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The Inhibitory effect of Placenta Fetal Mesenchymal stem cells on Prostate cancer cell line

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Mesenchymal stem cells (MSCs) have shown antitumor effects against various cancers. However, the mechanism of PF-MSCs and their extracts related to prostate cancer cell lines 22Rv1 remains undetermined. Therefore, the above study investigated the suppressive effects of PF-MSCs extract, including PF-MSCs/CM and PF-MSCs/CL, against 22Rv1 cells in vitro. Firstly, the characteristics of MSCs were investigated, followed by the preparation of PF-MSCs/CM and PF-MSCs/CL and the inhibitory effects against 22Rv1 cells. Finally, we used flow cytometry to assess how extracts affect the cell cycle of 22Rv1 cells and real-time polymerase chain reaction to measure the expression of inflammatory genes. Our study uncovered some interesting findings about PF-MSCs: they have the same markers as MSCs, can differentiate into bone and fat cells, and can multiply faster. When we treated the cells with 100% PF-MSCs/CM and 100 µg/ml PF-MSCs/CL, we observed an improvement in cell viability. We also noticed that the extracts caused a decrease in the sub-G1 population. On top of that, specific genes related to cell death, like BAX and CASP3, were more active, while genes associated with inflammation, such as IFN-γ, TNF-α, IL-1β, IL-6, IL-8, and IL-12A, were less active. In contrast, the expression of anti-inflammatory genes like IL-4 and IL-10 increased. Based on our findings, it seems that PF-MSCs can enhance the stimulation of 22Rv1 cells, which might be why they can promote cancer.

Keywords: Prostate Cancer, Mesenchymal Stem Cell, Differentiation, Gene Expression, Secretome

INTRODUCTION

Prostate cancer (PCa) affects a large number of men worldwide, ranking as the second most widespread type of cancer. Around 1.3 million new cases are reported annually (Bergengren et al. 2023). In Saudi Arabia, it is the fifth most prevalent cancer among men. (American Cancer Society 2018; Saudi Health Council Saudi Cancer Registry 2018). PCa is a life-threatening malignant tumor with high morbidity and mortality rates attributed to burden and metastatic potential (Adedapo et al. 2012; Voinea et al. 2021). Although not the most common cancer among males in the Middle East, the future prognoses for incidence and mortality impact have been more foreboding in the region; however, its etiology remains largely unclear (Saleh et al. 2020).

In the adult prostate, cellular homeostasis is maintained by a hierarchical arrangement of cells with varying proliferative potentials, mirroring mechanisms observed in other epithelial tissues (Chen et al. 2013). Somatic stem cells (SCs) exhibit several distinct properties, including self-renewal capability and differentiation potential across several cell lineages, confined proliferation in specific physiological microenvironments (niches), and remarkable proliferative potential despite their typical quiescent state (Reya et al. 2001). According to the hierarchical SC

carcinogenesis model, PCa is caused by changes in the genetic and epigenetic variables governing the proliferation of normal SCs (Kerr and Hussain 2014). These aberrantly expressed pathways transform normal SCs into cancer stem cells (CSCs), which are believed to drive tumor genesis, development, relapse, and metastasis, sharing certain traits with their non-malignant counterparts. CSCs confer resistance to traditional radiotherapy and chemotherapy (Tang et al. 2007; Yu et al. 2012). Therefore, the treatment of PCa poses significant challenges, underscoring the imperative for novel therapeutics to improve remission rates, reduce relapse rates, and eliminate the CSC population (Giridharan, Rupani, and Banerjee 2022; Portillo-Lara and Alvarez 2015).

Mesenchymal stem cells (MSCs) are brilliant in many areas because they come from different sources, are easy to grow, and have interesting biological properties. They can differentiate into different types of cells and keep multiplying. MSCs also have other excellent abilities like fighting tumors, reducing inflammation, helping the immune system, preventing fibrosis, stopping cell death, promoting blood vessels, protecting nerves, fighting bacteria, and attracting chemicals that can help treatment. The convergence of these characteristics has garnered significant research interest, resulting in numerous therapeutic trials based on MSC utilization (Gopalarethinam et al. 2023; Kumar et al. 2019).

