Potential of Unengineered and engineered Wharton's Jelly Mesenchymal Stem cell as cancer inhibitor agent

by Wahyu Widowati

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Abstract: Cancer is one the most common life-threatening diseases. Cancer cases worldwide are forecast to rise by 75% in the future. Although cancer therapies have been improved, many tumors remain unresponsive to conventional treatments, such as radiation and chemotherapy. Therefore, novel strategies in treating and managing the disease are urgently needed. Wharton's Jelly-derived mesenchymal stem cells (WJ-MSCs) have recently been shown to possess anti-cancer activities as well as tumor-homing ability. These cells are able to migrate to sites of neoplastic growth *in vivo* and their secretory products display tumoricidal activity both *in vitro* and *in vivo* models of human cancer. Recent reports also suggest that the anti-cancer potential of WJ-MSCs can also be enhanced through genetic engineering. This review will summarize the current understanding on the interactions between WJ-MSCs and tumor cells, as well as the potential use of both un-engineered and engineered WJ-MSCs as an anti-cancer agent.

1. INTRODUCTION

Cancer remains one of the leading causes of mortality and morbidity throughout the world [1, 2], accounting for an approximate of one in four human deaths in all age groups in the United States alone in 2010 [2]. A total of 1,660,290 cancer cases and 580,350 cancer-related deaths were projected to occur in the United States in 2013 [3]. This incidence has been increasing, and the number of new cases is expected to have doubled by the year 2020, reaching an approximate of 20 million cases, with the current annual mortality rate rising from about 6.6 million to more than 10 million [4, 5]. Each year, more than 700,000 new cases of cancer occur in ASEAN countries and this number is expected to increase [6]. Based on the GLOBOCAN (*International Agency for Research on Cancer* (IARC)) cancer incidence database for the year of 2012, breast cancer was the most frequent cause of malignancy-related mortality in women (43.3%), while in men, lung cancer was the predominant cause of cancer-related deaths, being responsible for 12.9% of the total death toll [6, 7]. The data collected by the Dharmais Cancer Hospital, Jakarta, Indonesia showed that cancers of cervical, breast, lung, ovarian, rectal, thyroid, hepatoma, intestinal, and nasopharyngeal origin were the most prevalent types of malignancy in Indonesia in 2012-2013 [7].

Cancer has severe impacts on individuals and communities, leading to both disability and death. Its treatment costs and the disability-associated loss of income can quickly undermine family finances [8].

Despite recent improvements in treatment modes, many tumors remain unresponsive to current conventional cancer therapies (surgery, chemotherapy and radiotherapy) [1]. The efficacy of such therapies is low, however, they impose a significant financial burden on the healthcare system [9, 10]. Therefore, a novel therapeutic strategy must be devised, which could directly target tumor cells in both primary and metastatic sites, and which possesses the ability to act locally in the tumor microenvironment over an extended period of time [11].

Mesenchymal stem cells (MSCs) represent an appealing source of adult stem cells for cell therapy and tissue engineering [12]. MSCs are the first type of stem cells to be utilized in clinical regenerative medicine. MSCs have an advantage of simple acquisition and harvesting procedures, as well as rapid *ex vivo* expansion under properly defined culture conditions. They are also capable of multipotent differentiation and trophic paracrine secretion, making them a potential mediator of tissue repair during cell transplantation [13, 14]. In the context of tumorigenesis, MSCs have been shown to invariably migrate and incorporate into sites of tumor growth both in the context of *in vitro* co-culture systems and *in vivo* xenograft models, independent of tumor type, host immunocompetence, and cell delivery route [1, 11, 12, 15-17]. This

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tumor-tropic capability and the relative ease of performing transduction in MSCs render them a promising vehicle for cell-based therapy of various invasive cancers [18, 19]. Indeed, engineered MSCs have shown some success in delivering various therapeutic molecules to treat multiple metastatic cancers including lung, breast, squamous cell, colon, pancreas, and cervical cancers [20, 21]. Here, we highlight current advances in the use of both naive and genetically engineered MSCs, with a special emphasis on WJ-MSCs, in cancer therapy.

