BILINGUAL PUBLISHING CO.
Advances in Modern Oncology Research
doi: 10.30564/amor.v4i6.196

REVIEW

The testis: an accessible Mesenchymal Stem Cells source

Myret Ghabriel[1], Asma Amleh[1,2]

[1] Biotechnology Program, American University in Cairo, AUC Avenue, P.O.Box74, New Cairo 11835, Egypt
[2] Department of Biology, American University in Cairo, Egypt

***Correspondence Author:** Dr. Asma Amleh, Biotechnology Program, American University in Cairo, AUC Avenue, P.O.Box74, New Cairo 11835, Egypt. E-mail: aamleh@aucegypt.edu

Abstract: Mesenchymal stem cells (MSCs) are adult stem cells that are predominantly found in bone marrow but also cord blood, peripheral blood, lungs, and adipose tissue. They are considered multipotent cells, which can differentiate to form multiple cells types—adipocytes, bone, cartilage, skin, and muscle. This potential elicits a great deal of attention because it appears to hold great promise regarding regenerative cell-based therapy to treat various diseases, including cancer. They are considered powerful allogeneic nonhematopoietic transplantation stem cells due to their low immunogenicity. Recently, several researchers have identified and isolated MSCs from primary human testes cultures. This review aimed to highlight the latest advances in MSC biological properties and characteristics studies, focusing on testicular mesenchymal stromal stem cells (tMSC), their differentiation capacity, and immunosuppressive properties—all of which have the potential to establish MSCs as powerful therapeutic agents for various diseases, including cancer.

Keywords: Testicular mesenchymal stromal stem cells; MSC sources; MSC isolation; multilineage potential; therapeutic application

Received: 22th March 2019; ***Accepted:*** 22th August 2019; ***Published Online:*** 22th August 2019

1. Introduction

The versatility of mesenchymal stem cells (MSCs) and their ability to differentiate into multiple mesenchymal lineages make them an important cell source for regenerative therapies. This review aims to summarize the terminology timeline, developmental origin, primary tissue source, differentiation capacity, and therapeutic potential of MSCs. This review encourages researching MSCs that are derived from testicular tissue—which is currently insufficiently investigated—to understand their differentiation potential and regenerative capabilities.

1.1 MSCs' nomenclature and developmental origin

Initially, MSCs were isolated from bone marrow as colony-forming unit fibroblasts (CFU-Fs) and osteogenic stem cells [1]. However, different terms have subsequently been used to describe them. In 1988, Maureen Owen et al. proposed the term "stromal stem cells," which emphasized that the cells are found in the bone marrow's stromal compartment [2]. Conversely, in 1991, Arnold Caplan developed the term "mesenchymal stem cells" to highlight the cells' self-renewing capability and differentiation potential rather than focusing on their locational origin [3]. James Dennis et al. challenged this nomenclature, arguing that the cells should be considered progenitors instead of stem cells—thereby introducing them as "mesenchymal progenitor cells" [4]. Almost a decade later, Paolo Bianco and Pamela Gehron Robey coined the term "skeletal stem cell" to reflect the fact that the cells give rise to the skeletal system's tissue types [5]. Two years later, Yuehua Jiang and colleagues suggested "multipotent adult progenitor cells" to

again emphasize the cells' multipotent nature and potential progenitor status [6]. Finally, in 2006, the International Society for Cellular Therapy (ISCT) proposed the term "multipotent mesenchymal stromal cells" (see Figure 1). Further, ISCT proposed a minimal criteria scheme to define MSCs:

1. plastic-adherent when cultured in vitro

2. express specific cell surface markers—CD105, CD90, and CD73 and do not express others—CD45, CD14, CD19, CD34, CD11b, CD79alpha, and HLA-DR

3. able to differentiate to osteoblasts, chondroblasts, and adipocytes in vitro (see Table 2) [6].

These criteria were found to be constraining at times because different MSCs populations have been isolated from multiple tissue types, each of which possesses unique properties [7]. Several studies have reported MSCs successful isolation from adipose tissue [8], muscle [9], umbilical cord blood [10], synovial membranes [11], and dental pulp [12], among others. However, ISCT's proposed criteria are not consistent across different species; for example, mouse-derived MSCs exhibit different marker expression profiles and culture characteristics from human mesenchymal stem cells (hMSCs) and different mouse-derived MSCs strains [17, 18]. The active search for MSCs postnatal sources continues, with several groups identifying and isolating MSCs from primary human testes cultures [13–16]. Gonzalez and colleagues isolated a stem cell population from adult human testes that has similar characteristics of MSCs—named gonadal stem cells (GSCs) [13]. This review aimed to highlight the latest advances in MSC biological property and characteristic studies, focusing on the most recent understanding of testicular mesenchymal stromal stem cells, their differentiation capacity, and their potential effect in therapeutic applications.

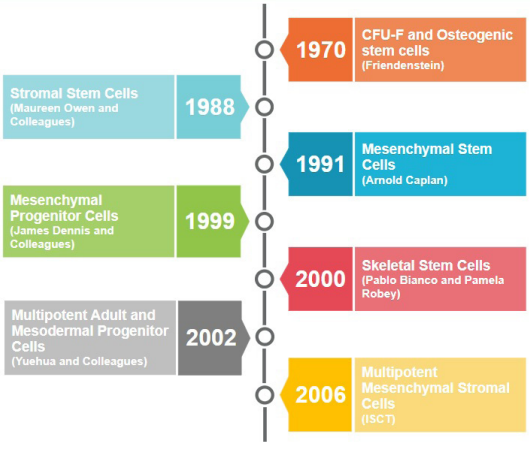

Figure 1. The MSCs nomenclature timeline.

It is widely accepted that MSCs arise from the mesoderm. However, Takashima et al.'s recent study discussed the earliest lineage providing MSC-like cells: the neuroepithelium, rather than the mesoderm, generates MSC-like cells through a neural crest intermediate stage during embryonic development [19]. These cells are subsequently replaced by MSCs from other origins in later development. Similarly, Nagoshi et al.'s study determined that neural crest–derived cells migrate to the bone marrow and can differentiate in vitro into neurons, myofibroblasts, and glial cells [20]. Notably, the most interesting hypothesis regards the pericyte origin of MSCs. Pericytes are mural cells that coat the endothelial cells that line a body's capillaries and venules and communicate with the blood vessel's endothelial cells through either direct physical contact or paracrine signaling [21]. Pericytes express MSC markers, such as CD146 and PDGFRβ, and cells positive for MSC markers express pericyte markers and possess similar multilineage differentiation potential [22, 23]. Lineage-tracing experiments reported that the cells that originated from the primary vascular plexus gave rise to mural cells with the capacity to differentiate into adipocytes and osteoblasts [24]. These findings suggest that MSCs and endothelium progenitor cells could arise from a common progenitor.

1.2. Major MSCs sources

Over the past few years, reports have increased regarding isolating adult MSCs from different sources. Despite bone marrow–derived MSCs remaining as the most frequently investigated cell type (often considered the gold standard), research groups have isolated MSCs from various adult tissues, such as adipose tissue [8], skeletal muscle [9], umbilical cord blood [10], synovial membranes [11], dental pulp [12], and the testis [13, 16]. This chapter briefly discusses various sources of MSCs and how their inherent characteristics and properties may differ depending on the tissue of origin, while also examining the testis as a MSCs source.

