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Therapeutic Effects of Wharton's Jelly Mesenchymal Stem Cells: from Laboratory to Clinical Application

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HIGHLIGHTS

- hWJSCs have Therapeutic effects without Tumorigenesis.
- hWJSCs is useful for treating burns and wounds.
- hWJSCs have therapeutic effect on MS.

Abstract: Stem cells are a promising solution for repaired damage tissue. Isolation of this cell from tissue, proliferation in vitro, and implanting them in damaged tissue microenvironment are the basic strategy of Regenerative medicine. Mesenchymal stem cells (MSCs) have unique therapeutics function such as homing to target tissue and stimulating host cell regeneration, repairing damaged cells, autocrine/paracrine effects, immune modulation and several lineages differentiation. For this reason, MSCs are appropriate choice for tissue engineering and cell based therapy. Umbilical cord as a commonly discarded tissue, which are obtained non-invasively. Umbilical cord contains Wharton's jelly (WJ), a gelatin substance surrounding umbilical cord vessels. WJ, a rich source of self-renewal MSCs with extended proliferation and multilineage differentiation potential can be a best option for Regenerative medicine based on cell- therapy. In this chapter, we will discuss an outline of the recent findings related to Wharton's jelly Mesenchymal stem cells (WJ-MSCs) and its therapeutic effects and possible advantage they possess.

Keywords: Wharton's jelly; mesenchymal stem cells; umbilical cord; Cancer; wound.

INTRODUCTION

The human umbilical cord connects a developing embryo or fetus to the placenta. This young tissue represents the bond between the mother and the fetus during pregnancy. Its main function is to supply blood to the fetus and to expel fetal waste. The umbilical cord contains two arteries and one vein. The umbilical cord arteries are embedded in a gelatinous tissue called Wharton's jelly (WJ). Wharton's jelly is a mucosal connective tissue that was first described in 1656 by Thomas Wharton, an English physician and anatomist [1]. The main role of Wharton's jelly is to protect the umbilical cord vessels, by preventing them from being compressed, twisted or bent. This connective tissue includes the perivascular region, the intravascular region, and the amniotic subcutaneous region and is mainly composed of glycosaminoglycan's, especially hyaluronic acid and chondroitin sulfate. While collagen fibers make up its main fibers, there are no elastic fibers in its composition. It is noteworthy that the extracellular matrix (ECM) of the umbilical cord has different types of cells with specific characteristics. Wharton's jelly cells include fibroblasts, myofibroblasts, smooth muscle cells and mesenchymal stem cells [2-5].

Wharton's jelly mesenchymal stem cells originate in early fetal development, with primary hematopoiesis occurring in the yolk sac and later in the aorta-gonad-mesonephros region (AGM). Hematopoietic cells and MSC mesenchymal stem cells migrate from the yolk sac and AGM through the umbilical cord to the placenta during the first human development between days 4-12. The second wave of migration of these cells starts from the placenta through the umbilical cord to the fetal liver, and they are finally located in the bone marrow of the fetus. During migration through the placenta and umbilical cord, some MSCs are trapped in Wharton's jelly (or reside there as planned) and remain there throughout pregnancy. In their new environment, they evolve, and then proliferate to eventually become the population observed in Wharton's jelly that are known as human Wharton's jelly stem cells (hWJSCs) and human umbilical cord mesenchymal stem cells (HUMSCs) [6]. Like other MSCs, hWJSCs are characterized by their ability to regenerate and their multipotent capacity. They can support stem cell origins and synthesize various cytokines, and have immunomodulation and homing properties Studies on the hWJSC immunophenotype have shown that they are positive for mesenchymal cell markers and negative for hematopoietic markers. HWJSCs express CD73, CD90, CD105, CD10, CD13, CD29, and CD44, and are negative for CD14, CD31, CD33, CD34, CD45, CD56, CD11b (Integrin alpha M), CD79α, CD19, and HLA-II[7].

HWJSCs are precursor cells of bone marrow mesenchymal stem cells and can be distinguished into different types of laboratory cells, including bone, cartilage, fat, muscle, nerve cells, hepatic cell and pancreatic β cells [8]. Despite their physical and functional similarities to other adult-derived MSCs such as bone marrow mesenchymal stem cells, umbilical cord-derived mesenchymal stem cells have the ability to last up to 3 weeks at the graft site. What has also distinguished these cells from their counterparts is their high flexibility, higher mesenchymal stem cell content compared to bone marrow (BM-MSC) and high similarity to chimeric cells due to expression of some embryonic markers such as Oct3 / 4, Nanog, Rex1, their anti-inflammatory properties and other characteristics such as improving host immune tolerance and repairing umbilical cord stem cell tissue [9].