2. OVERVIEW OF WHARTON'S JELLY MESENCHYMAL STEM CELLS (WJ-MSCS)

MSCs are a group of adult stem cells which can be isolated from fetal or fetal-associated tissues, for instance amniotic membrane, amniotic fluid, chorionic membrane, chorionic villi, decidua, placenta, cord blood, umbilical cord, and Wharton's Jelly, as well as adult tissues, such as bone marrow, peripheral blood, adipose tissue, and dental pulp [1, 12, 22-26]. *In vitro*, MSCs are characterized based on the following defined characteristics: (a) adherence to the plastic surface of cell culture flasks, (b) expression of mesenchymal surface markers, including cluster of differentiation (CD) 105, CD73, CD90 and absence of hematopoietic surface markers, such as CD45, CD34, CD14, CD19, and human leukocyte antigen type II (HLA-II), and (c) ability to differentiate into adipocytes, osteoblasts, and chondroblasts *in vitro* [27-29].

One of the most convenient sources of MSCs for *in vitro* expansion is the umbilical cord (UC). Since UC is usually discarded after delivery and its isolation process poses minimal ethical concerns and health risks for the stem cell donors, compared to the controversial nature of embryonic stem cell (ESC) isolation procedures and the highly invasive bone marrow (BM) extraction method, it has since become an attractive source of MSCs [26, 30-36]. Moreover, owing to its low proliferative potential, BM-derived MSCs (BM-MSCs) expansion typically requires a long period of *in vitro* culture, which elevates the risks of microbial contamination and raises the probability of the cells' losing their stemness properties, as well as accumulating deleterious chromosomal changes [37]. However, unlike BM-MSCs, which suffer from a significant age-related retardation in growth and differentiation potentials, UC-derived MSCs (UC-MSCs) are relatively unaffected by donor age and retain their proliferative capacity *in vitro* [26]. Furthermore, in contrast to ESCs, the high proliferative potential and multipotency of UC-MSCs is not associated with an increased risk of teratoma formation or immunogenicity in cell transplant recipient [30, 38].

Different types of MSCs have also been successfully isolated from individual parts of the UC, the most common of which are the umbilical cord blood (UCB) and Wharton's Jelly (WJ) [39, 40]. The UC is composed of two arteries and one vein, surrounded by mucoid connective tissues know as WJ [41-43]. WJ appears to serve the function of adventitia, which the UC vessels lack, by binding and encasing the umbilical vessels [32- 35].

WJ-MSCs are more primitive than MSCs isolated form most other tissue sources and do not express the major histo-

compatibility complex (MHC) class II protein, which contributes to their hypoimmunogenicity in transplantation settings [36, 44]. Possibly due to this primitive nature, WJ-MSCs exhibit a greater self-renewal and immunosuppressive potential compared to the MSCs isolated from older tissues, such as BM and adipose tissue (AT). Furthermore, WJ-MSCs are able to differentiate into mature cells of all three germ layer lineages *in vitro* [45, 46] A number of studies have revealed that in addition to the conventional trilineage differentiation potential displayed by all types of MSCs, WJ-MSCs are also able to differentiate into cardiomyocytes [41, 47, 48], endothelial cells [49], neuron-like cells [50], germ-like cells [45], and insulin-secreting cells [36] under suitable culture conditions. This high proliferative and differential potential may be employed to mediate tissue repair and regeneration in the context of *in vivo* cell therapy for a broad range of degenerative diseases [13]. The ability of WJ-MSCs to rapidly expand and generate dopaminergic neurons *in vitro* suggests that they may be beneficial for the treatment of Parkinson's disease. It appears that WJ-MSCs are capable of long-term survival and integration into recipient's brain [51]. WJ-MSCs transplantation was also found to promote the regeneration of corticospinal fibers and locomotor recovery following experimental spinal cord transection in rats [31]. Another study confirmed the ability of WJ-MSCs to alleviate the symptoms of stroke and myocardial infarction [52]. A few reports have also suggested that WJ-MSCs may be used to treat type I diabetes [53, 54].

The aforementioned positive influences exerted by MSCs on tissue repair are in part mediated by their capacity to home into sites of inflammation and injury [19]. Upon their arrival at damaged tissues, MSCs can begin to secrete trophic factors responsible for cell proliferation and the re-establishment of tissue homeostasis [55]. As previously alluded to, in the context of cancer, this ability to migrate to sites of neoplastic growth is indispensable for MSCs anticancer activity, the mechanisms of which will be the subject of subsequent sections.

