentially offer an actual solution for shortage of organ donors and complications related to allograft transplantation, soon in future [69].

7.5 Conclusion

Stem cell therapy for treatment of bladder dysfunction is an interesting approach which seems work through the ability of stem cells including self renewal, differentiation and also their paracrine effect. Inhibiting the bladder tissue fibrosis and restoring the detrusor muscle contractility seem to be the main stem cells' mechanisms of action in recovery of bladder dysfunction. Furthermore, this fact that stem cells potentially can differentiate into detrusor smooth muscle cells, offers new approaches for treatment of bladder dysfunction such as bladder regeneration and bladder bioprinting.

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Chapter 8
Transplant and Kidney Repair

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Abstract Kidney injury including acute and chronic types will end in transient or permanent renal failure. Even patients with an episode of acute kidney injury are at a higher risk of developing chronic kidney injury in future.

Stem cells have an interesting ability to migrate into an injured tissue and repair it via different mechanisms. So, stem cell therapy can be a novel alternative of different current treatment for renal failure such as hemodialysis and kidney transplant. In addition, by immunoregulatory mechanisms stem cells can improve graft survival in kidney transplantation.

In this chapter we aim to present a summary on the stem cells application in various kidney diseases as well as in kidney transplant.

Keywords Renal failure · Stem cells · Kidney transplant

Renal failure is a condition that can result in impaired homeostasis. Evidence shows kidney is cab be affected by various diseases (diabetes, hypertension, glomerulonephritis, etc.) and can trigger or exacerbate such pathophysiological disorders, like cardiovascular diseases, if its physiology is compromised [1, 2]. Chronic kidney injury (CKI) is defined as a long-standing disorder with a decline in the rate of glomerular filtration as well as increase in albuminuria. CKI can be correlated with an increased risk of cardiovascular morbidity, reduced quality of life, and early death [1]. CKI is often asymptomatic and ESRD needs dialysis or kidney transplantation (KTx), both are very costly for health care systems [2]. Stem cell therapy in this area may theoretically lead to a better outcome of nephropathy.

Embryonic stem cells (ESCs) are pluripotent cells with limitless proliferative lifespan, originating from the blastocyst's inner mass [3]. Friedstein described them

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as colony-forming unit fibroblasts nearly half a century ago from the cell cultures of the murine bone marrow [4]. An ability for differentiation into all cell lines makes them really appealing for approaches to cell therapy. The fact that the methods used for MSC isolation (enzymatic or nonenzymatic), selection (plastic adherence, cell sorting, etc.), expansion (cultural media, oxygen stress, etc.) and evaluation are not yet fully standardized is an important aspect to consider for the clinical use of MSC. In 2006, the ISCT (International Society for Cell Therapy) proposed a series of minimal MSC isolation and cultivation criteria. Further attempts have been made to standardize the MSC characteristics used in the clinic since [5–7].

Additionally, induced pluripotent stem cells (iPSCs) have been formed as differentiated cells have been reprogrammed genetically to induce an ESC-like state, but with a high probable risk of developing tumorigenicity [8, 9].

MSCs can be derived from a wide range of fetal or adult sources such as bone marrow, adipose tissue (ASC), dental pulp, periosteum, synovium, fetal tissue, placenta, and amniotic fluids umbilical cord blood (UCB), [10, 11]. MSCs are currently being used in a number of clinical settings based on the potential for differentiation of MSCs, stromal support, immunomodulatory properties and trophic secretion [12] For regeneration of the tissue, immunomodulation or strengthening of the graft. Nonetheless, some of the MSC properties can alter in vitro during the expansion process [13]. Nevertheless, it was also identified in the early stages as genetic instability, possibly due to the initial adaptation of the cells from their native niche to the conditions of culture [14]. This creates a question after the MSC implantation about the possible tumorigenic effect.

