Wharton’s Jelly Stem Cells as Agents for Cancer Therapy

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Abstract: Stem cell based therapy has significant potential to treat various diseases including primary and metastatic cancers. The umbilical cord matrix stem cells (UCMSC) derived from human umbilical cord Wharton’s jelly (also termed WJMSC) have been shown to exhibit low immunogenicity, which potentially negates immune consequences after cytotherapy. The homing ability of human and rat UCMSC to inflammatory tissues, including cancer tissues, further confers upon these cells the potential for targeted cancer therapy. Our previous studies demonstrated that un-engineered human and rat UCMSC significantly attenuated the growth of multiple cancer cell lines in vivo and in vitro through multiple mechanisms. We have also demonstrated that these cells can be engineered to express cytotoxic cytokines before being delivered to the tumor and can be preloaded with nanoparticle payloads and attenuate tumors after homing to them. In this review, intrinsic stem cell-dependent regulation of cancer growth, potential mechanisms involved in this unique biological function, delivery of exogenous anti-cancer agents, and the potential for clinical applications will be discussed.

Keywords: Cytotherapy, Immunogenicity, Tumor homing, Tumor tissue tropism, Umbilical cord matrix stem cell, Wharton’s jelly.

INTRODUCTION

Wharton’s jelly is a gelatinous tissue within the umbilical cord that contains myofibroblast-like stromal cells. These stromal cells originate from extra-embryonic mesoderm which is derived from the epiblast [1-3]. Wharton’s jelly is largely made up of mucopolysaccharides (hyaluronic acid and chondroitin sulfate). First named by Thomas Wharton in 1656, this gelatinous substance's function is to provide support and cushioning for the vein and arteries of the umbilical cord. The primitive mesenchymal-like cells of Wharton’s jelly are trapped at a very early embryological age and retain the properties of primitive stem cells. Stem cells are suggested to possess the ability to self-renew throughout life to maintain the stem cell population and the capacity to differentiate to become specialized progeny cells. Cells in Wharton’s jelly express several stem cell characteristic genes, and have a high telomerase activity [4, 5]. They possess stem cell properties and can be more easily obtained and processed than embryonic and mesenchymal stem cells (MSC) such as bone marrow-derived stem cells (BMMSC) [6, 7]. Although research shows the potential of umbilical cord matrix stem cells (UCMSC) for therapy in nerve injuries, diabetes mellitus, and several organ type cancers, the impact of naive UCMSC on tumorigenesis and tumor growth has not yet been established.

Regarding the impact of MSC from sources other than umbilical cords on tumorigenesis, several studies have shown that MSC from other sources promote tumor growth and metastasis by multiple mechanisms [8-10]. Furthermore, it has been revealed that BMMSC induce many pro-tumorigenic effects, such as host immunosuppression [11], tumor vascular formation [12], and reinforcement of the epithelial-mesenchymal transition [13]. In allogeneic transplantation models, MSC-dependent immune suppression plays a critical role; thus, tumors are frequently formed, but only in the presence of embryonic, BMMSC [14]. On the other hand, a few studies report that MSC from sources other than umbilical cords can suppress tumor growth. For example, Khakoo et al., reported that BMMSC could exert anti-tumor effects on Kaposi’s sarcoma, but only if there was cell-cell contact [15]. In this review, the intrinsic tumoricidal ability of naive UCMSC, potential mechanisms involved in this unique biological function, delivery of exogenous anti-cancer agents, and the potential for clinical applications will be discussed.

POTENTIAL APPLICATION OF UCMSC FOR VARIOUS DISEASES

Among the features of UCMSC, they have high proliferative potential and differentiation capacity into cells of multiple mesenchymal lineages. For example, Yang et al. have shown that transplantation of human UCMSC into the lesion site of a spinal cord injury is beneficial to wound healing in rats [16]. Parkinson’s disease is a potential target for the UCMSC therapy since transplanted UCMSC can be differentiated into dopaminergic neuron-like cells [17]. In addition, Chao et al., have reported that human UCMSC can be differentiated into mature islet-like cell clusters, and they possess an insulin-producing ability and significantly alleviated diabetic conditions in rats [18]. Taken together,
these results indicate that UCMSC could be used for multiple targeted therapies including cancer therapy.