3. WJ-MSCS AS *IN VITRO* AND *IN VIVO* ANTI-CANCER AGENTS

Naive MSCs have been consistently shown to exert tumoristatic and tumoricidal activities *in vitro* co-culture experiments. MSCs inhibit the growth of several tumor cell lines, including lung cancer cell line (A549), rectal cancer cell line (HT29), and breast cancer cell line (MCF-7) [18]. One possible mechanism, which may be responsible for this inhibition, is cell cycle arrest, since UC-MSCs were found to significantly halt tumor DNA synthesis in a dose-dependent manner when co-cultured with the human breast carcinoma cells MDA 231 [32]. BM-MSCs were also found to suppress angiogenesis in the setting of an MSC and glioma co-culture experiment. In the presence of BM-MSCs, there was a notable reduction in the secretion of pro-angiogenic factors, such as platelet-derived growth factor (PDGF)-BB and Interleukin (IL)-1β, as well as an attenuation of signaling through the pro-angiogenic PDGF/PDGFR axis. This inhibition of angiogenesis is accompanied by an increased production of anti-angiogenic factors. In addition, there was a decrease in Akt signaling and cathepsin B expression, which were responsi-

Table 1. WJ-MSCs modes of actions in various type of cancer.

Type of Cancer	Possible Mode of Action	Cell Line Models and Experiment	Reference
Breast cancer	Inhibition of anchorage-independent cell growth and DNA synthesis, induction of cell cycle arrest at G2 phase, and attenuation of Akt and MAPK phosphorylation	MDA-MB 231 (human breast carcinoma cells capable of metastasis to the lung) both *in vitro* co-culture and *in vivo* transplant in female CB-17 SCID mice.	Ayuzawa, *et al.* (2009) [32]
Breast cancer	Inhibition of proliferation and induction of apoptosis	Rat mammary carcinoma cell line (MAT B III) both *in vitro* culture and *in vivo* transplant in female F344 rats.	Ganta, *et al.* (2009) [57]
Breast cancer	Induction of cell cycle arrest at G2/M phase and apoptosis.	MDA-MB 231 and MCF 7 human breast carcinoma cell lines , along with the breast cancer stem cells sorted from those cell lines, both *in vitro* and *in vivo* transplant in nude mice and CB17 SCID female mice.	Ma, *et al.* (2012) [91]
Breast cancer	Induction of apoptosis upon WJ-MSC migration into tumor mass	MDA-MB 231 human breast carcinoma cell line *in vitro* and *in vivo* transplant in NOD/SCID mice.	Chao, *et al.* (2012) [92]
Lymphoma cell	Secretory product-mediated anti-tumor activity	human Burkitt's lymphoma cell lines (Rams, CRL 1596) co-cultured with WJ-MSCs.	Lin, *et al.* (2015) [93]
Pancreatic cancer	Cell-contact-dependent and cell-contact independent inhibition of proliferation.	an intraperitonial mouse pancreatic ductal carcinoma model (PAN02).	Doi, *et al.* (2010) [59]
Lung cancer	Attenuation of cell division and increase in apoptosis.	human bronchioloalveolar carcinoma cells (H358) and human lung alveolar carcinoma cells (SW1573) studied in *vitro* and *in vivo* transplant in SCID mice.	Matsuzuka, *et al.* (2010) [68]
Lung cancer	Induction of G0/G1 cell cycle arrest, inhibition of both anchorage-dependent and independent growth, increase in apoptosis.	Lewis lung carcinoma (LLC) cells co-culture *in vitro*.	Maurya, *et al.* (2010) [60]
Lung metastatic breast carcinoma	Secretory product-mediated growth inhibition and apoptosis induction.	MDA-MB 231 lung metastatic breast carcinoma-bearing SCID mice which received either intra-tumoral or intra-venous injection of WJ-MSCs.	Tamura *et al.* (2011) [94]

ble for mitogenic activity and invasion-associated matrix remodeling, respectively [56].