As previously mentioned in Chap. 2, the minimum requirements for identifying human derived-MSC (hMSC) are as follows: (1) plastic adherence must occur under standard conditions of culture and (2) For those antigens that are missing in the majority of hematopoietic cells, such as CD105, CD73 and CD90, more than 95% of the cell population must be positive. In addition, CD45 (leukocyte), CD34 (hematopoietic progenitor), CD14 or CD11b (monocytes and macrophages), CD79α or CD19 (B cells) and HLA-DR (cell and lymphocyte presenting antigen) may be expressed by up to 2% of the population (3) Potential for adipocyte, chondrocyte and osteoblast differentiation [15].

In response to combination signals, MSC has the ability to migrate into damaged tissues. This process is called homing and was first recorded in trafficking in leukocytes. Upon injury, MSCs migrate to inflammatory sites where they move through the endothelium and enter the wounded tissue bed. Homing is the result of an interaction between signaling molecules, such as chemokines, adhesive molecules, and matrix metalloproteinases, which are released from the damaged tissue and receptors expressed on the MSC surface [16].

MSCs transfer to inflammatory sites, damage, and tumors after their systemic administration to mediate healing [17]. Nevertheless, it also realized that most MSCs can be lodged in the lungs, spleen, and liver after intravenous injection (IV) [18–20]. It decreases the number of cells in the target organ capable of homing and grafting. Appropriate imaging techniques for monitoring and detecting the exact location of MSC in the tissues could therefore support to further understand homing.

Although preliminary results on MSC's therapeutic mechanisms suggested a significant role in homing, grafting, and cell differentiation at the injury site, several additional studies indicate very limited replacement of injured tissue by transdifferentiation ability and replacement potential. In particular, renal repair mechanisms found after damage to ischemia-reperfusion do not include replacing tubular cells with infused MSC [21, 22].

The justification for the MSC application is to cure or restrict renal diseases through factors that decrease fibrosis, support angiogenesis, inhibit apoptosis, minimize adverse inflammatory events by their capacity to immunomodulate and lead to the regeneration of the renal tissue [17, 23, 24]. MSC immunomodulation induces an immunotolerant state and decreases the effector cells 'immune response as monocytes/macrophages, dendritic cells, T or B cells [25–27]. Paracrine-mediated effects of extracellular vesicles (EVs) and the function of exosomes further enhance the role of kidneys [23, 24].

8.1 MSC Immunomodulation

Current evidence shows the value of the MSC's interactions with their environment as other immunomodulatory properties come into effect in a paracrine/endocrine fashion. MSC can release hundreds of active biological factors acting on local cell dynamics by decreasing apoptosis, reducing the development of inflammation and fibrosis, fostering angiogenesis and recruiting resident progenitor cells, and stimulating mitosis and/or differentiation processes. By the secretion of the following, MSC mediates these effects [16].

Immunomodulation is a main characteristic of MSCs, relating to the processes of therapy as well as their application as allogeneic cells. Despite expression of HLA/MHC class I and HLA/MHC class II inducible expression, MSCs are hypo-immunogenic and tend to be transplantable through allogeneic/xenogeneic barriers [28]. Many allogeneic studies of MSC animals indicate a poor allogeneic immune response [29].

Several studies reported that after allogeneic/xenogeneic MSC administration, there was no activation of the immune system [30]. Le Blanc et al. have indicated immunosuppressive properties of MSC in vitro, but only a limited number of studies support the in vivo translation [31].