1.2.1 Bone marrow MSCs

Friedenstein et al. first isolated MSCs from bone marrow, describing them as nonhematopoietic cells that support in vitro hematopoietic stem cell expansion and that can differentiate into various connective tissue cells. Bone marrow mesenchymal stem cells (BM-MSCs) are a subpopulation of the stromal cells that line the endosteal surface of the marrow space [1]; they are easily harvested and found in relatively high amounts. For these reasons,

10.30564/amor.v4i6.195

the bone marrow remains as a most commonly used MSC sources. BM-MSCs can be identified by positively expressed CD29, CD73, CD44, CD105, CD90, and CD166 surface markers, and negatively expressed CD31, CD14, CD45, and CD34 surface markers [25].

1.2.2 Adipose tissue MSCs

Adipose tissue is considered a highly abundant MSC source. Zuk et al. isolated MSCs from adipose tissue as an alternative to bone marrow–derived MSCs [8]. Adipose-derived mesenchymal stem cells (AD-MSCs) are easy to collect, give high cell yields, have robust in vitro proliferative capacity, and show similar properties to BM-MSCs. Zuk et al. discovered a few differences between the two populations [8], with distinctions identified by immunofluorescence analysis and showing a Cluster of Differentiation (CD) MSC surface marker profile. AD-MSCs express CD29, CD44, CD90, CD71, CD105 (SH2), and SH3, but do not express CD34. These findings indicate that MSCs isolated from different sources exhibit different characteristics.

1.2.3 Umbilical cord MSCs

MSCs may also be obtained from umbilical cord blood. Umbilical cord-derived mesenchymal stem cells (UC-MSCs) display a gene expression profile that is similar to embryonic stem cells (ESCs) rather than BM-MSCs, establishing them as useful in clinical applications. They also express pluripotency markers Oct4, Nanog, Sox2, and Klf4, but at lower levels than those of ESCs [26]. This could be a contributing factor to their quicker self-renewal and improved expansion capability in vitro compared to BM-MSCs [27]. Further, they are easily retrieved after a newborn birth because the umbilical cord tissue is considered medical waste with no value. MSCs can be isolated from three different umbilical cord compartments: tissue surrounding umbilical vessels, Wharton's jelly [28], and umbilical cord blood [10]. Interestingly, a recent study observed that Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs) successfully differentiated into postmeiotic germ cells through a simple two-step induction protocol using retinoic acid and Sertoli cell-conditioned medium. Haploid cells were obtained but only developed up to initial tail formation stages [29]. There are differences between the cells obtained from various umbilical cord compartments, with diversity in the three lineages' differentiation capacity and the stem cell characteristics between these compartments—the Wharton's jelly region being the richest in these stem cell properties [30–31].

1.2.4 Skeletal muscle MSCs

Skeletal muscle tissue accommodates two stem cell populations: muscle-derived mesenchymal stem cells (MD-MSCs) and satellite cells, which function predominantly as muscle precursors and play an influential role in muscle regeneration [32]. However, satellite cells are considered unipotent and committed to myogenesis and do not spontaneously adopt adipogenic or other nonmyogenic fates in culture. MD-MSCs are present under the muscle fibers' basal lamina and have been isolated from canine and rat muscle. They exhibit similar stem cell characteristics [9]. MD-MSCs positively express MSCs markers thy1 (CD90) and CD59 and negatively express CD45 and CD34. They were positive for desmin, a muscle-specific type III intermediate filament, validating their skeletal muscle origin, and they expressed a stem cell growth factor receptor (also known as c-kit or CD117). Conversely, BM-MSCs were found to be negative for both markers [33].

1.2.5 Synovial MSCs

Synovial fluid-derived mesenchymal stem cells (SF-MSCs) are isolated from synovial fluid and the synovial membrane. Yoshimura et al. discovered that rat SD-MSCs were superior to BM-MSCs, MD-MSCs, and AD-MSCs in terms of colony formation capability [11]. Human SD-MSCs displayed considerable ability to proliferate in culture while maintaining their multilineage differentiation potential in vitro. They were positive for CD44 and negative for CD45, CD3D, CD20, and CD14. Synovium is an attractive MSC source because it is easy to isolate arthroscopically without causing donor site complications, and there is usually little, if any, invasiveness [34].

1.2.6 Dental pulp MSCs

Dental pulp mesenchymal stem cells (DP-MSCs) originate and develop from neural crest cells and mesenchymal cells. In the tooth formation, cells from the epithelial and dental papilla support differentiating DP-MSCs into odontoblasts. In vitro, DP-MSCs have differentiated into bone and neurons [35, 36]. Because neural crest cells share origins with the neural tissue progenitor cells, DP-MSCs can potentially differentiate into neural cell lineages and have been induced to express neural markers, such as nestin.

1.2.7 The testis as a source of MSCs

In 2009, Gonzalez et al. isolated MSCs from the human testis and labeled them GSCs [13]. They reported that

GSCs express CD73, CD105, and CD166, do not express CD45, HLA-DR, CD34, CD11b, and CD19, and positively express CD90, CD44, and STRO-1—which are represented specifically by MSCs. Their reverse transcription polymerase chain reaction (RT-PCR) analysis indicated that GSCs express Oct4 and Nanog but that they do not express Sox2. Notably, GSCs detected vimentin—a protein present in mesenchymal cells' intermediate filaments. They analyzed germ cell markers Vasa and DAZL to further confirm cell origin and the cells did not express either, which validated that they are not of germ cell origins [13].

In 2012, Chikhovskaya et al. isolated MSCs from human testes that were positive for CD90, CD73, and CD105 and negative for CD34, CD31, CD45, and HLA-DR [14]. Similarly, in 2014, Smith et al. identified that the testicular thy1 cell population contained MSCs. These cells could adhere to plastic and differentiate into adipocytes, chondrocytes, and osteocytes, express mesenchymal markers (CD73 and 105), but that they do not express CD45 [37]. Our laboratory generated mouse testis-derived multipotent stromal cells (tMSCs) that were positive for CD44, CD73, and CD29 and negative for CD45 and Vasa [16]. In 2018, Chiara et al. isolated MSCs from human testicular biopsies; these cells positively express CD105, CD73, and CD90 and do not express CD14 and CD34 [15]. The ISCT's criteria are not always applicable to MSCs derived from different sources or species. Therefore, it remains a crucial concern to develop a comprehensive criteria that can specifically and accurately identify MSCs.