The *in vitro* anti-tumorigenic activity of unmodified MSCs can also be observed *in vivo* in some instances. Both intra-tumoral and intravenous injections of rat-derived WJ-MSCs into MatBIII-tumor-bearing animals resulted in significant tumor regression without any evidence of recurrence at 100 days post-transplant. Furthermore, consistent with previous findings, intravenously injected WJ-MSCs were able to rapidly home to the tumor microenvironment during a mere 4-day period [57]. Another group has also reported that multiple injections of UC-MSCs were able to reduce the metastatic tumor burden in immunodeficient female CB-17 SCID mice transplanted with MDA231 human breast carcinoma cells [32]. The two major mechanisms through which MSCs may attenuate tumor growth *in vivo* are the production multiple cell cycle arrest and cell death-inducing secretory proteins and the enhancement of anti-tumor immune reactions. Indeed, WJ-MSCs had been found to suppress the growth of lung and bladder tumor cells in animal models through the induction of p53-dependent cell cycle arrest in conjunction with a down-regulation in cyclin A2-CDK protein expression level crucial for cell cycle progression through the G0/G1 phase [58-60]. A summary of several studies that investigate the tumoristatic and tumoricidal activities of un-engineered WJ-MSCs can be found in Table **1**.

Interestingly, MSCs' tissue of origin seems to influence their interactions with tumor cells. At least in one example, neonatal-derived UCB-MSCs and adult-derived AT-MSCs exert contrasting influences on glioblastoma cells *in vitro*: the former inhibited, while the latter promoted tumor growth, respectively [25]. To overcome this variability, as well as to elevate the tumor-killing activity of MSCs, researchers have resorted to genetic engineering. There are already several instances in which the efforts to incorporate sequences encoding conditionally activated oncolytic virus, cytotoxic prodrug-converting enzyme, or anti-tumor cytokines into MSCs have yielded promising results [19, 61-68]. This will be the focus of the subsequent sections.

4. THE ANTI-CANCER PROPERTIES OF CONDITIONED MEDIUM AND CELL LYSATE OF WJ-MSCS

The discovery that the beneficial effects conferred by MSCs can largely be attributed to their paracrine secretions leads to the novel possibility of using MSC-derived cell free lysate (MSC-CL) or conditioned medium (MSC-CM) for therapy. The use of MSC-CL and MSC-CM may circumvent some safety concerns regarding the tendency of MSCs to differentiate into tumor-promoting stromal fibroblasts upon sustained interactions with the tumor microenvironment [69-71].

Both MSC-CL and MSC-CM contain a broad variety of cytokines, growth factors, cell adhesion molecules, hyaluronic acid, glycosaminoglycan's, as well as putative (micro)RNA-containing microvesicles, which may possess anti-cancer activity [72-74]. *In vitro*, MSC-CL and MSC-CM were able to inhibit the growth of several cancers, including breast adenocarcinoma, ovarian carcinoma, osteosarcoma, benign neoplastic keloid cells, bladder tumor, and lymphoma [75]. Recently, a study by Gauthaman *et al.* (2012) also revealed that WJ-MSC-CM and WJ-MSC-CL, specifically, interfered with the growth of breast cancer cells (MDA-MB-231), ovarian cancer cells (TOV-112D), and osteosarcoma cells (MG-63) *in vitro* [76]. Some possible mechanisms of this inhibition includes cell cycle arrest and the induction of apoptosis, which results from the up-regulation of pro-apoptotic genes, the down-regulation of pro-survival protein expressions, as well as the stimulation of caspases [38, 58, 77]. It has also been reported that MSC-CM could jeopardize Vascular-Endothelial Growth Factor (VEGF) production by tumor cells, thereby interfering with tumor-sustaining angiogenesis [71].

The aforementioned anti-cancer activity of MSC-CL and MSC-CM can also be observed *in vivo*, in the context of mammary carcinoma, osteosarcoma, pancreatic cancer, and lung cancer. Interestingly, it was noted that WJ-MSC-CL was a more potent tumor-inhibitory agent compared to WJ-MSC-CM. This additional anti-tumor activity may be attributed to certain intracellular molecules that, due to size or cellular-trafficking constraints, were not able to be secreted into the cell culture medium [37, 76]. A summary of selected publications detailing the anti-cancer activity of WJ-MSC-CL and WJ-MSC-CM is provided in Table **2**.