Consequently, due to inconsistent outcomes and significant in vivo and in vitro settings discordances, further perception of allogeneic/xenogeneic MSC infusion is needed in the circumstance of kidney injuries. Some of the factors to consider when seeking MSC diagnosis are MSC number of administration, unique dose, and immune suppression activity. Due to the contact with the graft microenvironment, timing of MSC care is also of extreme importance [32]. Since this is a highly debated aspect, this analysis will clearly dissect the studies in allogeneic and xenogeneic settings using stem cell transplantation. MSC can also act as immunomodulators by inhibiting the activation and proliferation of lymphocytes [33]. MSCs affect all cell types of the immune system: the functional potential of the activated T cells is reduced, the regulatory T cell (Treg) phenotype is triggered, the monocytes are

polarized to the anti-inflammatory M2 phenotype and the maturation of the dendritic cells is disrupted, such as the B and NK cell functions [34]. M2 macrophages have been shown to secrete a number of factors that promote wound healing, angiogenesis, extracellular matrix (ECM) deposition, and tissue remodeling [35].

8.2 Renoprotective Modes of Action

Kidney fibrosis is an ECM deposition that contributes to ESRD in the renal parenchyma. ECM is usually degraded by the matrix of metalloproteinases (MMPs), but a change in the balance between MMPs and metalloproteinase tissue inhibitors (TIMPs) induces ECM accumulation and degradation and matrix restoration inhibition. MSCs tend to have a coercive effect on TIMPs expression, leading to resolution of fibrosis [36–39].

Oxidative stress is caused by an imbalance between free radical production in the mitochondrial electron transport chain and reduced antioxidant defenses [40]. There is also an accumulation of reactive oxygen species (ROS), which contributes to the upregulation of TGFβ1 and ECM proteins in the expression of glomerular mesangial cells [41]. MSCs can have an antioxidant effect, reducing oxidative damage to the organ [42]. MSCs, mediated by the soluble factor secretion, avoid ROS accumulation due to antioxidant upregulation and scavenging. MSC secretion of exosomes leads to inhibiting the main enzyme depletion of ROS metabolism, thereby minimizing oxidative stress injury [40, 41].

The role of MSCs in angiogenesis and vascular remodeling may include pro-angiogenic and pro-survival factor upregulation. These may include not only VEGF-a, IGF-1 and HGF, but also EVs [43, 44]. MSC secretion of VEGF, HGF, IGF-1 in conjunction with TGF-β, stanniocalcin-1, GM-CSF, and FGF-2 appears to have an anti-apoptotic effect [45]. The MSC-treated AKI mouse models reported a decrease in pro-apoptotic (Bcl-xs) and an increase in anti-apoptotic molecules (Bcl-2 and Bcl-xl) [46]. Anti-apoptotic ratio balance is needed to improve kidney recovery [47].

Apoptosis of tubular cells is a central pathomechanism in some kidney disease models, such as cisplatin-induced AKI. It has been demonstrated that MSCs have anti-apoptotic, pro-regenerative signs, such as inducing pro-regenerative/anti-apoptotic gene expression or passing mRNA/miRNA to damaged cells [42, 48].

8.3 Clinical Use of MSCs in Treatment of Kidney Diseases

The strong results obtained from various studies using MSC in vitro and in vivo have created great excitement in the scientific community, offering new cell-based therapy possibilities for a broad spectrum of diseases. Here in this part, clinical use of MSCs in some particular kidney diseases are discussed.

8.4 Acute and Chronic Kidney Injury

AKI is a clinical condition characterized by a rapid decrease of glomerular filtration rate(GFR), resulting in serum creatinine (sCr) and blood urea nitrogen (BUN) increase [49]. It is a global health issue with a high prevalence (about 20% of patients admitted to the hospital each year) [50]. AKI can lead to significant morbidity and also mortality because the lack of early detection biomarkers and limited fully successful therapeutic approaches [51].

Patients surviving an AKI episode have been shown to have an increased risk of developing CKI over the past few years. CKI leads to ESRD, where dialysis or transplantation are the only treatments available [52, 53]. All modalities have limitations and drawbacks of their own. Despite the fact that dialysis can cause high morbidity and mortality, Patients needing renal transplantation typically wait several years before an optimal renal allograft is available [54].