The testis homes at least two types of "stem-like" cells and it is crucial that researchers can efficiently distinguish them. Nestin-positive perivascular cells in the fetal testis have multipotent capabilities: the mesenchymal perivascular progenitors that comigrate with mesonephros endothelial cells into the testis gives rise to Leydig cells, pericytes, and smooth muscle cells [38]. Eildermann et al. identified reliable markers to differentiate tMSCs from cells of spermatogonial origin in culture [39]. Recent reports identified thy1 (CD90) and ITGA6 (CD49f) as specific markers for spermatogonia [40, 41]; however, as proven before, these markers are also expressed by MSCs [6, 42]. Using marmoset testis tissue sections, Eildermann et al. demonstrated that tMSCs do not lack the expression of the Androgen Receptor gene that Sertoli, Leydig and myoid cells usually express. Therefore, this concludes that tMSCs do not originate from any of these testis-specific cell types [39]. Like their human counterparts, marmoset tMSCs express the MSC-characteristic cell surface antigens CD105, CD166, CD44, and CD90 but moderately express MSCs negative markers CD45 and CD19. Moreover, for the first time, they expressed TRA-1-81, GPR125, and GFR-a by tMSCs like that of spermatogonia [37]. Hence, the question of which markers are truly and unequivocally specific for tMSCs remains an enigma.

Very few reports describe isolating pure MSCs devoid of germ cells from the testis—two on hMSCs [13,14], one from the marmoset monkey [37], and one from the mouse testis [16]. Isolating comparatively rare cells, such as MSCs, remains problematic in most tissues, let alone in a tissue such as the testis—where there is a spermatogonial stem cells abundance. Nonetheless, researchers were able to isolate tMSCs by adopting and minorly moderating Gonzalez et al.'s isolation method (see Table 1). The source tissue is briefly dissociated in two [14, 16, 39] or three enzymatic digestion steps [15] by using a combination of trypsin, collagenase type I, and hyaluronidase with the option to filter through a 40 µm [13] or 70 µm [16] strainer after digestion. tMSCs ability to adhere to plastic cells allows them to be cultured on plastic plates for several days; the nonadherent cells are discarded but the adherent cells are cultured and expanded. Based on positive selection by the extracellular matrix (ECM) ligand–laminin, a simple method was introduced to enrich MSCs from mouse testicular cell suspensions. Laminin can bind surface integrins on MSCs (see Figure 2) [16]. Cells of interest can be positively selected from seeding the cells on laminin-coated culture plates. The laminin selection step's initial cell yield (16 percent) is regarded as high, considering that the yield generated from plastic adherence is between 0.01 percent and 0.001 percent of nucleated cells isolated from bone marrow aspirates [43,44].

Table 1. tMSCs' differentiation potential across different mammalian species.

Mammalian species	Documented or reported differentiation potency	Reference
Human	Osteogenesis, adipogenesis, and chondrogenesis	[13]
	Osteogenesis, adipogenesis, and chondrogenesis	[14]
	Osteogenesis and adipogenesis	[15]
Marmoset monkey	Osteogenesis, adipogenesis, and chondrogenesis	[39]
Mouse	Osteogenesis and adipogenesis	[16]

10.30564/amor.v4i6.195

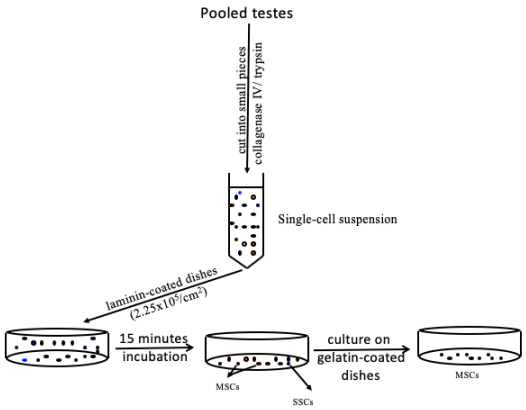

Figure 2. Representation of tMSCs' isolation and enrichment procedure[16].

2. MSCs' proliferation and differentiation potential

Although MSCs are capable of considerable proliferation in culture, this is not without limitations. After several cell divisions, they tend to assume irreversible growth arrest status—known as senescence, which is a major challenge when culturing MSCs for prolonged periods because senescence deteriorates MSCs' fitness for therapeutic purposes. Unfortunately, the lack of freshly extracted MSCs' high homogeneity necessitates extensive in vitro expansion following isolation, which may cause cell senescence. A recent study isolated BM-MSCs, SF-MSCs, AD-MSCs, and SF-MSCs from the anterior cruciate ligament and compared their self-renewal capability [45]. MD-MSCs and AD-MSCs displayed a lower proliferation capacity than other MSCs. Conversely, SD-MSCs displayed high expansion ability, comparable to that of BM-MSCs. Gonzalez et al. reported that GSCs were propagated for 17 passages. GSCs had a clonogenic efficiency of 35±1.8 percent and a doubling time of 33.8±6.5 hours [13] and tMSCs results reflected those of cell populations with average doubling times of 44.1–6.5 hours. tMSCs' clonogenic capacity was demonstrated through successful colony generations. Moreover, tMSCs were propagated for eight passages [16].

At present, there are no universal phenotypic or morphologic criteria to characterize MSCs, since they remain somewhat morphologically heterogeneous, ranging from narrow spindle-shaped cells to large polygonal-shaped cells [13]. An important but undefining tMSCs feature is their ability to be plastic-adherent and form colonies when seeded at a low density. tMSCs are morphologically highly heterogeneous, consisting of both round- and spindle-shaped cells. Interestingly, colonies composed of cells that resembled ESCs were observed in confluent passage one cells cultures (see Figure 3A). Population heterogeneity decreased with subsequent passaging and the cell population became almost homogeneous by the third passage. In the early passages' course (passages 1–6), tMSCs maintained their bipolar spindle-shaped morphology status (see Figures 3B & 3C). However, they disappeared with subsequent passaging as cells were flattened and assumed an irregular morphology with a circumscribed nucleus and increased debris in the medium (see Figure 3D) [16]. Eildermann et al. also found that tMSCs formed a cohesive monolayer consisting of spindle-shaped cells [39].

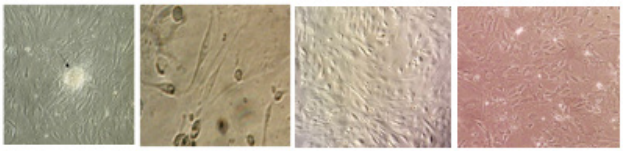

Figure 3. Mouse tMSCs' enrichment and propagation in culture[16].

A key MSCs feature is their innate ability to differentiate along several mesenchymal cell lineages. Although it is a fundamental property of MSCs, it greatly depends on the tissue source and culturing techniques, which may cause a substantial loss of potency in vitro. Numerous studies have discussed significant discrepancies in MSCs features, due to experimental variation and the cell source. MSCs' differentiation potential is highly dependent on prolonged culture conditions, which may cause a substantial loss of multipotentiality in vitro (most likely due to cellular senescence). A gradual reduction in MSCs' differentiation capacity has been demonstrated, especially in late passages, as they assume a senescent nature [46]. Thus, timing is critical when assessing MSCs' true differentiation potential.