**UCMSC POSSESS LOW IMMUNOGENICITY**

Although UCMSC are relatively easy to prepare in large quantities and can be banked until use, their practical application would be in allogeneic transplantation. One important requirement for allogeneic cell transplantation therapy is low immunogenicity. UCMSC apparently escape the host immune responses by multiple mechanisms. They express MHC class I (HLA-ABC) but not class II (HLA-DR) antigens. They are negative for other co-stimulatory antigens such as CD80 and CD86 [5, 19, 20]. This characteristic is similar to other MSC, since MSC are generally MHC class II antigen negative [21]. UCMSC produce large amounts of tolerogenic IL-10 and TGF-β and express HLA-G at higher levels than BMMSC [5, 20, 22]. HLA-G induces the expansion of regulatory T cells (Treg), which would contribute to the suppression of effector responses to alloantigens [23, 24]. It has also been shown that UCMSC suppress T cells and NK cells by stimulating Treg activation [22] and expressing Nanog and Oct3/4A [5]. UCMSC showed very weak T cell activation with non-significant increases in IFN-gamma and IL-2 [22]. Furthermore, UCMSC derived prostaglandin E2 (PGE2) modulates monocytes to suppress T cells [25, 26]. Adhesion molecules, such as inflammatory cytokine-induced intercellular adhesion molecule-1 (ICAM-1) [20] and vascular cell adhesion molecule-1 (VCAM-1), play critically important roles for the MSC-dependent immunosuppression of T cells. These molecules are inducible by the parallel presence of IFN-γ and inflammatory cytokines such as IL-1 and TNF-α [5, 20]. Furthermore, UCMSC express IL-6 and vascular endothelial cell growth factor (VEGF), molecules which are thought to suppress the immune system [21]. The immunosuppressive properties of UCMSC are significantly stronger than those of BMMSC. In fact, un-stimulated UCMSC suppress mitogen-induced lymphocyte-proliferation responses to a greater extent than BMMSC. In a co-culture of 1:100 UCMSC:PBMC, suppression of lymphocyte proliferation responses was observed [20]. Additionally, priming with inflammatory stimuli can enhance only BMMSC, not UCMSC, to stimulate mitogen-induced lymphocyte proliferation [20]. These UCMSC characteristics are very important and convenient properties as these cells are applied to cyotherapeutic treatment of various diseases including cancer.

Despite the immunosuppressive properties of UCMSC, repeated injections of UCMSC could induce MHC class II

![Fig. (1). Transplanted human or rat UCMSC exhibit tumor tropism. Panels A and B, MDA-MB 231 lung metastatic breast carcinoma-bearing SCID mice were treated systemically via intravenous (IV) injection with human UCMSC labeled with SpDiI red fluorescent dye (5 x 10^5 cells) [32]. One week after the last treatment, lungs or pancreatic tumors were dissected and subjected to immunohistochemical analysis. Sections were counterstained with Hoechst 33342 nuclear stain (DAPI, blue). Panel A, MDA-MB 231 cells in the lung were identified by anti-human mitochondrial antibodies conjugated with FITC (Mt, green) to pinpoint the location of SpDiI labeled human UCMSC within the tumor. Panel B, Morphology of SP-DiI-labeled cells in the lung section indicated that the SP-DiI-labeled cells appear to be UCMSC. Panels C and D, Pan02 murine pancreatic carcinoma sections from peritoneal tumors of mice treated via intraperitoneal injection with rat UCMSC labeled with SP-DiI (5 x 10^5 cells) [33]. Sections were visualized with the fluorescent microscope or in bright field after H&E staining. These figures clearly indicate that systemically or intraperitoneally administered human or rat UCMSC selectively engrafted within the tumors or in close proximity to the tumors. Scale bar =100µm. Figure is modified from the original figures, Rachakatla et al., Cancer Gene Ther, 14: 828-835 2007, and Ayuzawa et al., Cancer Lett, 280: 31-37, 2009.](image-url)
growth factors are secreted by tumor cells and their mediated by the interaction of cytokines/growth factor production sites by sensing these cytokine gradients [14, 31, 39, 40]. For example, a subpopulation of BMMSC was shown to express CXCR4, the receptor for stromal cell-derived factor 1 (SDF-1), and CXCR2, the receptor for fractalkine [41]. Tumors secrete SDF-1, PDGF, EGF, etc. that attract cells from surrounding tissues as well as from the bone marrow to provide support of tumor growth. Due to the over-expression of IL-8 receptor, CXCR1, and CXCR2, UCMSC have a greater capacity to migrate towards glioma than BMMSC [39]. In NPSC, which also exhibit tropism to gliomas, hypoxia is another key factor affecting tropism. SDF-1/CXCR4, uPA/uPAR, VEGF/VEGFR2, and hepatocyte growth factor/c-Met signaling pathways mediate increased NPSC-to-glioma tropism under hypoxic conditions [11]. However, the effect of hypoxia on UCMSC tumor tropism has not been reported. Hypoxic conditions may be one way in which tumor tissue provides a niche for stem cells. The tumor tropic properties of UCMSC and other MSC is apparently not disturbed by loading of various therapeutic genes [29, 42-45], drugs [46, 47], and nanoparticles into the cells. Thus, it is possible to utilize UCMSC as tumor or inflammatory tissue-targeted delivery vehicles for various therapeutics.