While bone marrow MSCs (BM-MSCs) have been the primary focus of many preclinical studies, they may not necessarily represent the best feasible cell source. BM-MSCs have been employed in several clinical trials targeting glioma, ovarian, and prostate cancer (Jafari et al. 2021). However, employing these cells poses particular difficulties, including the requirement for invasive procedures to extract them from the bone marrow, limitations on the quantity of cells that can be collected, and decreased differentiation potential with advancing patient age (Jafari et al. 2021). Alternative places to obtain MSCs exist, like adipose tissue, placenta, umbilical cord, and Wharton's jelly (Kalamegam et al. 2018).

Placental tissue, easily obtained as medical waste, provides a convenient source for obtaining placenta fetal MSCs (PF-MSCs) from the discarded placental tissue post-birth without ethical concerns surrounding their use in research. The placenta is complicated. Therefore, it can be logically segmented into separate components: the fetal side (amnion, chorion, umbilical cord) and the maternal side (decidua). Many studies have reported MSCs from placental components (Abomaray et al. 2016; Wu et al. 2018). Perinatal sources offer several advantages over adult sources, including greater availability, reduced donor site morbidity, cell naivety, having more stem cells in tissues, and exceptional proliferative capability (Yang et al. 2013).

MSCs release a vast spectrum of protective bioactive substances called the secretome, which modulate cell-environment cross-talk to influence biological processes (Madrigal, Rao, and Riordan 2014). Therefore, the MSC secretome has garnered considerable attention from researchers and can be potentially applied in cell-free therapeutic settings. Furthermore, the secretome can protect host cells by stopping cell death, reducing inflammation, stopping scar formation, having immunomodulatory effects, encouraging blood vessel growth, and stopping tumor growth (Huwaikem et al. 2021; Kološa et al. 2015; Vaiasicca et al. 2024).

Various studies have proved the antitumor properties of MSCs derived from numerous sources when tested against different types of cancer cells (Fang et al. 2023; Gauthaman et al. 2012; Gondi et al. 2010; Khakoo et al. 2006). Sun et al. showed that MSCs suppressed the spread of cancer cells to the lungs and stimulated programmed cell death in breast cancer cells. Their research also revealed that the transplantation of human umbilical cord blood and adipose tissue into a rat metastatic cancer model did not stimulate tumor growth or the spread of cancer cells (Sun et al. 2009). Furthermore, Takahara et al. reported that adipose-derived stromal cells promoted apoptosis in commercial PCa cell lines, including LNCaP and PC3 cells, via specific signaling pathways (Takahara et al. 2014). Further research revealed that umbilical cord MSCs prevented tumor growth and exhibited antitumor potential against PC3 PCa cells (Safari et al. 2021). In a recent study, human amnion MSCs induced cellular apoptosis in LNCaP cancer cells in a co-culture system (Safari et al. 2021).

Previous studies involving MSCs have not definitively determined whether all types of human SCs possess anticancer properties nor identified which cancers may respond to these anticancer effects. Therefore, this research examined PF-MSCs' cancer-inhibiting effects on a PCa cell line *in vitro*. We sought to characterize MSCs derived from the human placenta and evaluate their anticancer properties against PCa using *in vitro* assays with the 22Rv1 cell line, focusing on parameters such as cell proliferation, the cell cycle, and related gene expression.