In their review, Torres Crigna et al. [55] describe two types of induced kidney injury: AKI induced by ischemia/reperfusion and AKI induced by chemotherapy**.**

8.5 AKI Induced by Ischemia/Reperfusion

Ischemia/Reperfusion (I/R) is the primary cause of AKI caused by a decrease of blood supply to the kidney accompanied by a return of perfusion. This reduced oxygenation results in depletion of ATP, metabolic dysfunction, apoptosis, ROS development and worsening during the reperfusion phase, resulting in sterile inflammation, vasoconstriction and oxidative damage [56, 57]. AKI I/R is usually the product of arterial occlusion, heart obstruction or kidney transplantation during surgery [58]. For decades, AKI I/R in animal models have been commonly used in various species. The most commonly used technique is to clamp both the renal artery and vein for a specific duration of time and then release the clamps [59]. The optimum pacing and dosage is the secret to the therapy. Studies on BM-MSCs conclude that beginning care an hour after the injury leads to a better outcome, as cells can graft to a higher degree into the kidney [60, 61]. Interestingly, before the induction of AKI I/R, a study using human adipose stromal vascular fraction (SVF) revealed increased cell retention in the kidney and increased the proliferation tubular cell [62]. The researchers declare that mostly because cell emboli formation, “the higher the dose, the worse the outcome” [63, 64].

Stem cells derived from various sources tend to overcome the key pathophysiological events responsible for AKI I/R in an efficient manner. Nevertheless, the mechanisms of observed beneficial effects remain indefinable. Before the effective implementation of MSC therapy into clinical practice, further research focusing on both the outcome and the mechanisms of action is required.

8.6 AKI Induced by Chemotherapy

Cisplatin is a chemotherapeutic drug that is commonly used to treat some solid organ tumors and is believed to be a gold standard of treatment in the field of oncology [65]. For decades, cisplatin has been used in animal experiments to induce AKI and CKI and is a common model for testing the therapeutic effects of MSCs [66].

The earliest study concerning MSCs as a therapeutic approach to cisplatin-induced AKI treatment dates back to 2004, when on a mouse model, Morigi et al. identified the impact of stem cell administration [67]. In this study, hematopoietic stem cells (HSCs) derived from BM-MSCs and BM have been tested and compared, demonstrating that only BM-MSCs contribute to kidney restoration both functionally and morphologically. Many studies have reported the usefulness of allogeneic MSC in treatment of cisplatin-induced AKI, where different cell origins, routes of administration and strategies were tested to achieve a clear understanding of the involved action mechanisms [68–70].

8.7 Chronic Kidney Disease

An approximately 8–16% of the general population has CKD and its prevalence in people over 70 years of age increases to about 30%. The number of people affected by chronic kidney disease (CKD) is growing globally, largely due to a significant rise in atherosclerosis and type 2 diabetes [71]. CKD is characterized by a reduced capacity for renal regeneration. Several in vivo studies suggest beneficial cell-based therapy regenerative effects in CKD animal models [72].

8.8 Diabetic Nephropathy

Diabetic nephropathy is one of the most expensive complications of long-term hyperglycemia, which is the most common cause of ESRD (more than 35% in the U.S.) (https:/www.usrds.org/2017/view/Default.aspx). ESRD's onset and progress is mostly silent and gradually evolving. Abnormal function of the kidney is often found by chance 5–10 years after diagnosis of diabetes [73–75]. The pathological aspects of diabetic nephropathy are well defined and adopt a progressive trend where the main findings are the deposition of ECM in the base membrane of glomeruli and tubular tissues [76].

The current treatment model is based on early detection, glycemic control and strict monitoring of blood pressure with preferential use of blockage of the renin-angiotensin system [77].