2.1 In vitro differentiation capacity

2.1.1 Osteogenic differentiation

When first identified by Alexander Friedenstein, MSCs were described as osteogenic stem cells. Thus, osteogenesis would be the first step in testing newly identified MSCs potency. tMSCs' osteogenic differentiation is induced in vitro by treating cultured cells with osteogenic media. Usually, the osteogenic media consists of dexamethasone, ascorbic acid 2-phosphate, and beta-glycerophosphate. Although mineralized deposits appear within seven days of incubating MSCs in osteogenic media, treatment is

continued for up to 21 days to allow osteoblasts, which produce and deposit calcium, to mature. After the treatment is complete, cells can be fixed and stained with von Kossa silver nitrate or alizarin red S solution, which highlight calcium phosphate deposits. Further, expressing osteogenesis-specific genes that can be detected at the RNA and protein levels—DLX5, RUNX2, osteopontin, and osteocalcin, mediates osteogenic differentiation [47]. Under appropriate conditions, tMSCs can be induced to differentiate into osteocytes using osteogenic media. This is evident by positive alizarin red S solution staining, which detects calcium deposit accumulation [13–16] and increases expressing osteocalcin, DLX5 [13], RUNX2, and SPP1 [14], indicating osteogenic differentiation compared to noninduced controls.

2.1.2 Adipogenic differentiation

The next step is tMSCs' adipogenic differentiation, which occurs by treating a monolayer culture with adipogenic media. This media is supplemented with dexamethasone, isobutylmethylxanthine, and insulin and changed twice weekly for three weeks. Alternatively, MSCs can be processed through three treatment cycles—each cycle containing three days of culture in differentiation medium, followed by two days in maintenance medium. Indomethacin is included in the differentiation medium, whereas the maintenance medium contains insulin. Mature adipocytes' appearance is characterized by lipid-filled droplets (which can be detected by staining with Oil red O) and detected by the RNA expression of adipsin, lipoprotein lipase, and Peroxisome proliferator-activated receptor (PPAR) gamma [47]. Under adipogenic differentiation-inducing conditions, tMSCs successfully differentiate toward the adipogenic lineage by displaying lipid vacuoles stained with Oil red O [13-16]. Moreover, they display increased expression of adipocyte-specific markers—lipoprotein lipase [13,14], PPARγIso2 [13], and adipsin [14], suggesting that the cells have high adipogenic potential.

2.1.3 Chondrogenic differentiation

The last step is chondrogenic differentiation, which is traditionally achieved by treating tMSCs with chondrogenic medium. Usually, culture protocols state that chondrogenic media should contain dexamethasone, ascorbic acid phosphate, and ITS+ supplement. Throughout treatment, chondrogenic markers—such as collagen II, aggrecan, collagen XI, and syndecan—increase expression and can be detected at RNA and protein levels. After three weeks in culture, staining with Safranin O, Toluidine blue, and Alcian blue, respectively, detect acid mucopolysaccharides, glycosaminoglycans, and proteoglycans [47]. Similarly, tMSCs differentiated toward chondrogenic lineage, confirmed by accumulating ECM components (acid mucopolysaccharides), sulfated proteoglycans stained with Alcian blue, in addition to expressing cartilage-specific markers, aggrecan [13,14], link protein [13], and collagen IX [14]. This data clearly demonstrates that tMSCs possess trilineage differentiation potential (see Table 2) [48].

Table 2. Trilineage differentiation markers.

Differentiation potency	Differentiation Marker
Osteogenesis	Alkaline Phosphatase/ ALPP/ALPI
	BAP1
	Collagen I
	Collagen I α1
	Collagen II
	RUNX2/CBFA1
	DLX5
	Osteocalcin
Adipogenesis	Adiponectin/Acrp30
	FABP4/A-FABP
	Leptin/OB
	PPARγ/NR1C3
	Glut4
	Adipsin
	Lipoprotein Lipase
	PPARγ/NR1C3
Chondrogenesis	Aggrecan
	Collagen II
	Collagen IV
	Annexin A6
	IBSP/Sialoprotein II
	CRTAC1
	CD44
	CD151
	DSPG3

2.2 In vivo differentiation capacity

Several studies demonstrate MSCs' in vivo differentiation potential. Horwitz et al.'s clinical trial involved pediatric patients with Osteogenesis imperfecta undergoing an allogeneic BM-MSCs transplant. These cells engrafted and generated functional osteoblasts, which led to bone structure and function improvement [49]. Moreover, numerous animal models have successfully injected MSCs directly into tissue to repair various cartilage and bone defects [50]. A study by Pagnotto et al. showed inducting hMSCs by transforming growth factor-β1 led to successful chondrogenesis and improved cartilage repair [51].

Further, bone marrow cells can engraft into cardiac tissue, differentiate into cardiac myocytes, and improve cardiac function [52]. However, controversy has surrounded MSCs' in vivo potential. This, in part, has been attributed to a majority of studies using an undefined cell popula-

tion extracted from bone marrow and making it debatable whether their properties are of mesenchymal or hematopoietic origin. Therefore, it is essential to define and enrich the MSCs population before addressing their differentiation potential, and tMSCs' in vivo differentiation capacity is yet to be determined.

3. MSCs' therapeutic potential

3.1 MSCs and immunomodulation

Several studies reported that MSCs can affect almost all cells of both innate and adaptive immune systems, as well as inducing an anti-inflammatory phenotype [55,56]. MSCs modulate the immune response through producing soluble factors and cell–cell interaction. Adult BM-MSCs possess a potent mechanism, which allows cells to evade allogeneic rejection [56]. Moreover, recent studies have reported an additional MSCs therapeutic benefit that exists in their paracrine secretion. In 1996, Haynesworth et al. first determined MSCs' paracrine secretion [57]. They demonstrated that MSCs produce and release a broad range of growth factors, chemokines, and cytokines that can attune neighboring cell action [58].

3.2 MSCs and cancer

Moreover, studies reported that MSCs have cancer therapeutic potential. They inhibit tumor progression and metastasis by suppressing cell proliferation signaled by the Akt and Wnt pathways and induce apoptosis or cell cycle arrest in the G0 and G1 phase. This healing ability is verified using WJ-MSCs encapsulated in sodium alginate beads. Administering the beads exhibited anticancer activity against breast cancer stem cells by impeding the Wnt pathways, which was triggered by Wnt antagonism through increasing sFRP4, DKK1, and GSK-3β expression[59]. This activity associates with the downregulation of β-catenin [60]. Another potential mechanism of MSCs tumor suppression ability relates to its immunosuppressive effects. The MSCs inhibitory effects decreased multiple malignant cell (of both immune and nonimmune origin cell) proliferation by arresting the cell cycle's early stages [61].

MSCs have been used to deliver chemotherapeutic drugs or nanoparticles. A recent study focused on incorporating nongenetically modified MSCs with nanoparticles containing a paclitaxel anticancer drug. This incorporation aimed to create cellular drug depots capable of targeting tumors and sustain-release the drug. When the drug releases within the MSCs it diffuses out of the cell and affects cancer cells. Nanoengineered MSCs deliver paclitaxel, which results in decreasing tumor cell proliferation, increasing apoptosis and inhibiting angiogenesis within the tumor matrix at a considerably reduced drug dose [62]. Previous studies employed nongenetically modified MSCs, but a second approach for cell-based cancer therapy involves using genetically engineered MSCs. Genetically modified human AD-MSCs producing IP-10—a chemoattractant cytokine with pleiotropic antitumor effects—displayed increased activated T cells trafficking to the tumor site and reduced angiogenesis in mice that are bearing melanoma lung metastasis [63].