MATERIALS AND METHODS

PF-MSCs Culture *In Vitro*

The harvesting and cultivating methods of PF-MSCs were followed according to Gauthaman et al. (2012). The Embryonic Stem Cell Unit at Jeddah's King Fahd Centre for Medical Research kindly provided placenta-derived cells. Placenta-derived cells were seeded in a humidified incubator at 37 °C using Dulbecco's modified Eagle's medium – Low Glucose (CN# 11965-092; Gibco, USA) supplemented with 5% fetal bovine serum (FBS)

(CN# 10099133; Gibco, USA), 1% L-glutamine (CN# 35050-038; Gibco, USA), and 1% penicillin/streptomycin (CN# 15070-061; Gibco, USA). Cells were sub-cultured and proliferated *in vitro*, following standard passaging procedures. The cells were cultured in vitro and allowed to undergo proliferation until they reached confluence. The trypsin-EDTA (CN# 25200056, Gibco, USA) was used to detached cells at a concentration of 0.25%. PF-MSCs from passages 4–6 were used upon reaching 90% confluence. Cell morphology was visualized using a phase-contrast microscope (Nikon Corporation, Tokyo, Japan).

22Rv1 Cell Line Culture

The human prostate cancer cell line 22Rv1 was cultured in Roswell Park Memorial Institute medium (RPMI-1640, CN# 21875091; Gibco, USA) containing 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin in a 5% CO_2 humidified atmosphere. The media was replaced every 48 hours until it reached 90% confluence. Cells were detached using trypsin-EDTA and then transferred to a new medium in culture flasks for subculturing. Cell morphology was examined using phase-contrast microscopy.

CD Marker Expression in PF-MSCs

Surface CD marker expression in passage 3 PF-MSCs was evaluated by flow cytometry. For each antibody treatment, PF-MSCs were plated at 1×10^5 per Fax tube. Cells were blocked with 100 µl PBS, pH 7.4 (CN#10010015; Gibco, USA) and 3% FBS after centrifugation to prevent nonspecific binding. PF-MSC indications were identified utilizing dual CD marker antibody combinations. Mix 1 had CD29 APC, CD90-FITC, CD73-PERCP, and CD45-PE, whereas Mix 2 included CD44-APC, CD105-FITC, and CD34-APC. In the dark, groups were incubated with CD marker cocktails at 4 °C for 20–30 min. After washing twice with PBS containing 3% FBS, the cells were centrifuged at 1000 rpm for 5 min, and the supernatant was aspirated. A fluorescence-activated cell sorting study was conducted on each cell pellet in 500 µl of 3% FBS (FACS Aria II, BD Biosciences, USA).

Differentiation of PF-MSCs

Adipogenesis and osteogenesis differentiation kits (CN# A10070-01 and CN# A10072-01; StemPro® et al. USA) can turn PF-MSCs into adipocytes and osteocytes. A 5×10^3 cells per well were seeded in 6-well plates and incubated overnight in a complete culture medium. PF-MSCs were grown in a differentiation basal medium with appropriate supplement (StemPro® kit components; Thermo Fisher Scientific, USA) and refreshed every 72 h. Cultured cells in the differentiation basal medium only act as a control. PF-MSCs that become adipocytes were stained with oil-red O (Abcam, UK) per manufacturer instructions. PF-MSCs that developed into osteocytes were stained with alizarin red (Sigma, St. Louis, USA) per the manufacturer's instructions, followed by imaging using a light microscope.

Experimental Design

We separated 22Rv1 cells into three groups. . A group served as the untreated (control) group. The remaining two groups were treated with PF-MSCs: one was treated with PF-MSCs/conditioned medium (PF-MSCs/CM) (30%, 50%, 70%, and 100%), and the second was treated with PF-MSCs/cell lysate (PF-MSCs/CL) (30, 50, 75, and 100 µg/ml). Phase contrast microscopes were used to detect morphological changes from various treatments.