The creation of models of diabetic animals such as the model STZ (diabetes type 1, T1D) or defective models of leptin receptors (diabetes type 2, T2D) is essential to

understand the progression that could occur in different types of diabetic patients. It is not new to use MSCs to alleviate this complication. In 2006, Lee et al. showed that hBM-MSC intracardiac (IC) infusion had a double benefit for the STZ diabetic mice by reducing the deposition of the mesangial matrix and restoring pancreatic damage [78]. In the latter tests, the restoration of insulin secreting tissue was not confirmed. Ezquer et al. administered BM-MSCs in a similar STZ model and found a significant reduction in glomerulosclerosis and ECM deposition due to pancreatic regeneration in presence or absence of glucose normalization [79–81]. The advantages of stem cell therapies in this environment are accompanied by common histological endpoints [82–88]. With regard to functional parameters, most studies reported an increase in albuminuria in severely damaged kidneys, in both models of STZ and models of type 2 diabetes [89, 90].

Obviously, Nagaishi et al. demonstrated repeatedly the advantages of BM-MSCs in models of insulin-resistant and insulin-depleted diabetic nephropathy with positive results using not only cells but also CM [89]. This indicates that EVs and cytokine release may be more important than the cells themselves for therapeutic actions [91]. Also, the immunomodulatory properties are reported to have decreased macrophage infiltration, tissue expression of MCP-1, and inflammatory cytokines [81, 89, 92–94]. These results strongly indicate that the metabolic environment has a substantial impact on the performance of MSCs (by deactivating or changing their properties detrimentally). More studies in vitro and in vivo are required to answer the questions that have not yet been answered [90, 95]. Yet different concentrations have been used [78, 91, 96, 97]. The positive results found in most studies are similar where ECM deposition, urinary albumin/creatinine ratio, and less mesenchymal transition epithelial (EMT) are observed. There is a clinical trial conducted by Packham et al. in 2016 showed no adverse allogeneic effects compared to placebo in patients treated with BM-derived mesenchymal precursor cells [98]. Nonetheless, the findings of renal functional change were inconclusive, indicating that the problem could involve larger populations and long-term studies. Until discussing the benefits mentioned in the literature, alloreactivity and the immune responses of patients are critical aspects to evaluate. Nevertheless, in the field of preclinical research, these topics are scarcely investigated indicating that further studies are required.

8.9 Focal Segmental Glomerulosclerosis

A uncommon but major cause of ESRD is focal segmental glomerulosclerosis (FSGS). The risk of recurrence in children is higher than in adults and in subsequent kidney transplants in patients. In addition, approximately 30–40% of patients with FSGS develop recurrent FSGS after kidney transplantation. The incidence is rising throughout the world [99].

Very few preclinical studies investigating the beneficial effects of MSC infusion in in vivo FSGS models can be found in the literature, but all showed promising

results, resulting in a clinical translation [100]. A 2013 article by Belingheri et al. describes the first allogeneic bmMSC treatment in a pediatric kidney transplant recipient with a type of FSGS that does not respond to traditional and unconventional treatments [101].

8.10 Polycystic Kidney Disease

Polycystic kidney disease (PKD) is a kidney disease which can cause ESRD and is the fourth most common cause of chronic kidney failure. The disease can occur sporadically, but most manifestations are inherited [102]. Two types of PKD are autosomal dominant polycystic kidney disease (ADPKD), usually found in adults, and autosomal recessive polycystic kidney disease (ARPKD), which is associated with significant mortality and morbidity and primarily affects infants [102, 103]. The most likely mechanism is a protein alteration signaling the cell and primary cilia function. As a result, the cells that line the renal tubules can grow and divide abnormally, resulting in numerous cysts developing [104–106].

There are actually only a few treatments available for patients with PKD. Patients with end-stage PKD typically undergo dialysis or kidney transplantation. Due to poor quality of life, there is a growing need for more effective medical approaches in the treatment of long-term dialysis, high insurance costs and rising waiting lists for organ transplants. So far, various types of PKD animal models have been described. In this sense, the characteristic mutation was either caused or spontaneously formed in these animals [107–110].