Non-invasive resection method, lower risk of viral infection and higher proliferation, and differentiation potential in mesenchymal cell transplantation have made this tissue a suitable option for cell therapy [10-12]. In addition, the possibility of teratoma formation is very low. Since this tissue is commonly considered a lesion in the clinic, there is no medical ethical concern about its use. Umbilical cord mesenchymal stem cells are anti-inflammatory, anti-cancer with anti-fibrotic properties and are tolerated by the immune system [13, 14]. Recent advances show that WJ-MSCs reinforced with micro particles and scaffolds can be used effectively for a variety of clinical applications. Previous studies suggest that WJ-MSCs can be used for a wide range of treatment plans including neurological disorders, kidney injury, lung injury, orthopedic injury, and liver injury, and cancer therapy [15]. In this review, we present an evaluation of the successful and failed cell therapy efforts using Wharton's jelly stem cells in the past few years, which can provide a new framework for the enhancement of future research in this area.

CANCER

Tumorogenesis is the most important barrier to cell therapy using human embryonic stem cells[16] and human induced pluripotent stem cells (IPSC). Therefore, researchers have to look for a new source of ESC for cell therapy. Multiple mesenchymal stem cells (MSCs) have recently become a viable option for tumor treatment. Previous research has shown that Wharton's jelly mesenchymal stem cells can be more useful in treating cancer than other adult stem cells [17] .

At 36 weeks of gestation, embryonic cells are found in the bloodstream of 100% of pregnant women, which decreases by 22-75% after delivery. Because cell transfer between mother and fetus is possible, it is conceivable that cancer cells can pass through the placenta to reach the fetus. However, studies have confirmed the spread of metastasis in the placenta, but not in the fetus. Also, in cancers of trophoblast origin, fetal metastasis, despite maternal metastasis, is rare. Therefore, there must be a defense mechanism during pregnancy that blocks the metastasis of these cancer cells in the fetus. Hence, studies on the interaction between hWJSCs and cancer cells such as lung, prostate, breast, ovary[18, 19] glioblastoma multiforme (GBM) [20], bladder, kidney, lymphoma, leukemia, esophagus, osteosarcoma, liver and squamous cell carcinoma, etc [21] In these studies, human Wharton's jelly stem cells (hWJSCs), cell lysate (hWJSC-CL), conditioned medium (hWJSC-CM), exosomes and Wharton's jelly mesenchymal stem cell microvesicles (hWJMSC-MV) were used. Most of these studies showed an improvement in cancer, but in other studies such as Du and coauthors, in which Wharton's jelly mesenchymal stem cell microvesicles were used, the rate of cancer cell invasion increased [22]. In another study, hWJSC-CM and hWJSCs led to the proliferation of AC-LCSC cancer cell lines 1[6] .The results of another study showed that the inhibitory effect of Wharton's jelly stem cell lysate on the tumor was greater than that of the conditioned medium of these cells[19] .These studies show that Wharton's jelly mesenchymal stem cells do not have the same effect on different types of cancer. Conditioned medium under normal conditions (WJMSCs-norCM) and under hypoxia conditions (WJMSCs-hypoCM) can inhibit the proliferation of various cancer cells such as cervix (HeLa), liver (HepG2), prostate (PC3), ovary (SKOV3) and oral squamous cell (Manassas) with different activities, but have no toxicity for normal cells. To date, hWJSCs have been injected in a variety of ways, including subcutaneously (SC), intramuscularly (IM), intraperitoneally (IP), intravenously (iv), intratumorally (it) or intratracheally, indicating the ability of these cells to migrate towards tumors. HWJSCs showed their homing ability to breast cancer 6 weeks or only 7 days after tumor formation. Treatment with hWJSCs was relatively effective in both in situ and metastatic breast cancer, and the cancer-free phase could last up to 50 days[23].Many chemokines, including VEGF, TGF-β, FGF, PDGF, MCP-1, EGF, and IL8, are secreted by tumors, and hWJSCs express chemokine receptors such as SDFR1, TGFBR3, and FGFR2, which push hWJSCs toward the tumor[24].MMP-2 and MMP-9 are two important proteins associated with tumor cell invasion and metastasis. The activity and protein levels of MMP-2 and MMP-9 decreased in the presence of hWJSCs. When hWJSCs reach a tumor tissue, they secrete cytokines to kill that tumor. This secretion is local and their serum levels are low compared to the systematic administration of cytokines, and this function of hWJSCs suggests that stem cell-based gene therapy can prevent their systematic side effects.