**UCMSC HOME TO TUMOR TISSUES**

MSC possess tropism to inflammatory tissues such as cancer tissues [28-31]. Both human and rat UCMSC also possess a strong tropism to various tumor tissues [32, 33] and other inflammatory lesions [17, 34, 35]. Both human and rat UCMSC were specifically localized in the periphery of the tumor tissues when they were transplanted (Fig. 1) [32, 33]. The homing ability of stem cells seems to be mediated by the interaction of cytokines/growth factors and their receptors [36]. Large amounts of various cytokines and growth factors are secreted by tumor cells and their associated stroma [37, 38]. Since UCMSC and other MSC express various cytokine and growth factor receptors on their membrane surfaces, they are likely to migrate towards cytokine/growth factor production sites by sensing these cytokine gradients [14, 31, 39, 40].

Table 1. Studies of Tumor Growth Attenuation by UCMSC and Other MSC. This Summary Table Lists Only Studies Describing Tumor Cell Growth Attenuation by UCMSC and Other MSC [15, 32, 33, 45, 49, 50, 59-61]

| Author           | Cell type and experiment                                                                 | Results                                         | Possible mechanism                                                                 |
|------------------|------------------------------------------------------------------------------------------|------------------------------------------------|------------------------------------------------------------------------------------|
| Ayuzawa et al. [7] | Pulmonary metastatic xenograft of MDA231 (breast cancer) in SCID mice<br>IV injection of UCMSC | Tumor size decreased                             | 1. G2 arrest 2. Attenuation of proliferation                                         |
| Cousin et al. [14]| Subcutaneous xenograft of Capan-1 (pancreatic cancer) in athymic nude mice<br>IT injection of ATMSC | Tumor size decreased                             | 1. G1 arrest 2. Induction of proliferation                                           |
| Doi et al. [20]  | Orthotopic xenograft of PAN02 (pancreatic cancer) in C57/BL mice<br>IP injection of UCMSC | Tumor size decreased and survival time increased | 1. G0/G1 arrest 2. Attenuation of proliferation                                     |
| Ganta et al. [24]| Orthotopic graft of Mat B III (breast cancer) in F344 rats<br>IT and IV injection of UCMSC | Tumor abolished and no evidence of metastasis or recurrence 100 days after post injection | Induction of apoptosis                                                              |
| Khakoo et al. [30]| Subcutaneous xenograft of KSIMM (Kaposi’s sarcoma) in athymic nude mice<br>IV injection of BMMSC | Tumor size decreased                             | Attenuation of AKT signaling                                                        |
| Matsuzuka et al. [35]| Orthotopic xenograft of H358 (lung cancer) in SCID mice<br>IV injection of UCMSC | Tumor multiplicity and size decreased           | 1. Attenuation of proliferation 2. Induction of apoptosis                             |
| Maurya et al. [36]| Orthotopic graft of LLC (lung cancer) in C57/BL mice<br>Intratracheal injection of UCMSC | Tumor multiplicity and size decreased           | 1. G0/G1 arrest 2. Decrease of cyclin A and CDK2                                   |
| Otsu et al. [40]| Subcutaneous graft of B16F10 (melanoma) in C57/BL mice<br>IT injection of BMMSC | Tumor size decreased                             | 1. Inhibition of angiogenesis 2. Induction of apoptosis                              |
| Secchiero et al. [48]| Intraperitoneal xenograft of SKW6.4 or BJAB (non-Hodgkin’s lymphoma) in SCID mice<br>IP injection of BMMSC | Tumor size decreased                             | Induction of apoptosis of endothelial cell (in vitro result)                         |