5. ENHANCING THE ANTI-CANCER ACTIVITY OF WJ-MSC THROUGH GENETIC ENGINEERING

Genetic manipulations of MSCs for the purpose of anti-cancer therapy usually involve the introduction of exogenous genes, the expression products of which confer additional anti-tumor activity against cancers of various origins, into naive MSCs [61]. The ability of engineered MSCs to home into sites of neoplastic growth, as mentioned in previous sections, will ensure that these anti-angiogenic, anti-proliferative, and pro-apoptotic molecules are directly secreted into the tumor microenvironment, thereby increasing their tumor-killing efficiency [19, 57, 61]. Several examples of these molecules include interferon-α (IFN-α), IFN-β, IL-2, IL-12, IL-24, and chemokine C-X$_3$C motif ligand 1

(CX$_3$CL1) [78]. In general, MSCs are engineered to express the following classes of molecules: (a) immunostimulatory cytokines, such as IFNs; IL-2; IL-7; and IL-12, (b) enzymes capable of facilitating cytotoxic pro-drug conversion, such as cytosine deaminase (CD) and herpes simplex virus thymidine kinase (HSV-TK), and (c) apoptosis-inducing ligands, such as IL-8; natural killer-4 (NK4); and tumor necrosis factor-related apoptosis inducing ligand (TRAIL) [19, 63, 65]. Recently, however, scientists have succeeded in utilizing MSCs transduced with conditionally replicating adenovirus to reduce tumor growth and spread *in vivo* [15, 16, 19]. To further enhance the tumor-targeting specificity of engineered MSCs, there have also been some efforts to increase specific homing to target tissues and to control transgene expressions by subjecting them under the regulation of tissue-specific or tumor-stromal-specific promoters [62]. In one instance, polyurethane nanocompostites loaded with gold nanoparticles (PU-Au) were found to enhance WJ-MSCs migration in response to tissue and blood vessel injury. PU-Au promoted the expression of matrix metalloproteinase-9 (MMP-9) and C-X-C chemokine receptor type 4 (CXCR4), which in turn contributed to cell mobility towards microenvironment rich in VEGF and stromal cell-derived factor 1 (SDF-1) [79].

In vitro, transfection of MSCs with plasmid carrying the tumor suppressor gene phosphatase and tensin homolog (MSC^PTEN) enables these cells to eliminante Panc-1 tumor cells in the setting of an *in vitro* co-culture experiment [63]. Likewise, MSCs engineered to secrete recombinant tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) gained the ability to induce caspase-mediated apoptosis in established glioma cell lines as well as CD133-positive primary glioma cells *in vitro* [66].

The application of engineered MSCs to treat cancer *in vivo* has also yielded some encouraging results. One example is the transduction of MSCs with lentiviral vector carrying murine IL-15 (MSC-IL-15). These cells were shown to migrate towards tumor sites in a mouse model of pancreatic cancer and mediate anti-tumor immunity. Depletion of NK and CD8$^+$ T cells abolished the MSC-IL-15-dependent anti-tumor activity, suggesting an important role for these immune cells in tumor-killing. Importantly, the animals which showed tumor remission after MSC-IL-15 treatment eventually became resistant to re-challenge with syngeneic tumors. Adoptive transfer of lymphocytes from these mice into naive mice was also sufficient to cause the latter to reject Pan02 pancreatic tumor cell transplantation. These data implied that MSC-IL-15 was able to initiate tumor-specific T-cell-mediated memory response [64]. Another group has reported that WJ-MSCs engineered to express IFN-β (WJ-MSC-IFN-β) were able to trigger apoptosis in human breast adenocarcinoma *in vitro* and *in vivo* [15, 61]. In another experimental context, WJ-MSC-IFN-β was able to kill MDA-231 cells transplanted in SCID mice. Interestingly, transducing the cells with a greater number of plaque forming units could increase the amount of IFN-β secretion. In this way, more copies of IFN-β sequences might be introduced per genome [67].

Table 2. Anti-cancer activity of conditioned medium (CM) and Cell Lysate (CL) from WJ-MSCs.