Cancer cell death and cancer tissue volume reduction in the presence of hWJSCs occur through mechanisms such as apoptosis [19] (Table 1), autophagy [25] (Table 1), oxidative stress pathway, immune cell death (ICD), phagocytosis, and a new phenomenon called known as cic-apoptosis etc[4]. Also, unlike bone marrow mesenchymal stem cells, hWJSCs do not convert to tumor-associated fibroblasts (TAFs). TAFs are involved in tumor growth and metastasis [26]. Therefore, hWJSCs are good carriers for the delivery of specific genes. HWJSCs were designed to secrete a therapeutic protein *IFN-β*[*27*] and *ILZ-sTRAIL* [20], which successfully killed cancer cells. HWJSCs have been used in combination with chemotherapy drugs such as doxorubicin, and taxol [27, 28]. By adding their effects to the anti-cancer effect of chemotherapeutic agents, hWJSCs can reduce the dose of cytotoxic drugs and reduce side effects. HWJSCs can be used in both allogeneic and autological contexts. Undifferentiated hWJSCs are hypoimmunogenic and do not induce teratoma or other tumors in vivo and are thus safe for use in allogeneic clinical settings [16].

THERAPEUTIC EFFECTS OF hWJSCs ON BURNS AND WOUNDS

Wound healing and the spread of fibrosis of the limbs both follow the cascade of processes beginning with inflammation and unfortunately usually ending with the formation of fibrotic scar tissue. Wound healing requires cell migration, inflammation, angiogenesis, granular tissue formation, re-epithelialization, and extracellular matrix (ECM) regeneration [29]. Wound healing is associated with the coordination of growth factors and extracellular proteins and intercellular interaction [34,35]. Thus far, in most cases, the use of topical medications, dressings or skin grafts has not had satisfactory results in the treatment of wounds, but a promising new method to stimulate wound healing, which has received special attention in recent years, is the use of mesenchymal stem cells (MSCs) [30, 31]. MSC-based therapies induce interactions between countless cell types, extracellular matrix components, and signaling molecules in a complex process. Previous studies have reported that MSC-based therapy improves wound healing, maintains cutaneous homeostasis, and reduces scar formation [32, 33].

MSCs have also been shown to have a high ability to repair damaged tissue. They play a role in responding to skin damage by collagen deposition, wound shrinkage, angiogenesis and regeneration of skin

appendages and increased epidermal cell growth In addition, MSC modulates the immune response in damaged tissue by interacting with T cells, B cells, NK cells, dendritic cells, macrophages, and neutrophils. The umbilical cord is considered the optimal source of MSC. Expression of pro-angiogenic molecules and their growth factors and angiogenesis-related cytokines have made these cells a viable option for wound healing (scheme 1) [33, 34]. Compared to other transfection strategies, the hWJSC-based approach is safer and more efficient because it simulates the endogenous mechanism for cellular communication. HWJSCs have a stronger immune system immunity than their adult counterparts due to the release of large amounts of anti-inflammatory molecules such as TGFβ, IL-10, IDO, TSG-6 and PGE2 compared to bone marrow MSC [35, 36].