UCMSC: umbilical cord matrix-derived stem cell, BMMSC: bone marrow mesenchymal stem cell, ATMSC: adipose tissue-derived mesenchymal stem cell.
TUMORICIDAL ABILITY OF UCMSC

Although many studies have demonstrated that MSC can have a stimulatory effect on tumor growth [48], naïve MSC, including UCMSC, BMMSC, and ATMSC, have been shown to be capable of attenuating the growth of tumor cells both in vitro and in vivo (Table 1). Both un-engineered human and rat UCMSC significantly attenuated growth of cancer cell lines in vitro and in vivo with human breast cancer cells [32], rat mammary tumor cells (Fig. 2) [49], human lung cancer cells (Fig. 4) [45], mouse Lewis lung carcinoma (LLC) cells [33], and mouse pancreatic carcinoma cells [33]. Detailed analysis with multiple assay methods including MTT assay, thymidine uptake assay, direct cell counting, and tumor colony assay clarified that this UCMSC-dependent growth attenuation is mediated by secretory proteins/peptides and is associated with decreased MAP kinase activity. The secretory proteins/peptides are produced by either the UCMSC or the tumor cells in response to the UCMSC secretory products. Analysis with flow cytometry further revealed that co-culture with human UCMSC caused G2 arrest of MDA 231 human breast cancer cells [32], whereas rat UCMSC caused G0/G1 arrest of LLC [50] and murine pancreatic carcinoma cells [33]. The G0/G1 arrest is evident in the decrease of CDK2 expression [50]. In vivo studies showed that both human and rat UCMSC treatment significantly decreased tumor weights [32, 33, 49, 50]. In particular, intratumoral or systemic administration of rat UCMSC caused complete regression of orthotopic Mat B III grafts in the female F344 rat mammary gland to undetectable levels by 34 to 38 days (Fig. 2). There was no evidence of metastasis or recurrence 100 days post–tumor cell inoculation (Fig. 2). Histological studies revealed that intracheally, intraperitoneally, or systemically administered rat UCMSC homed to tumor areas and survived for at least 3 weeks without any evidence of differentiation or adverse effects [33, 50]. Therefore, the findings described above suggest that UCMSC may represent a new therapeutic modality and will have important implications for the treatment of patients with breast cancer, lung cancer, pancreatic cancer, and other types of cancer.

POSSIBLE MECHANISMS BY WHICH UCMSC ATTENUATE TUMOR GROWTH

The mechanisms by which naïve UCMSC attenuate tumor growth have yet to be fully clarified, however, two potential mechanisms are suggested.

The first potential mechanism is that the UCMSC produce multiple secretory proteins that induce cell death of cancer cells and cell cycle arrest. This possibility is suggested by multiple reports that UCMSC stimulate caspase activities and arrest the cell cycle even in the absence of direct contact with cancer cells [7, 18, 22, 31]. In support of this mechanism, our recent study via microarray analysis of rat UCMSC discovered over-expression of multiple tumor suppressor genes when they were co-cultured with Mat B III rat mammary tumor cells in a Transwell culture system. In this culture system, the two cell types were divided by a porous membrane with 0.4 μm pores and were devoid of direct contact. The comparison of gene expression profiles between untreated and co-cultured rat UCMSC identified five up-regulated genes ( follistatin (FST), sulfatase1 (SULF1), glucose phosphate isomerase (GPI), HtrA serine peptidase (HTRA1), and adipocyte differentiation-related protein (ADRP)) and two down-regulated genes (transforming growth factor, beta-induced, 68kDa (TGFBI) and podoplanin (PDPN)) [51].