Two research that demonstrate the potential therapeutic impact of allogeneic stem cells in rats have been released in the past few years. Franchi et al. demonstrated that a single injection of BM-MSCs extracted from healthy SD rats could enhance the function of the kidneys in PCK rats and partially restore renal function [111]. This research highlights two potential effects of stem cells: paracrine effects, by releasing cytokines such as SDF1, VEGF, and HGF, and cells 'ability to acquire endothelial cell characteristics after injection, indicating transdifferentiation of these cells. After the transplantation, the authors reported a strong grafting of the donor stem cells, leading to a decrease in the overall cyst volume and fibrosis and improving the vasculature, resulting in improved oxygen and nutrient distribution. The above studies have indicate that MSCs boost renal function and restrict the development of cysts in the animal model of PKD. In order to assess whether hMSCs can also be safely implemented in PKD, further studies should be carried out. Ironically, given the shortage of preclinical evaluations, one phase I clinical trial has already been completed with the goal of investigating the safety and efficacy of autologous hBM-MSCs to enhance renal function in ADPKD patients [112]. Here, the patients were given cultivated BM-MSCs with an 18-month follow-up. There are currently various clinical studies in the literature on the protection and tolerability assessment of infusion of MSCs under different pathological conditions. Nevertheless, as suggested by the writers, the progression of PKD such as

inflammation, cyst proliferation, and apoptosis is linked to several pathways. Because the mechanisms that illustrate the positive effects are not yet completely understood, in relation to a long-term MSC injection therapy an assessment of these reported mechanisms of action is recommended.

8.11 Autoimmune Disease: Systemic Lupus Erythematosus

SLE is a chronic autoimmune disease characterized by a wide range of clinical symptoms, with severe morbidity and mortality that can affect multiple organs in the body. Nephritis is the most important form of SLE, and normal therapies include high doses of corticosteroids, cyclophosphamides, and other biological and immunosuppressive agents. Some patient outcomes improve significantly after treatment, although strong side effects such as cancer, ovarian failure and secondary malignancy can exacerbate the prognosis and lead to patient death [113]. While the effectiveness of MSC therapy in preclinical models varies and seems to depend on the model and the MSC population used, multiple studies have shown that MSC's anti-inflammatory immunomodulatory effects can be beneficial to SLE patients [114].

8.12 MSCs in Kidney Transplantation

Kidney transplant in patients with ESRD provides the best chance of survival and increases health-related quality of life relative to dialysis remaining [16]. Nevertheless, the grafts 'long-term survival is still not optimum [115–117]. In addition, the major cause of kidney transplant failure is the occurrence of a progressive kidney disease associated with interstitial fibrosis and tubular atrophy, as well as vascular occlusion and glomerulosclerosis [118, 119].

Stem cell treatment therefore poses the possibility of creating new therapies in conjunction with immunosuppressive drugs or reducing the doses needed to prevent rejection while maintaining the renal function [11, 120]. MSC therapy has a positive effect on renal function and survival of the graft [17, 18]. Since the first clinical success of MSCs in 2004 [3], MSC therapeutic technology work has grown to the point where so many clinical trials have now been registered (http:/clinicaltrials.gov/). Despite the limited amount, for use in kidney transplantation, transfer of MSCs from the bench to the bedside is highly possible.

The key points of interest in kidney transplant stem cell therapy are their ability to modulate the immune response and the interstitial fibrosis. Due to their low immunogenicity and immunoregulatory properties, in the context of kidney transplantation, MSC can potentially prove beneficial. Many in vivo studies have shown that MSC is capable of successfully controlling immune response and promoting kidney repair [121].

BM-MSCs can maintain the renal function and minimize interstitial fibrosis and tubular atrophy by reducing the activation of T cells and macrophages in a Fisher to Lewis KTx model within 24 weeks of transplantation [122].