Preparation of PF-MSCs Extracts

PF-MSCs/CM and PF-MSCs/CL were prepared according to previously published protocols (Gauthaman et al. 2012). Early passages (P4-P6) of 80% confluent PF-MSCs cultured in PF-MSC medium for 48 hours at 37 °C and 5% CO_2 yielded PF-MSCs/CM. After separating and filtering the medium with a 0.22 mm syringe (Millipore, MA, United States), it was kept at −20 °C for immediate use or −80 °C for storage. After lysing the pellet with radio immuno precipitation assay lysis solution and protease and phosphatase inhibitors (Solarbio, Beijing, China), the mixture was incubated on ice for 30 min and then centrifuged at 14,000 g for 20 min. Aliquots of the resulting supernatant were stored at -80 °C for future use. The nanodrop™ spectrophotometers measured the PF-MSCs/CL protein (Nanodrop Technologies, DW, USA).

Cell Metabolic Activity (MTT Assay)

MTT was used to examine the proliferation of PF-MSCs and 22Rv1. The metabolic activity of the experimental groups was monitored at 24 h, 48 h, and 72 h by the instructions provided by the manufacturer. After the treatment, 10 µl of 5 mg/ml MTT reagent (Solarbio, Beijing, China) was added to each well. Additionally, 100 µl of fresh culture medium was added, and the cells were cultured for 4 hours at 37 °C in a 5% CO_2 incubator. For dissolving formazan crystals, 100 µl of dimethylsulfoxide (Sigma, MO, USA) was added to each well after removing the supernatant and incubated for 5 min in the dark. A wavelength of 570 nm was detected using spectrophotometry (SpectraMax i3 Multimode Reader; Molecular Devices, USA).

Cell cycle analysis

The effect of PF-MSCs/CM (100%) and PF-MSCs/CL (100 µg/ml) treatments on the 22Rv1 cell cycle was evaluated following 72 h of culture using flow cytometry. Untreated 22Rv1 cells were used as the controls. In brief, 22Rv1 cells were collected, washed three times with PBS, and fixed in droplets with 70%

cold ethanol to reduce cell clumping (Sigma-Aldrich, Darmstadt, Germany). The samples stayed at -20°C overnight. The fixed cells were washed twice in PBS after 5 min of 1,000 rpm centrifugation. Cells were suspended in 400 µl of staining solution containing 50 µg/ml propidium iodide (CN# ab14038, Abcam, UK) and 10 µg/ml RNAse, incubated for 15 minutes in darkness. The marked cells were examined using the flow cytometer.

Gene Expression Analysis via Real-Time Polymerase Chain Reaction

For the gene expression study, the 22Rv1 cells treated with PF-MSCs/CM at 100%, PF-MSCs/CL at 100 µg/ml, and untreated cells (control) were examined in real-time polymerase chain reaction for 72 h, following the protocol instructions. The RNA extraction was done using the RNeasy Mini Kit (Qiagen, Germany). The RNA quality was evaluated by measuring the 260/280 ratio using a NanoDrop 2000/2000c spectrophotometer. Random hexamers reverse transcriptase kit (Promega, WI, USA) were employed to reverse transcribe first-strand cDNA. The SYBR Green master mix (Life Technologies, Thermo Fisher Scientific, MA, USA) was used in qRT-PCR. The expression of apoptotic (BIRC5, BAX, BCL2, CASP3) and inflammatory genes (IL-4, IL-10, IFN-γ, TNF-α, IL-1β, IL-6, IL-8, and IL-12A) were analyzed using the Step One Plus™ real-time PCR device (Thermo Fisher Scientific, MA, USA). GAPDH was used as the reference gene. The forward (F) and reverse (R) primer sequences obtained from previously published studies are listed in (Table 1).

Statistical Analysis

Data are expressed as mean ± standard deviation. The statistical analysis was performed using SPSS 22.0 (IBM Corp. in Armonk, New York, USA). An assessment of the normality of the value distribution was conducted using the Shapiro-Wilk test. One-way analysis of variance and Tukey's test were used for data analysis and group comparisons. The statistical significance level is below 0.05.