By injecting hWJSCs, the healing process is accelerated by activation of tissue repair / M2 macrophages, re-epithelialization, and granular tissue formation. These M2 macrophages modulate the process of endogenous wound healing by activating invasive anti-inflammatory cytokines such as IL-10 and tissue-repairing cytokines such as TGF-β 1[37, 38] .On the other hand, it should be noted that blood supply is the key to wound healing. By expressing more IL-8 than their counterparts, hWJSCs act directly on endothelial cells and are involved in cell proliferation, migration and survival of these cells. They also induce the secretion of PDGF-AA, a mesenchymal angiogenic agent, and VEGF-A, which is the most important factor studied for angiogenesis [39, 40] .Finally, it can be argued that hWJSCs have almost all the growth factors required for wound healing, including vascular growth factor, epidermal growth factor (EGF), platelet-derived growth factor, hepatic growth factor (HGF), and growth factors IGF-1, EGF, and TGF, which are essential for wound healing [41, 42]. Collagen is involved as a pivotal molecule at each stage of wound healing to regenerate the extracellular matrix (ECM) and create scaffolding in connective tissue. In wounds treated with hWJSCs, wound closure is accelerated due to the accumulation of modified collagen types. Umbilical cord stem cells can accelerate the accumulation of collagen I and III. In addition, smooth muscle actin positive stromal cells and Wharton's jelly myofibroblast-like cells improve the regeneration process of the extracellular matrix. Bonding of hWJSCs to the burn site closes the wound in severe burns by modulating the inflammatory environment and promoting the formation of a highly vascularized granular matrix and collagen scaffold[33].

On the other hand, in patients with deep burns, the damage can reach muscle tissue and sweat glands. In most cases of deep burns, stem cells in the injured cannot rearrange the sweat glands. In addition, after wound healing, new epidermal stem cells are unable to differentiate into sweat gland cells[43] .According to recent experimental results, a new potential for umbilical cord stem cells has been considered to treat burns and regenerate sweat glands in damaged skin[44]. The use of hWJSCs by producing the anti-inflammatory cytokines IL-10 and TNF-α significantly reduces the inflammation of the burned skin and promotes angiogenesis in this tissue. Gland-like cells created under the induction system can be used to regenerate damaged sweat glands [44] (Figure 1).

NEUROPROTECTIVE EFFECTS OF UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS

In recent years, the use of mesenchymal stem cells as an effective treatment tool for a variety of neurodegenerative diseases such as Alzheimer's, Parkinson's, Myotrophic sclerosis, Huntington's and MS has received much scholarly attention. Mesenchymal stem cells can affect neurodegenerative damage in two ways: 1- These cells are able to differentiate into neurons under certain conditions 2- They have neuroprotective and immune regulatory effects. HWJSCs have many applications in the treatment of neurological defects and diseases, and when exposed to CSF[45], these cells are able to express neuronal markers and neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor cells (GDNF). Mitchell and coauthors (2003) showed that Wharton's jelly stem cells can be differentiated into neurons under certain conditions[46]. Transplantation of these cells is very effective in improving the damage caused by stroke and ischemia[46, 47] .

Real-time and immunocytochemical results have shown that these differentiated neurons are able to express markers of motor neurons such as nestin, PAX6, NF-H, Islet 1, HB9 and acetylcholine transferase. These cells can be differentiated into dopaminergic neurons and then transplanted to the striatum and substantia nigra to treat Parkinson's disease[48, 49].On the other hand, the use of enriched culture medium obtained from hWJSCs increases the viability and proliferation of Schwann cells, increases the expression of brain growth and neurotrophic factors by Schwann cells, and promotes the growth of neurites from the dorsal ganglion of the spinal cord. Stem cells are very useful for transplantation due to their reproducibility and high proliferation. Stem cells promote angiogenesis and neurogenesis, reduce inflammation, and increase oxygen delivery to the brain. Therefore, by transplanting these cells into a brain tissue suffering from a stroke or ischemia, the tissue can be partially restored to its original shape [49]. Previous studies have shown that transplanting hWJSCs in mice with ischemic injury leads to the differentiation of these cells into

neuron-like cells and improves neurological and behavioral functions in them[50].It has also been shown that hWJSCs can improve motor function and cholesterol metabolism in the brain. These cells also prevent the destruction of Purkinje cells through anti-apoptotic and anti-inflammatory mechanisms. Injection of these cells increases the number of dentate gyrus cells by activating the PI3K / Akt and Jak2 / STAT3 signaling pathways [51, 52]. Min and coauthors showed that treatment with hWJSCs improves motor and cognitive function in children with cerebral palsy[53].

THERAPEUTIC EFFECTS OF hWJSCs IN MS

Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system. Damage to the protective myelin sheath around nerve fibers in the brain and spinal cord, following damage and loss of oligodendrocytes, impairs nerve conduction. Recent studies suggest that stem cell-based remilination induction approaches are promising treatments for demyelinating diseases like MS [54].