When discussing MSCs' therapeutic potential it is important to highlight their tumorigenic potential. Several studies have recently observed potential MSCs tumorigenic effects, such as metastasis, tumor growth, and transformation into cancer cells. MSCs have been reported to interact with cancer cells at primary tumor sites and induce tumor cells to become more invasive [64]. Additionally, research data shows that coculturing BM-MSCs with breast or gastric cancer cells results in upregulating EMT markers (vimentin, N-cadherin, and Twist) and downregulating E-cadherin in cancer cells. This creates increased migration and invades cancer cell capabilities. [65, 66]. A study by Duda and colleagues suggests that, in some cases, tumor cells depart the primary site as "heterotypic tumor fragments," consisting of metastatic cancer cells accompanied by tumor stromal cells, which promote tumor growth at the metastasis site [67]. This indicates a potential role for MSCs in creating and possibly maintaining a metastatic niche. These effects may vary depending on the MSC source; for example, BM-MSCs could only support breast cancer and not ovarian cancer growth [68]. This difference is greatly important because MSCs can be used as therapeutic vehicles with disease-specific effectiveness.

Further, it is reported that MSCs support tumor vasculature directly by differentiating into pericytes, and indirectly by secreting vasculogenic growth factors. Thus, generating a growth-supporting microenvironment promoted by trophic factors secretion, such as transforming growth factor β, vascular endothelial growth factor, platelet-derived growth factor, and epidermal growth factor [69]. Compelling evidence supports MSCs' capacity to differentiate into pericytes. Transplanted MSCs have engrafted in the perivascular niche with proximal contact with the underlying endothelial cells [70]. Pericytes isolated from the stromal-vascular compartment contains an MSC-like population, which express CD10, CD13, and CD90 surface markers and have the capacity to differentiate into tissues of mesenchymal lineage [23, 71].

MSCs can also be therapeutically applied in infertility. Wang et al. transplanted MSCs isolated from goat bone marrow into busulfan-treated mice's seminiferous tubules, showing their potential to restore spermatogenesis [72]. When transplanted into damaged ovaries, BM-MSCs enhanced germ cell survival and diminished granulosa cell apoptosis [73]. Other MSCs sources have been studied in this regard; UC-MSCs demonstrated the potential to differentiate into germ-like cells, and improve damaged testicular tissue in busulfan-treated mice [74]. Moreover, BM-MSCs have diminished ovary cell apoptosis and enhanced folliculogenesis in premature ovarian failure models [75]. Lai and colleagues reported that skin-derived MSCs transplanted into chemotherapy-treated females migrated to the damaged ovaries and almost certainly played a role in regulating proinflammatory cytokines, which resulted in function restoration [76]. These findings suggest that MSCs could be a suitable option for infertility treatment [77], but, MSCs' role in tumor progression is context dependent and remains debatable.

3.3 MSCs and regenerative medicine

The in vitro and in vivo differentiation capacity establishes MSCs as a promising candidate for regenerative medicine, but there are additional features that reaffirm their potential. MSCs can respond to inflammatory factors by migrating to injury sites, reaching the sites through the circulatory system and exerting their functional effects locally in the target tissue. Various studies reported that MSCs selectively migrate to the injured tissue from the assistance of several cytokines, such as receptor tyrosine kinase-dependent growth factors and chemokines, which are secreted by injured cells and/or immune cells [53,54]. Efficient cell delivery is a critical regenerative therapy step and these factors would greatly facilitate MSCs' therapeutic delivery to target the injured tissue [54]. MSCs can also exert an anti-inflammatory effect through the secretion of many molecules, which subsequently stimulate injured cell recovery.

3.3.1 MSCs' extracellular vesicles contribute to tissue regeneration

It is reported that MSCs release extracellular vesicles, which contribute to regenerating tissue by carrying informational signals to damaged cells or tissue [78]. The membrane bound extracellular vesicles receive various cell type secretion and play an essential role in cell–cell communication. They have been implicated to participate in essential processes, such as immune responses, coagulation, homeostasis maintenance, inflammation, angiogenesis, cancer progression, and antigen presentation. MSCs enhance the repair of injured tissues via the production of paracrine signals, and several secreted molecules such as microvesicles (MVs)[79]. These highly important extracellular vesicles MVs originate from the cell membrane external budding and range in diameter from 100–1000 nm. They act as vehicles to carry proteins, lipids, mRNAs, and microRNAs and are abundant in the surface marker CD40 [80, 81]. MVs may change some target cell functions, such as promoting tumor suppression activity by delivering specific intracellular proteins[82].

MSCs respond to damaged tissues, and tumor sites deem them as an appropriate vehicle for delivering therapeutic agents to tumors and metastatic niches. MSCs can be genetically modified to encode tumor suppressor genes or immunomodulate cytokines, or a combination of both. Studies explored that BM-MSCs - EV caused cell cycle arrest in G0 and G1 phases, apoptosis of Kaposi's sarcoma, and ovarian cancer cells [83]. Lee and colleagues reported angiogenesis suppression in tumor cells through the downregulation of VEGF by MSCs - EV [84]. Conversely, Vallabhaneni et al. demonstrated that BM-MSCs - EV contained small, tumor - supporting RNA (e.g., miRNA - 21 and 34a) and multiple factors recognized as tumor - supporting proteins (e.g., TIMP - 1 and TIMP - 2) [85]. MSCs - EV also stimulates breast cancer cell migration and growth through enhancing Wnt/β - catenin signaling [86]. However, MSCs - EV's role in tumor development is still controversial and molecular mechanisms are not yet entirely established [87].

3.3.2 Characterizing MVs derived from tMSCs

Chiara et al.'s experiment isolated MVs from tMSC culture after serum starvation for 24 hours and labeled them with PKH26 dye. Next. the tMSC-derived MVs were incubated with inner medullary collecting duct cells for 4 hours, 12 hours, and 24 hours. Uptake of renal cell's MVs were evident at 4 hours' post incubation and continued time-dependently, finally reaching maximum uptake at 24 hours. Researchers performed real-time MVs PCR analysis to evaluate mRNA composition and verify mesenchymal origins, revealing that tMSC-MVs do possess mesenchymal origin and express high levels of vimentin, CD90, and mRNA. This data is influential because it is the first to discover that tMSCs can secrete MVs that are readily occupied by renal cells and can be used as a regenerative medicine applications avenue, specifically in renal injury repairs [15].

4. Conclusion

MSCs are adult, nonhematopoietic stem cells initially isolated from bone marrow. Their ability to self-renew and differentiate into multiple cell lineages establishes them a desirable cell source in a new generation of cell-based regenerative therapies. In the early 1950s, Friedenstein et al. described these cells, but, to this day, researchers are still attempting to unlock their secrets. In 2006, the criteria used to identify MSCs involved their plastic adherence, ability to express specific cell surface markers, and multilineage differentiation capacity. Despite MSCs of various tissue sources sharing many characteristics and almost fulfilling the established criteria, there are observed differences. We are increasingly learning that these criteria should be revisited to include newly discovered species- and tissue-specific markers and allow more therapeutic options. Researchers have isolated MSCs from various tissue types—bone marrow, adipose tissue, umbilical cord, skeletal muscle, synovial membrane, dental pulp, and testes. Researchers have demonstrated that tMSCs comply with minimal MSCs criteria—adhere to plastic, express MSC-specific surface markers and potency through successful differentiation into multiple lineages. This renders them a good choice for treating various diseases, including cancer.