Table 1: The genes and primer sequences used for qRT-PCR

Genes	Primer Sequence
GAPDH	F: 5'-GCACCGTCAAGGCTGAGAAC-3' R: 5'-GGATCTCGCTCCTGGAAGATG-3'
BIRC5	F: 5'-ACCAGGTGAGAAGTGAGGGA-3' R: 5'-AACAGTAGAGGAGCCAGGGA-3'
BAX	F: 5'-GGCTGGGATGCCTTTGTG-3' R: 5'-CAGCCAGGAGAAATCAAACAGA-3'
CASP3	F: 5'-TGACTGGAAAGCCGAAACTC-3' R: 5'-AGCCTCCACCGGTATCTTCT-3'
IL-4	F: 5'-TGGATCTGGGAGCATCAAGGT-3' R: 5'-TGGAAGTGCGGATGTAGTCAG-3'
IL-10	F: 5'-GCTCTTACTGACTGGCATGAG-3' R: 5'-CGCAGCTCTAGGAGCATGTG-3'
IFN-γ	F: 5'-CCCTCACACTCAGATCATCTTCT-3' R: 5'-GCGTTGGACATTCAAGTCAG-3'
TNF-α	F: 5'-GGTGCTTGTTCCTCAGCCTC-3' R: 5'-CAGGCAGAAGAGCGTGGTG-3'
IL-1β	F: 5'-CTGTCCTGCGTGTTGAAAGA-3' R: 5'-TTGGGTAATTTTTGGGATCTACA-3'
IL-6	F: 5'-CCACTCACCTCTTCAGAA-3' R: 5'-GCGCAAAATGAGATGAGT-3'
IL-8	F: 5'-AGACAGCAGAGCACACAAGC-3' R: 5'-ATGGTTCCTTCCGGTGGT-3'
IL-12A	F: 5'-CACTCCCAAAACCTGCTGAG-3' R: 5'-TCTCTTCAGAAGTGCAAGGGTA-3'

RESULTS

Growth Characteristics of PF-MSCs and 22Rv1 and their Morphology

Visualization using the phase-contrast microscope revealed that PF-MSCs exhibited fibroblast-like features (Figure 1a), whereas 22Rv1 cells displayed epithelial-like characteristics (Figure 1b).The MTT assay revealed insignificant changes in proliferation levels between the 22Rv1 cell line and PF-MSCs at 24 h ($p = 0.137$) and 48 h ($p = 0.059$). However, at 72 h, the proliferation level was significantly lower in PF-MSCs than in 22Rv1 cells ($p = 0.005$) (Figure 1c).