The second potential mechanism of tumor attenuation is the enhancement of an immune reaction to cancer cells by a mixture of UCMSC, malignant tumor cells, and dead tumor cells. Histopathology of the tumors of orthotopic Mat B III grafts in the female F344 rat mammary gland treated with rat UCMSC indicated that a large portion of tumor was replaced with extracellular matrix, and a large number of lymphocytes had infiltrated into tumor tissues (Fig. 3). Immunohistoche-

Fig. (2). Rat UCMSC completely eliminate mammary tumors without recurrence. Figure 2A shows Mat B III mammary tumor growth (volume) with time. Mat B III mammary tumor-bearing F344 rats were treated with rat UCMSC, either systemically via IV injection (1.5 x 10⁶ cells, at days 4 and 6 post tumor cell transplantation) or intratumorally (IT, 1 x 10⁶ cells, bolus injection at day 4 post tumor cell transplantation). Rats bearing tumors reaching beyond 2±0.2 cm in diameter were sacrificed. *Significantly different from PBS treated rats. Figure 2B shows representative examples of rat mammary tumors after treatment with PBS or IV rat UCMSC. Figure is modified from the original figure, Ganta et al., Cancer Res, 69: 1815-1820, 2009 [49].
Histopathologic analysis of orthotopic Mat B III mammary tumor grafts revealed that rat UCMSC treatment stimulates lymphocyte infiltration in the tumors. Control PBS treated mammary tumors in female F344 rats contained mostly anaplastic tumor cells (arrows) (A), whereas rat UCMSC–treated rat tumors contained island-like clusters of tumor cells (arrow) surrounded by connective tissues with moderate to large numbers of lymphocytes (B). Figure is modified from the original figure, Ganta et al., Cancer Res, 69:1815-1820, 2009 [49].

mistry revealed that the majority of infiltrating lymphocytes in rat UCMSC-treated tumors were T cells. The treatment of rat UCMSC apparently increased CD 8+ T cell infiltration throughout the tumor tissue. CD68+ cells were occasionally observed in the tumors of the PBS control group but not observed in the rat UCMSC treated group. DNA microarray analysis suggested that monocyte chemotactic protein-1 (MCP-1 also known as CCL2), which is associated with lymphocyte infiltration, was the most likely candidate to induce migration of lymphocytes by rat UCMSC treatment. Although these results contradict results described above which show the low immunogenicity of human UCMSC, the immunogenicity of UCMSC in tumor-bearing animals may be dependent upon the microenvironment of UCMSC and tumor cells.

UCMSC AS CELLULAR VEHICLES FOR TARGETED CANCER THERAPY

Developing targeted cancer therapy requires cancer tissue-targeted delivery of therapeutics such as tumor suppressor genes, anticancer drugs, and/or therapeutic nanoparticles. UCMSC are very durable, have a large loading capacity, and have marked tumor tissue tropism. In addition, human UCMSC are useful human postnatal stem cells because a relatively large number can be harvested, propagated without any feeder cells, and stored after birth without any risks to the donor. As a result, they are ideal vehicles for targeted therapy. In fact, our previous reports have proven that human UCMSC are suited for tumor-targeted delivery of various anticancer agents, such as the...
cytotoxic cytokine interferon (IFN)-β [42, 45] (Fig. 4) and the anticancer prodrug irinotecan (unpublished results). Our recent study has demonstrated that human UCMSC do not form any teratomas when injected into SCID mice [42]. Other types of MSC, such as BMMSC [29,30], adipose tissue-derived mesenchymal stem cells (ATMSC), and neuronal stem/progenitor cells (NSPC) are also shown to be usable for cancer-targeted delivery of various therapeutics [52, 53]. Such applications cause significant attenuation of the growth of difficult tumors such as malignant glioma, metastatic melanoma, etc.