Source of CM or CL	Results	Possible Mechanisms of Anti-cancer Activity	Author
Conditioned medium (CM) was obtained from human WJ-MSCs grown under normoxic condition (norCM) or hypoxic condition WJMSCs (hypoCM) at passage 4 and 8.	• Both hypoCM and, to a lesser extent, hypoCM collected at Passage 4 and 8 exhibited toxicity against cervical (HeLa), liver (HepG2), prostate (PC3), ovarian (SKOV3), oral squamous (HSC3) cancer cell lines *in vitro*. • Both types of CM were well tolerated by non-malignant cell lines, including mouse fibroblast cell line (NIH-3T3), primary human fibroblast cells, and primary cultured human WJ-MSCs.	• Cell-contact-independent secretory factors may be responsible for the tumoricidal activity of hypoCM and norCM	Widowati, *et al.* (2015) [34]
CM and CL were collected from early passage (Passage 3 to 5) human WJ-MSC culture.	• Both CM and CL induced apoptosis and cell cycle arrest in human Burkitt's lymphoma cell line Rams and CRL1596.	• Secretory-product-mediated induction of cell cycle arrest at subG1 phase and reduction in entry to G2/M • Increase in oxidative stress indicated by an elevation in SOD activity.	Lin, *et al.* (2015) [93]
CM and CL were obtained from human WJ-MSC and human foreskin fibroblast (CCD-112sk) culture at early passages (P3 and P4).	• CM and CL from WJ-MSCs interfered with the proliferation of human keloid cells *in vitro* with an apparent decrease in the number of mitotic keloid cell numbers. • WJ-MSC CM and CL also induced apoptosis and autophagy in keloid cells, resulting in a positive TUNEL and autophagy-related marker staining, respectively. • CM and CL significantly down-regulated the expression of TAF (TBP-associated factor) in keloid cells.		Fong, *et al.* (2014) [95]
• CM was collected from WJ-MSC at Passage 3.	• WJ-MSC-CM inhibited the proliferation of U251 glioma cells, A459 lung cancer cells, HT29 colon cancer cells, and MCF-6 breast cancer cells. • WJ-MSC-CM induced apoptosis in U251 cells, as indicated by an increase in the proportion of Annexin-V and PI double positive cells, an elevation in caspase 3 and caspase 9 protein expression levels, and a decrease in survivin and XIAP (X-linked inhibitor of apoptosis protein) expressions in U251 cells upon incubation with WJ-MSC-CM. • WJ-MSC-CM induced G0/G2 growth arrest in U251 cells • Incubation with WJ-MSC-CM promoted the acquisition of a normal differentiated glial phenotype by U251 cells and caused a decrease in tumor infiltration.	• Certain paracrine factors secreted by early passage WJ-MSCs may abolish the malignant phenotype of glioma cells by inducing the cells to acquire a more mature phenotype.	Yang, *et al.* (2014) [18]

Table 2. contd….

Source of CM or CL	Results	Possible Mechanisms of Anti-cancer Activity	Author
Microvesicles (MVs) obtained from the WJ-MSC culture.	• T24 bladder tumor cells displayed an altered morphology (cell shrinkage, membrane damage, blebbing, cell debris formation) upon contact with MVs, indicating an induction of apoptosis. • Incubation with MVs resulted in an increase in the percentage of Annexin-V positive T24 cells, a downregulation in Akt phosphorylation, and an elevation in Caspase 3 activation. MVs induced a G0/G1 cell cycle arrest and reduction in S phase entry in T24 cells	• Putative miRNAs present in the MVs may be responsible, at least in part, for the suppressive effects on tumor cells.	Wu, *et al.* (2013) [58]
CM and CL were collected from WJ-MSC culture at early passages (P3 to P7).	• Incubation with CM (at differing concentrations) and CL resulted in an increased cell death in human ovarian cancer cells (TOV-112D), osteosarcoma cells (MG-63), and breast adenocarcinoma cells (MDA-MB-231), while no toxicity was observed in the case of a normal human fibroblast cell line (CDE-112sk) • This induction of apoptosis was accompanied by an up-regulation of pro-apoptotic BAX gene and a down-regulation of the pro-survival BCL-2 and SURVIVIN genes. • CL was found to be a more potent inhibitor of tumor growth than CM, probably owing to the presence of additional non-secreted factors in CL. • There was also evidence of the induction of cell cycle arrest in the sub-G1 phase	• Secretory product-mediated apoptosis induction	Gauthaman, *et al.* (2012) [76]
CM was collected from the culture of IFN-β-treated WJ-MSCs.	• CM was found to significantly attenuate the growht of both H358 human bronchio-alveolar carcinoma and SW1573 human lung alveolar carcinoma cells. • This was probably mediated by an increase in caspase 3 protein expression level.	• Secretory factor-induced activation of death caspases.	Matsuzuka, *et al.* (2010) [68]