Numerous studies have used hWJSCs to modulate the immune response in autoimmune diseases, including diseases such as rheumatoid arthritis, type 1 diabetes, and encephalomyelitis. Umbilical cord blood stem cells have the potential to differentiate into damaged and demyelinated neurons with minimal potential for glioma cancer cells [55-58].

Hence, considering the options available in the treatment of neurological diseases, their superior cellular properties, their ability to reproduce easily, ethical issues, and other characteristics of nerve / glial cells, it seems that cord stem cells and cord blood stem cells are preferred over other mesenchymal stem cells. Numerous reports indicate that chemical and neural factors can induce hWJSCs to differentiate into neuronal stem cells and oligodendrocyte progenitor cells[59, 60].These cells not only have the morphology and phenotype of oligodendrocyte progenitor cells but also exhibit their function. These cells can promote axonal growth by secreting a variety of neuronal growth factors and reduce axonal delamination and degradation[61]. In addition to their potential for neuronal repair, differentiated cells derived from hWJSCs have toxic effects on glioma cancer cells and inhibit glioblastoma cell proliferation in several ways.

These cells play their role by inhibiting the Wnt signaling pathway via secreting DKK1 or initiating apoptosis in tumor cells by increasing the expression level of TNF-related apoptosis-inducing ligand (TRAIL) and decreasing the expression of CXCL12 which inhibits TRAIL. On the other hand, stopping the growth of K562 cells (human erythromyeloblastoid cell line) in G0 / G1 proliferation stage in the culture medium of these cells, is another activity that inhibits tumor growth in cell therapy and transplantation using these cells[62].

Transplantation of hWJSCs combined with minimally invasive hematoma aspiration can significantly reduce p53 expression and neuronal damage and can be useful in restoring neuronal cell function. These cells, in combination with methylprednisolone, can improve MS patients' condition by changing (Th1) to (Th2) or enhancing Th2 differentiation and inhibiting Th1 differentiation in simple T cells [52].

Results of different studies have shown improved regulatory T cell function in MS, increased regulatory cytokines such as growth factor TGF-β1, prostaglandin E2, and IL-10, and decreased production of IFN-γ, an anti-inflammatory cytokine in single-cell hWJSCs Nuclear peripheral blood (obtained from MS patients) in a ratio of 1: 5 [62, 63].

Therapeutic outcomes of injecting these cells are stable up to one month in some treatment procedures and up to 1 year in some other procedures, in comparison with the current medical treatment methods for MS. In addition, side effects have been seen with the use of current MS medications, whereas no specific side effects have been observed after injection of hWJSCs for up to 1 year after treatment[64].

According to previous studies, intravenous injection of hWJSCs over several days in people with MS, in addition to being safe, brings benefits such as improved bowel and bladder function, reduced sexual dysfunction, improved upper limb function, reduced gait disturbance, improved energy consumption, reduced fatigue, and improved physical health and quality of life for these patients[65].

Therefore, due to the ability of hWJSCs to differentiate into neurons and oligodendrocyte-like cells in vitro, they can be a major candidate in cell therapy in MS.