However, there is not an established protocol for isolating a pure population of tMSCs—the protocol currently used produces a heterogeneous population that differs in growth kinetics and differentiation potential. Further consideration is required to develop highly efficient extraction methods that can generate a pure tMSCs population. Further, tMSCs represent a versatile adult stem cell population that demands further molecular characterization and functional investigation to be effectively utilized for therapeutic purposes. Future research should focus on further defining their potential in clinical applications as an autologous and allogeneic stem cell source.

Additionally, researchers must develop a new culturing technique that allow tMSCs to bypass senescence and maintain their self-renewal capabilities in prolonged in vitro cultures and search for more suitable markers to isolate source-specific MSCs. Addressing these issues establishes a pure tMSC culture possibility and subsequently prepares further research to investigate and unlock MSCs' differentiation potential capacity and their efficient use as a new regenerative medicine and different therapeutic approaches cell source. tMSCs can secrete MVs that have been successfully occupied by renal cells and can potentially be utilized in renal injury repair. However, further analysis is required to assess tMSC-derived MVs' relevant biological activity and the possibility to exploit their role for restoring various chronic diseases in vitro, such as cancer and acute diseases .

List of Abbreviations

List of abbreviations	
HSCs	Hematopoietic stem cells
MSCs	Mesenchymal stem cells
CFU-F	Colony forming unit fibroblasts
ISCT	International Society for Cellular Therapy
GSCs	Gonadal stem cells
BM-MSCs	Bone marrow mesenchymal stem cells
AD-MSCs	Adipose derived mesenchymal stem cells
ESCs	Embryonic stem cells
UC-MSCs	Umbilical cord-derived mesenchymal stem cells
MD-MSCs	Muscle derived mesenchymal stem cells
MVs	Microvesicles
SD-MSCs	Synovial derived mesenchymal stem cells
DP-MSCs	Dental pulp derived mesenchymal stem cells
TMSCs	Testis-derived multipotent mesenchymal stromal cells (Marmoset Monkey)
tMSCs	Testis-derived multipotent mesenchymal stromal cells (Mouse)

Ethics Statement

Not applicable. No ethical approval was sought for producing the review of published literature.

Author Contributions

Conceived: A. Amleh

Wrote the paper: M. Ghabriel and A. Amleh

Acknowledgements

The author(s) received no financial support for the research of this article.

References

[1] Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation. 1968;6:230–247.

[2] Owen, M. (1988). "Marrow stromal stem cells." Journal of Cell Science 1988 (Supplement 10): 63-76.

[3] Caplan AI. Mesenchymal stem cells. Journal of Orthopaedic Research. 1991;9(5):641-50.

[4] Dennis JE, Merriam A, Awadallah A, Yoo JU, Johnstone B, Caplan AI. A Quadripotential Mesenchymal Progenitor Cell Isolated from the Marrow of an Adult Mouse. Journal of Bone and Mineral Research. 1999;14(5):700-9.

[5] Bianco P, Gehron Robey P. Marrow stromal stem cells. The Journal of clinical investigation. 2000;105(12):1663-8.

[6] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315-7.

[7] Keating A. Mesenchymal stromal cells: new directions. Cell stem cell. 2012;10(6):709-16.

[8] Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Molecular biology of the cell. 2002;13(12):4279-95.

[9] Kisiel AH, McDuffee LA, Masaoud E, Bailey TR, Esparza Gonzalez BP, Nino-Fong R. Isolation, characterization, and in vitro proliferation of canine mesenchymal stem cells derived from bone marrow, adipose tissue, muscle, and periosteum. American journal of veterinary research. 2012;73(8):1305-17.

[10] Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. British journal of haematology. 2000;109(1):235-42.

[11] Yoshimura H, Muneta T, Nimura A,Yokoyama A, Koga H, Sekiya I. Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. Cell and Tissue Research. 2007;327:449-462.

[12] Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A. 2000;97(25):13625-30.

[13] Gonzalez R, Griparic L, Vargas V, Burgee K, Santacruz P, Anderson R, et al. A putative mesenchymal stem cells population isolated from adult human testes. Biochem Biophys Res Commun. 2009;385(4):570-5.

[14] Chikhovskaya JV, van Daalen SK, Korver CM, Repping S, van Pelt AM. Mesenchymal origin of multipotent human testis-derived stem cells in human testicular cell cultures. Molecular human reproduction. 2014;20(2):155-67.

[15] De Chiara L, Famulari ES, Fagoonee S, van Daalen SKM, Buttiglieri S, Revelli A, et al. Characterization of Human Mesenchymal Stem Cells Isolated from the Testis. Stem Cells International. 2018;2018:9.

[16] Ahmed M., Ghabriel M., Amleh A. Enrichment, Propagation, and Characterization of Mouse Testis-Derived Mesenchymal Stromal Cells. Cellular Reprogramming. 2017;19(1):35-43.

[17] Peister A, Mellad JA, Larson BL, Hall BM, Gibson LF, Prockop DJ. Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. Blood. 2004;103(5):1662-8.

[18] Sung JH, Yang HM, Park JB, Choi GS, Joh JW, Kwon CH, et al. Isolation and characterization of mouse mesenchymal stem cells. Transplantation proceedings. 2008;40(8):2649-54.

[19] Takashima Y, Era T, Nakao K, Kondo S, Kasuga M, Smith AG, et al. Neuroepithelial Cells Supply an Initial Transient Wave of MSC Differentiation. Cell. 2007;129(7):1377-88.

[20] Nagoshi N, Shibata S, Kubota Y, Nakamura M, Nagai Y, Satoh E, et al. Ontogeny and Multipotency of Neural Crest-Derived Stem Cells in Mouse Bone Marrow, Dorsal Root Ganglia, and Whisker Pad. Cell stem cell. 2008;2(4):392-403.

[21] Yamashita J, Itoh H, Hirashima M, Ogawa M, Nishikawa S, Yurugi T, et al. Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors. Nature. 2000;408(6808):92-6.

[22] Crisan M, Deasy B, Gavina M, Zheng B, Huard J, Lazzari L, et al. Purification and Long-Term Culture of Multipotent Progenitor Cells Affiliated with the Walls of Human Blood Vessels: Myoendothelial Cells and Pericytes. Methods in Cell Biology. 86: Academic Press; 2008. p. 295-309.

[23] Crisan M, Yap S, Casteilla L, Chen C-W, Corselli M, Park TS, et al. A Perivascular Origin for Mesenchymal Stem Cells in Multiple Human Organs. Cell stem cell. 2008;3(3):301-13.

[24] Brachvogel B, Moch H, Pausch F, Schlötzer-Schrehardt U, Hofmann C, Hallmann R, et al. Perivascular cells expressing annexin A5 define a novel mesenchymal stem cell-like population with the capacity to differentiate into multiple mesenchymal lineages. Development. 2005;132(11):2657-68.