The primary benefits of live cell-based delivery of these therapeutics include a reduction of side effects and increased efficacy of each therapeutic agent. For example, the use of adenoviral vectors is an effective strategy as they allow strong expression of transgenes in a variety of tissues, including tumor tissue [54, 55]. However, their clinical applications are limited by their efficacy in that viral vectors are rapidly cleared from circulation [56]. Viral vectors are also potentially pathogenic, as cases of viral infection have been reported [57]. Since naked plasmid DNA does not easily penetrate cellular membranes, non-viral gene delivery systems, such as nanoparticle encapsulation of therapeutic genes, may be an alternative procedure. However, since nanoparticles do not have tumor tropism, surface modifications that cause tumor-targeted accumulation are required. Loading nanoparticles encapsulating therapeutic genes or drugs into the stem cells and having these stem cells deliver the therapeutics to cancer tissues is a potentially viable alternative procedure.

IFN-β is known to have a strong ability to inhibit tumor cell growth and induce apoptosis in vitro. However, IFN-β
has not been successfully used in in vivo studies because of its short half-life and because the maximally tolerated dose is lower than the effective dose. Recent studies by our colleagues, Rachakatla et al., and Matsuzuka et al., have shown successful expression of IFN-β in human UCMSC (IFN-β-hUCMSC). They have demonstrated that systemically administered IFN-β-hUCMSC successfully homed to lung tumor sites and attenuated growth of lung-metastasized breast cancer [19] and human bronchioloalveolar carcinoma in mice (Fig. 4) [45]. A unique feature of these IFN-β-hUCMSC is that the efficacy of IFN-β-dependent tumor growth attenuation appears to be significantly enhanced by the intrinsic tumoricidal ability of the human UCMSC themselves [32]. Furthermore, poor immunogenicity [21, 58] and non-tumorigenicity [42] of human UCMSC make this cellular vehicle potentially applicable to human patients. This live cell-based cytokine gene therapy appears to be significantly safe and effective in controlling NSCLC, lung metastasis of other types of cancers, and various other types of primary cancers (Fig. 5). Cell-based cytokine gene and nanoparticles should also be applicable to various inflammatory diseases.

CONCLUSION

Naïve UCMSC have many potential advantages for cell-directed cancer therapy, since they have been shown to have the intrinsic ability to secrete factors that can result in cancer cell growth inhibition and/or apoptosis in vitro and in vivo. Among many tissue-originated multipotent stem cells, UCMSC are very usable due to their abundance, low immunogenicity, lack of CD34 and CD45 expression, and simplicity of the methods for harvest and in vitro expansion. These properties of UCMSC encourage their development as therapeutic tools or agents because they can potentially be used for allogeneic transplantation. In addition, human UCMSC engineered to express IFN-β produced sufficient amounts of IFN-β to induce death of human breast adenocarcinoma cells and bronchioloalveolar carcinoma cells in vitro and in vivo. Thus, the IFN-β-human UCMSC could also be a new therapeutic modality for the treatment of breast adenocarcinoma and bronchioloalveolar carcinoma and will have important implications for patients with breast cancer, lung cancer, or other types of cancers.

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CONFLICT OF INTEREST

None declared.

ABBREVIATIONS

UCMSC = Umbilical cord matrix stem cells  
MSC = Mesenchymal stem cells  
BMMSC = Bone marrow mesenchymal stem cells  
ATMSC = Adipose tissue-derived mesenchymal stem cells  
NSPC = Neuronal stem/progenitor cells  
PBMC = Peripheral blood mononuclear cells  
IL-6 = Interleukin-6  
IL-10 = Interleukin-10  
TGF-β = Transforming growth factor-beta  
HLA-ABC = Human leukocyte antigen-ABC  
HLA-DR = Human leukocyte antigen-DR  
HLA-G = Human leukocyte antigen-G  
Treg = Regulatory T cells  
ICAM-1 = Inflammatory cytokine-induced intercellular adhesion molecule-1  
VCAM-1 = Vascular cell adhesion molecule-1  
SDF-1 = Stromal cell-derived factor 1  
MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium  
IFN-β = Interferon-β  
IFN-γ = Interferon-γ  
EGF = Epidermal growth factor  
PDGF = Platelet-derived growth factor  
VEGF = Vascular endothelial cell growth factor  
CXCR3 = C-X-C chemokine receptor type 3 for CXCL9, CXCL10 and CXCL11  
CXCR4 = C-X-C chemokine receptor type 4 for stromal cell-derived factor 1 (SDF-1)IV, intravenous  
IT = Intra tumor  
IP = Intrapertitoneal

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