6. CONTROVERSIES IN MSC-MEDIATED ANTI-CANCER THERAPY

There are some lingering safety concerns concerning the use of un-engineered and engineered MSCs in cancer therapy. Several reports revealed that MSCs might facilitate tumor progression *in vitro* and *in vivo*. MSC-like cells have been identified at sites of neoplastic lesions in animal models of glioblastoma and breast cancer [81, 82] as well as in specimens of human head neck squamous carcinoma [83]. Furthermore, the factors secreted by MSCs played a role in enhancing tumor growth, promoting metastasis, and dampening anti-tumor immune response [8-87]. Indeed, MSC-associated secretomes isolated from various tissues had been shown to contain numerous cytokines and chemokines implicated in tumor growth and/or metastatic spread, such as the C-C motif-containing chemokines CCL2 and CCL5, IL-6, the immune-modulator transforming growth factor-β (TGF-β), and pro-angiogenic VEGF [88, 89]. These contradictions regarding MSC-tumor interactions, to some extent, might have arisen due to differences in cell sources being used (primary tumor cells or tumor cell lines), differences in culture methods, as well as differences in experimental designs and conditions [18]. Nevertheless, the use of engineered WJ-MSCs in combination with classical chemothera-

Table 3. List of genetic manipulations performed on WJ-MSCs with the aim of enhancing their anti-cancer activity.

Engineered WJ-MSCs Procedure	Effects		Authors
	WJ-MSCs	WJ-MSCs Secretory Agent	
Transduction of WJ-MSCs with an IFN-β encoding adenoviral vector	IFN-β-transduced WJMSCs significantly inhibited the growth of human bronchio-alveolar carcinoma cells (H358) and human lung carcinoma cells (SW1573) in a more potent manner compared to unmodified WJ-MSCs *in vitro* and *in vivo* transplant of H358 cells in SCID mice	IFN-β secreted in the medium played a role in the inhibition of the growth of H358 cell lines.	Matsuzuka, *et al.* (2011) [68]
Transduction of WJ-MSCs with an IFN-β encoding adenoviral vector.	MDA-231 tumor-bearing mice that received a combination of 5-fluorouracil (5-FU) and IFN-β secreting WJ-MSCs showed a greater reduction in tumor burden than those mice which only received either 5-FU or WJMSCs alone.	IFN-β secreted in the medium played a role in the inhibition of the growth of MDA-231 human breast carcinoma cells, in combination with 5-FU treatment.	Rachakatla, *et al.* (2007) [67]
WJMSCs were seeded on PU (Polyurethane), PU-Au (gold microparticles-laden PU) and TCPS (tissue culture polystyrene)	The elastic nanocomposite (Pu especialy Pu-Au) with distinct phase separation may bestow a greater migratory potential on WJ-MSCs.	-	Huang, *et al.* (2013) [79]

peutic drugs might be a good strategy, at least in the short-term, to maximize anti-tumor activity while suppressing the tumor-promoting tendency of MSCs [90].

CONCLUSION

WJ-MSCs represent a promising cell source alternative for MSCs-based therapies, especially for applications in anti-cancer treatments. WJ-MSCs exhibit a greater proliferative and immunosuppressive capacity than MSCs isolated from older, adult tissue sources such as the bone marrow or adipose, thereby making them a better alternative for cell therapy applications. WJ-MSCs have been shown to mediate tumor-killing via cell-contact-dependent and/or cell-contact-independent mechanisms, both *in vitro* and *in vivo*. Paracrine secretions of WJ-MSCs found in CM, as well as WJ-MSC-CL, contain various factors, including cytokines and putative (micro)RNA-containing vesicles, which were able to trigger tumor cell apoptosis. Although naive MSCs do inhibit tumor progressions in some instances, engineered MSCs turn out to be much superior in terms of their anti-tumor activity. Several efforts in introducing sequences that encode anti-tumor molecules into MSCs have been proven to be successful. Unfortunately, current studies that have utilized engineered WJ-MSCs for the purpose of anti-cancer therapy have been scarce at best. More research is still required in order to assess the safety and efficacy of both unengineered and engineered WJ-MSC-based treatments in cancer patients. As in the case of any chemical and biological-based drugs, stringent pre-clinical and clinical trials need to be conducted before WJ-MSCs-based anti-cancer therapy may reach the clinic.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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