[25] Boxall SA, Jones E. Markers for Characterization of Bone Marrow Multipotential Stromal Cells. Stem Cells International. 2012;2012:12.

[26] Arutyunyan I, Elchaninov A, Makarov A, Fatkhudinov T. Umbilical Cord as Prospective Source for Mesenchymal Stem Cell-Based Therapy. Stem cells international. 2016;2016:6901286.

[27] Baksh D, Yao R, Tuan RS. Comparison of Proliferative and Multilineage Differentiation Potential of Human Mesenchymal Stem Cells Derived from Umbilical Cord and Bone Marrow. STEM CELLS. 2007;25(6):1384-92.

[28] Troyer DL, Weiss ML. Wharton's jelly-derived cells are a primitive stromal cell population. Stem Cells. 2008;26(3):591-9.

[29] Dissanayake D, Patel H, Wijesinghe PS. Differentiation of human male germ cells from Wharton's jelly-derived mesenchymal stem cells. Clinical and experimental reproductive medicine. 2018;45(2):75-81.

[30] Mennan C, Wright K, Bhattacharjee A, Balain B, Richardson J, Roberts S. Isolation and characterisation of mesenchymal stem cells from different regions of the human umbilical cord. BioMed research international.

2013;2013:916136.

[31] Subramanian A, Fong C-Y, Biswas A, Bongso A. Comparative Characterization of Cells from the Various Compartments of the Human Umbilical Cord Shows that the Wharton's Jelly Compartment Provides the Best Source of Clinically Utilizable Mesenchymal Stem Cells. PloS one. 2015;10(6):e0127992-e.

[32] Relaix F, Marcelle C. Muscle stem cells. Current opinion in cell biology. 2009;21(6):748-53.

[33] Pavyde E, Maciulaitis R, Mauricas M, Sudzius G, Ivanauskaite Didziokiene E, Laurinavicius A, et al. Skeletal Muscle-Derived Stem/Progenitor Cells: A Potential Strategy for the Treatment of Acute Kidney Injury. Stem Cells Int. 2016;2016:9618480.

[34] De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent Mesenchymal Stem Cells From Adult Human Synovial Membrane. Arthritis Rheum 2001;44:1928-1942.

[35] Gronthos S, Brahim J, Li W, et al: Stem cell properties of human dental pulp stem cells. J Dent Res 2002;8:531–5.

[36] Yu J, Wang Y, Deng Z, Li Y, Shi J, Jin Y: Odontogenic capability: bone marrow stromal stem cells versus dental pulp stem cells. Biol Cell 2007;8: 465–74.

[37] Smith JF, Yango P, Altman E, Choudhry S, Poelzl A, Zamah AM, et al. Testicular niche required for human spermatogonial stem cell expansion. Stem Cells Transl Med. 2014;3(9):1043-54.

[38] Kumar DL, DeFalco T. A perivascular niche for multipotent progenitors in the fetal testis. Nat Commun. 2018;9(1):4519-.

[39] Eildermann, K., Gromoll, J., and Behr, R. Misleading and reliable markers to differentiate between primate testis-derived multipotent stromal cells and spermatogonia in culture. Hum. Reprod. 2012;27, 1754–1767.

[40] Lee JH, Engel W, Nayernia K. Stem cell protein Piwil2 modulates expression of murine spermatogonial stem cell expressed genes. Mol Reprod Dev 2006;73:173–179.

[41] He Z, Kokkinaki M, Jiang J, Dobrinski I, Dym M. Isolation, characterization, and culture of human spermatogonia. Biol Reprod 2010;82:363–372.

[42] Roncoroni LP, Maerz JK, Angres B, Steuer H, Benz K, et al. Adhesion to extracellular matrix proteins can differentiate between human bone marrow derived mesenchymal stem cells and fibroblasts. J. Tissue Sci. Eng. 2013; S11, 008.

[43] Muschler, G.F., Nitto, H., Boehm, C.A., and Easley, K.A. Age and gender related changes in the cellularity of human bone marrow and the prevalence of osteoblastic progenitors. J. Orthop. Res. 2001;19, 117–125.

[44] Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of Human Stem Cells Derived From Various Mesenchymal Tissue. Superiority of Synovium as a Cell Source. Arthritis Rheum 2005;52:2521-2529.

[45] Noer A, Boquest AC, Collas P. Dynamics of adipogenic promoter DNA methylation during clonal culture of human adipose stem cells to senescence. BMC Cell Biol. 2007;8:18.

[46] Marcantonio, N.A., Boehm, C.A., Rozic, R.J., Au, A., Wells, A., Muschler, G.F., and Griffith, L.G. The influence of tethered epidermal growth factor on connective tissue progenitor colony formation. Biomaterials 2009; 30, 4629–4638.

[47] Jackson L, Jones DR, Scotting P, Sottile V. Adult mesenchymal stem cells: differentiation potential and therapeutic applications. Journal of postgraduate medicine. 2007;53(2):121-7.

[48] R&D systems. Mesenchymal stem cell differentiation markers [Internet].2019. Available from https://www.rndsystems.com/research-area/mesenchymal-stem-cell-differentiation-markers

[49] Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WWK, Gordon PL, Neel M, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nature Medicine. 1999;5(3):309-13.

[50] Harris MT, Butler DL, Boivin GP, Florer JB, Schantz EJ, Wenstrup RJ. Mesenchymal stem cells used for rabbit tendon repair can form ectopic bone and express alkaline phosphatase activity in constructs. Journal of Orthopaedic Research. 2004;22(5):998-1003.

[51] Motaln H, Schichor C, Lah TT. Human mesenchymal stem cells and their use in cell-based therapies. Cancer. 2010;116(11):2519-30.

[52] Toma C, Pittenger MF, Cahill KS, et al. Human mesenchymal stem cells differentiate to a car-diomyocyte phenotype in the adult murine heart. Circulation. 2002;105:93–8

[53] Otto WR, Wright NA. Mesenchymal stem cells: from experiment to clinic. Fibrogenesis & tissue repair. 2011;4:20.

[54] De Miguel MP, Fuentes-Julian S, Blazquez-Martinez A, Pascual CY, Aller MA, Arias J, et al. Immunosuppressive Properties of Mesenchymal Stem Cells: Advances and Applications. Current Molecular Medicine. 2012;12(5):574-91.

[55] Li W, Ren G, Huang Y, Su J, Han Y, Li J, et al. Mesenchymal stem cells: a double-edged sword in regulating immune responses. Cell death and differentiation. 2012;19(9):1505-13.

[56] Fitzsimmons REB, Mazurek MS, Soos A, Simmons CA. Mesenchymal Stromal/Stem Cells in Regenerative Medicine and Tissue Engineering. Stem Cells International. 2018;2018:16.

[57] Haynesworth SE, Baber MA, Caplan AI. Cytokine expression by human marrow-derived mesenchymal progenitor

cells in vitro: Effects of dexamethasone and IL-1 alpha. J Cell Physiol. 1996;166(3):585–92.

[58] Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. Mol Ther. 2015;23:812–23.