In a model of KTx, however, using a full MHC discordant donor-recipient mixture, the use of BM-MSCs aggravates the transplant outcome, causing massive infiltration, thrombotic microangiopathy and increased expression of IL-2 and IFN- γ [123]. As predicted, administration time plays a major role, as in a completely inconsistent MHC mouse model in which the administration of the BM-MSCs derived from the recipient after 2 days of KTx resulted in progressive dysfunction of the graft and rejection of the graft within 20 days, nevertheless, MSCs injection 1 or 7 days before KTx extended the survival of the graft by moving the cells into the spleen [32]. The use of BM-MSCs together with the immunosuppressant cyclosporine-A was also investigated, showing safety of the graft feature but not an increase survival in animal model as strongly as the treatment of cyclosporine-A alone, indicating a possible association between this drug and MSCs [11]. BM-MSCs decreased ED1 + and CD8 + cells in an allogeneic rat transplantation model and could reduce interstitial fibrosis and TGF-β1 3 and 7 days after KTx [10, 124].

A normal dose of calcineurin inhibitors as well as a reduced dose (80 percent of the standard) were tested in patients treated with MSC to avoid organ toxicity. Patient evaluation at a 1-year follow-up found that removing the blockade of CD25 did not affect the survival of the graft [16].

BM-MSC-MVs also improved graft survival in an allogeneic mouse model by reducing the percentage of MHCII+, CD80 + and CD86 + cells. Ironically, pretreatment of MVs with miRNA-146a inhibitor also removed the valuable effect observed in the transplantation scenario. ASCs were tested by decreasing the CD4+/CD8 + ratio in a completely MHC disparate rat model indicating sustained survival of the graft. The mechanism involved would be factor-inducible gene 6 protein (TSG-6) upregulation of tumor necrosis by in vitro ASCs. This upregulation results in the allo-reactive T cells to be suppressed by CD44 regulation, resulting in T cell activation suppression and transplant infiltration [125].

To date, a few clinical trials have been conducted using MSCs in kidney transplantation. In all cases, the common factor is the use of MSCs throughout conjunction with immunosuppressive drugs to reduce the doses required, thereby reducing the occurrence of adverse effects associated with their use.

In a clinical trial which is performed by Trivedi et al., in pediatric recipients, in conjunction with cyclosporine-A and prednisolone, high doses of peripheral blood stem cells derived from donors were used. There was stable renal function and 100% survival as well as low incidence of opportunistic infections after 18 months of observation [126].

A double infusion of autologous BM-MSCs was administered in another clinical trial, conducted by Reinders et al. to allograft recipients with subclinical rejection. Six months after cell infusion five out of the six patients had a donor-specific immunity inhibition [127].

Also there are other clinical trials by the aim of investigating tolerability, efficacy and safety of different drugs administration in conjunction with MSCs infusion in recipients.

Completed clinical trials provide an outline of potential new treatment regimes. There are, however, many things on which no consensus exists, including the most appropriate administration time (before vs after KTx) and the most appropriate dose. Such knowledge is important for a suitable clinical practice translation. More research is needed to explain the above-mentioned issues and determine the possible synergistic or antagonistic effects of MSCs on the most widely used immunosuppressive drugs. The findings of kidney transplantation involving stem cells appear promising. Nonetheless, the transfer from animal models to clinical practice appears unlikely due to the overwhelming nature of the mechanisms involved. In conjunction with the most popular immunosuppressive drugs, it would be important to see more research using MSCs to examine potential synergic effects.

8.13 Conclusions

Stem cells are amazing sub group of cells with many instinctive ability like differentiation into many other types of cells and migration into damaged organs to induce repair and regeneration. They can be used for therapeutic aims in different kidney diseases. The mechanisms through which they act are mainly related to their immunomodulatory properties and their paracrine effects. However, clinical studies focused on this topic are yet limited. But existing data have suggested this novel MSC-based therapy in kidney diseases as an promising approach.

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