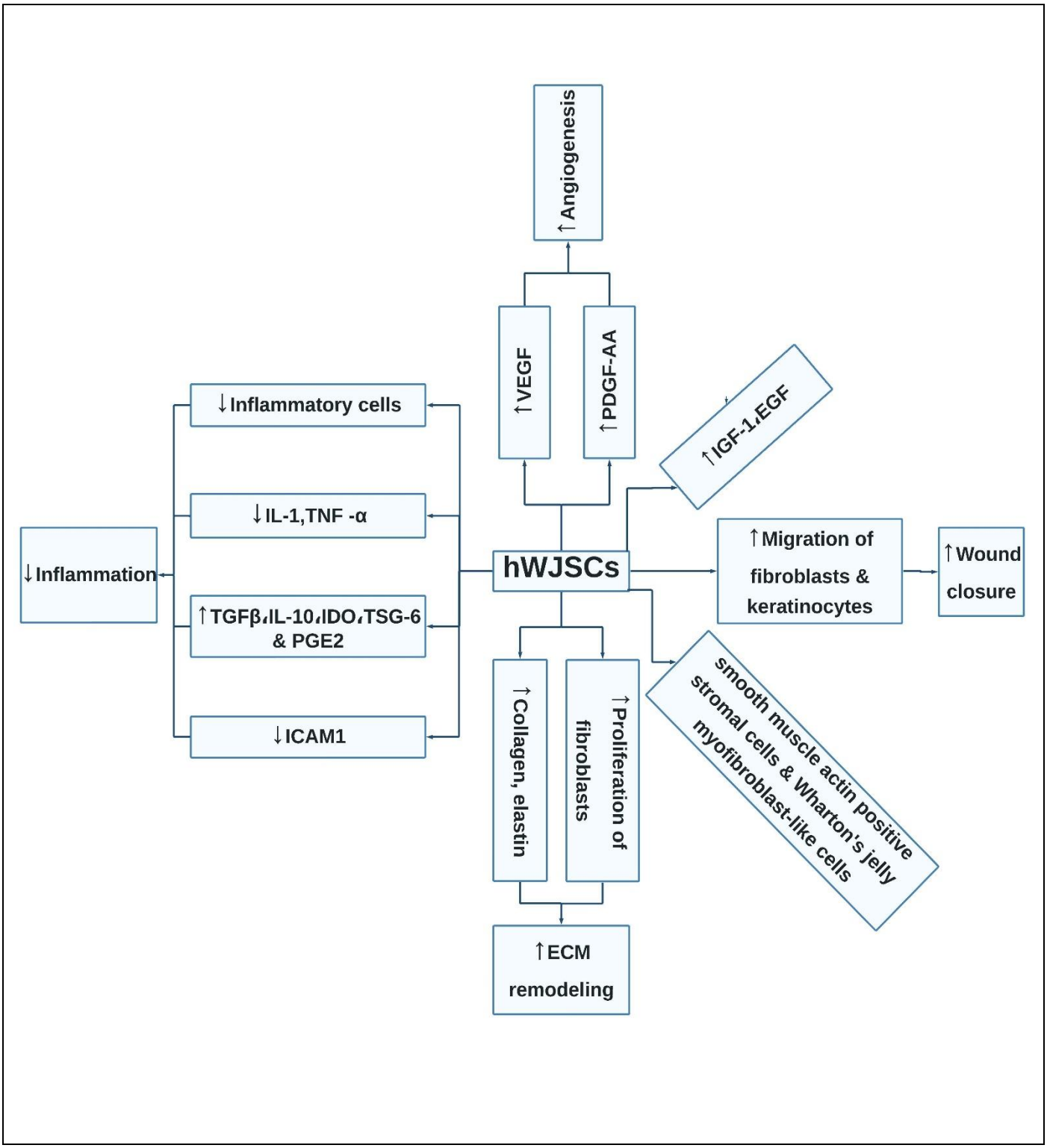

Figure 1. In wound healing, UMSCs reduce tissue damage, inhibit fibrotic regeneration and apoptosis, enhance angiogenesis, stimulate endogenous stem cell uptake, and proliferate and reduce immune responses to new vascular formation and precipitation of the collagen matrix

Table 1. Alterations of autophagic proteins and apoptosis in co-culture of cancer cells with hWJSC

MSC	Cell line of cancer	Proteins involved in apoptosis	Autophagy proteins	References
hWJSC-CM	HEP-2	p53↑&Bcl-2↓		[25]
hWJSC-CM	Lymphoma	Caspases3& 8& 9↑		[26]
WJMSCs	WHCO1 MDA-MB-231	Caspase s 3 & 9↑& MMP-2↓& MMP-9↓& Cyclin D1↓& Bcl↓& Bcl-xL↓		[27]
hWJMSC-MV	T24	Caspase 3↑ &p-p53 / p21↑		[28]
WJMSCs	TOV-112D MG-63	BCL2 ↓& SURVIVIN↓& BAX↑		[19]
hWJSC-CL	MDA-231	-	BECLIN - 1 ↑& LC3B↑	[19]
hWJSC-CL& hWJSC-CM	MG - 63	BAX↑	ATG-5↑ & BECLIN-1↑	[19]
hWJSC	SKES-1	BAX↑& Caspases 9&3 ↑ & PARP↑		[29]

CONCLUSION

The present report clarified an hWJSCs are useful approach to promote cancer, wound healing and prevent scar formation. Therefore, WJ-MSC can be applied safely, immediately, and on demand.

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