[59] Mandal S, Arfuso F, Sethi G, Dharmarajan A, Warrier S. Encapsulated human mesenchymal stem cells (eMSCs) as a novel anti-cancer agent targeting breast cancer stem cells: Development of 3D primed therapeutic MSCs. The International Journal of Biochemistry & Cell Biology. 2019;110:59-69.

[60] Konala VB, Mamidi MK, Bhonde R, Das AK, Pochampally R, Pal R. The current landscape of the mesenchymal stromal cell secretome: a new paradigm for cell-free regeneration. Cytotherapy. 2016;18:13–24.

[61] Ramasamy R, Lam EWF, Soeiro I, Tisato V, Bonnet D, Dazzi F. Mesenchymal stem cells inhibit proliferation and apoptosis of tumor cells: impact on in vivo tumor growth. Leukemia. 2006;21:304.

[62] Layek B, Sadhukha T, Panyam J, Prabha S. Nano-Engineered Mesenchymal Stem Cells Increase Therapeutic Efficacy of Anticancer Drug Through True Active Tumor Targeting. Molecular cancer therapeutics. 2018;17(6):1196-206.

[63] Mirzaei H, Salehi H, Oskuee RK, Mohammadpour A, Mirzaei HR, Sharifi MR, et al. The therapeutic potential of human adipose-derived mesenchymal stem cells producing CXCL10 in a mouse melanoma lung metastasis model. Cancer Letters. 2018;419:30-9.

[64] Albarenque SM, Zwacka RM, Mohr A. Both human and mouse mesenchymal stem cells promote breast cancer metastasis. Stem Cell Research. 2011;7(2):163-71.

[65] Martin FT, Dwyer RM, Kelly J, Khan S, Murphy JM, Curran C, et al. Potential role of mesenchymal stem cells (MSCs) in the breast tumour microenvironment: stimulation of epithelial to mesenchymal transition (EMT). Breast Cancer Research and Treatment. 2010;124(2):317-26.

[66] Xue Z, Wu X, Chen X, Liu Y, Wang X, Wu K, et al. Mesenchymal Stem Cells Promote Epithelial to Mesenchymal Transition and Metastasis in Gastric Cancer Though Paracrine Cues and Close Physical Contact. Journal of Cellular Biochemistry. 2015;116(4):618-27.

[67] Duda DG, Duyverman AMMJ, Kohno M, Snuderl M, Steller EJA, Fukumura D, et al. Malignant cells facilitate lung metastasis by bringing their own soil. Proceedings of the National Academy of Sciences. 2010;107(50):21677-82.

[68] Coffman LG, Pearson AT, Frisbie LG, Freeman Z, Christie E, Bowtell DD, et al. Ovarian Carcinoma-Associated Mesenchymal Stem Cells Arise from Tissue-Specific Normal Stroma. STEM CELLS. 2019;37(2):257-69.

[69] Nwabo Kamdje AH, Kamga PT, Simo RT, Vecchio L, Seke Etet PF, Muller JM, et al. Mesenchymal stromal cells' role in tumor microenvironment: involvement of signaling pathways. Cancer Biol Med. 2017;14(2):129-41.

[70] Rajantie I, Ilmonen M, Alminaite A et al. Adult bone marrowderived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. Blood 2004;104:2084–2086.

[71] Traktuev DO, Merfeld-Clauss S, Li J et al. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. Circ Res 2008;102:77–85.

[72] Wang F, Liu C, Zhang S-s, Liu W-s, Hua J-l. Transplantation of Goat Bone Marrow Mesenchymal Stem Cells (gMSCs) Help Restore Spermatogenesis in Endogenous Germ Cells-Depleted Mouse Models. Journal of Integrative Agriculture. 2013;12(3):483-94.

[73] Fu X, He Y, Xie C, Liu W. Bone marrow mesenchymal stem cell transplantation improves ovarian function and structure in rats with chemotherapy-induced ovarian damage. Cytotherapy. 2008;10(4):353-63.

[74] Chen H, Tang Q-L, Wu X-Y, Xie L-C, Lin L-M, Ho G-Y, et al. Differentiation of human umbilical cord mesenchymal stem cells into germ-like cells in mouse seminiferous tubules. Mol Med Rep. 2015;12(1):819-28.

[75] Wang S, Yu L, Sun M, Mu S, Wang C, Wang D, et al. The therapeutic potential of umbilical cord mesenchymal stem cells in mice premature ovarian failure. BioMed research international. 2013;2013:690491-.

[76] Lai D, Wang F, Dong Z, Zhang Q. Skin-derived mesenchymal stem cells help restore function to ovaries in a premature ovarian failure mouse model. PloS one. 2014;9(5):e98749-e.

[77] Fazeli Z, Abedindo A, Omrani MD, Ghaderian SMH. Mesenchymal Stem Cells (MSCs) Therapy for Recovery of Fertility: a Systematic Review. Stem Cell Reviews and Reports. 2018;14(1):1-12.

[78] Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013;200:373–83

[79] Fierabracci A, Del Fattore A, Luciano R, Muraca M, Teti A, Muraca M. Recent Advances in Mesenchymal Stem Cell Immunomodulation: The Role of Microvesicles. Cell Transplantation. 2015;24(2):133-49.

[80] Ratajczak J, Miekus K, Kucia M, Zhang J, Reca R, Dvorak P, Ratajczak M. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. Leukemia. 2006;20:847–56.

[81] Konala VBR, Mamidi MK, Bhonde R, Das AK, Pochampally R, Pal R. The current landscape of the mesenchymal stromal cell secretome: A new paradigm for cell-free regeneration. Cytotherapy. 2016;18(1):13-24.

[82] Seyhoun I, Hajighasemlou S, Muhammadnejad S, Ali J, Nikbakht M, Alizadeh AA, Hosseinzadeh F, Mirmoghtadaei M, Seyhoun SM, Verdi J. Combination therapy of sorafenib with mesenchymal stem cells as a novel cancer treatment regimen in xenograft models of hepatocellular carcinoma. J. Cell Physiol. 2019; 234(6): 9495-9503.

[83] Bruno S, Collino F, Deregibus MC, Grange C, Tetta C, Camussi G. Microvesicles derived from human bone marrow mesenchymal stem cells inhibit tumor growth. Stem Cells Dev 2013; 22: 758–71

[84] Lee JK, Park SR, Jung BK et al Exosomes derived from mesenchymal stem cells suppress angiogenesis by down-regulating VEGF expression in breast cancer cells. PLoS One 2013; 8: e84256.

[85] Vallabhaneni KC, Penfornis P, Dhule S et al Extracellular vesicles from bone marrow mesenchymal stem/stromal cells transport tumor regulatory microRNA, proteins, and metabolites. Oncotarget 2015; 6: 4953–67.

[86] Lin R, Wang S, Zhao RC. Exosomes from human adipose-derived mesenchymal stem cells promote migration through Wnt signaling pathway in a breast cancer cell model. Mol Cell Biochem 2013; 383: 13–20.

[87] Lee H. Y., & Hong I. S. (2017). Double-edged sword of mesenchymal stem cells: Cancer-promoting versus therapeutic potential. Cancer science, 108(10), 1939-1946.