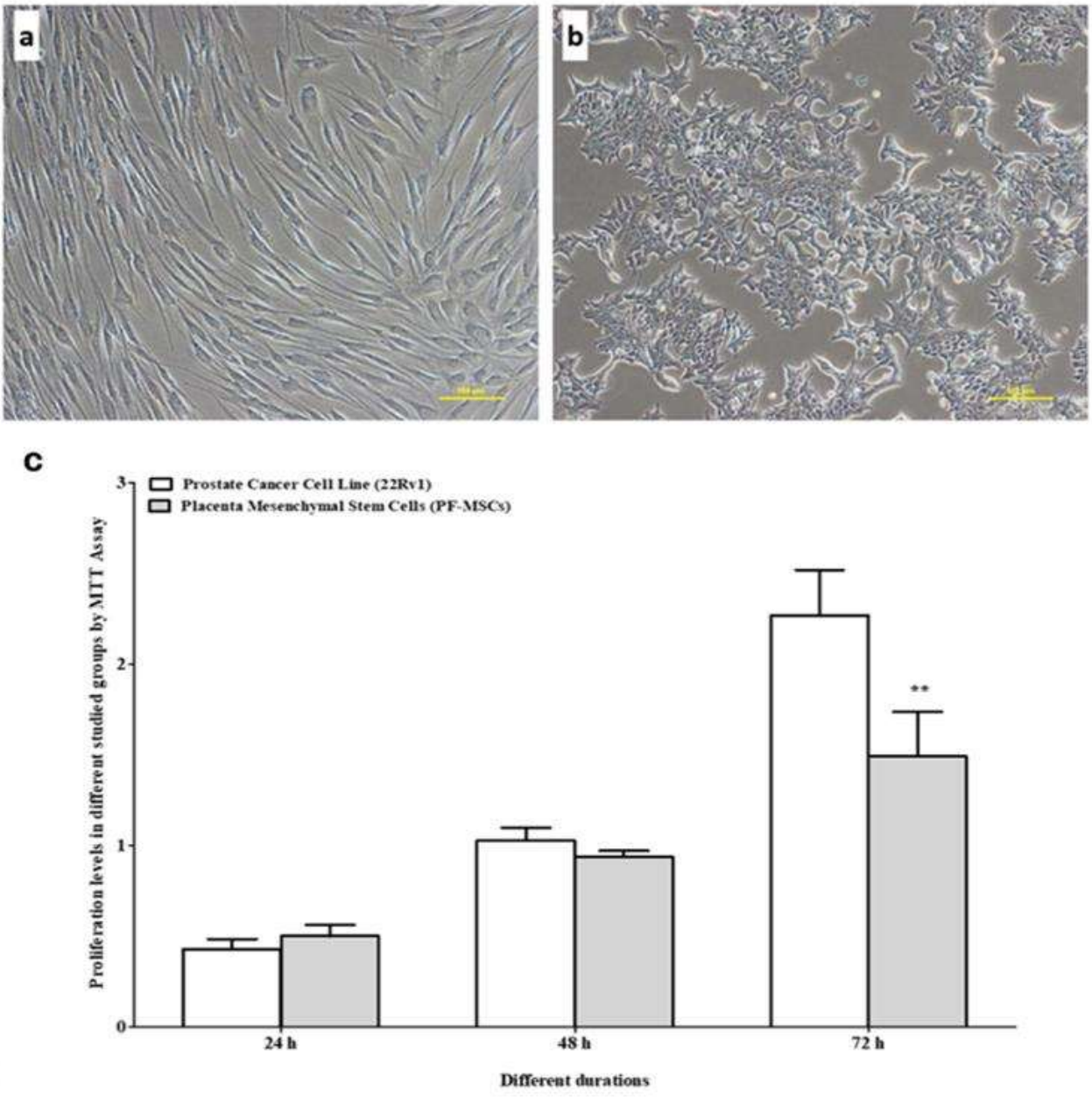

Figure 1: (a) Phase contrast micrographs showing the morphology of the PF-MSCs at passage (3) show fibroblast -like cells. (b) The 22Rv1 in culture showed epithelioid like cells. Scale bar is 100µm, Magnification 10x. (c) Comparison of Prostate Cancer Cell Line (22Rv1) and Placenta Fetal Mesenchymal Stem Cells (PF-MSCs) proliferation levels in different studied groups by MTT Assay *: significance versus 22Rv1-untreated (control) group. **: p <0.010

CD Marker Characterization of PF-MSCs

Analysis of FACS results shows that PF-MSCs have high levels of positive CD surface markers, such as CD105 (95.86%), CD90 (98.73%), CD73 (93.99%), CD44 (95.78%), and CD29 (80.93%), which are typically

associated with MSCs. The elevated levels of CD markers linked to MSCs validate the stemness potential of PF-MSCs. Moreover, PF-MSCs show reduced levels of CD34 (0.24%) and CD45 (0.23%), which are hematopoietic stem cell markers (Figure 2).

Differentiation Potential of PF-MSCs

PF-MSCs exhibited differentiation into osteocytes and adipocytes following culture in the differentiation medium. Specifically, in osteogenic differentiation, PF-MSCs exhibited positive alizarin red staining at 14 days, indicating calcium mineralization. However, cells cultured in the adipogenic medium demonstrated lipid droplets at 21 days, with differentiated adipocytes demonstrating positive staining of oil red O (Figure 3).

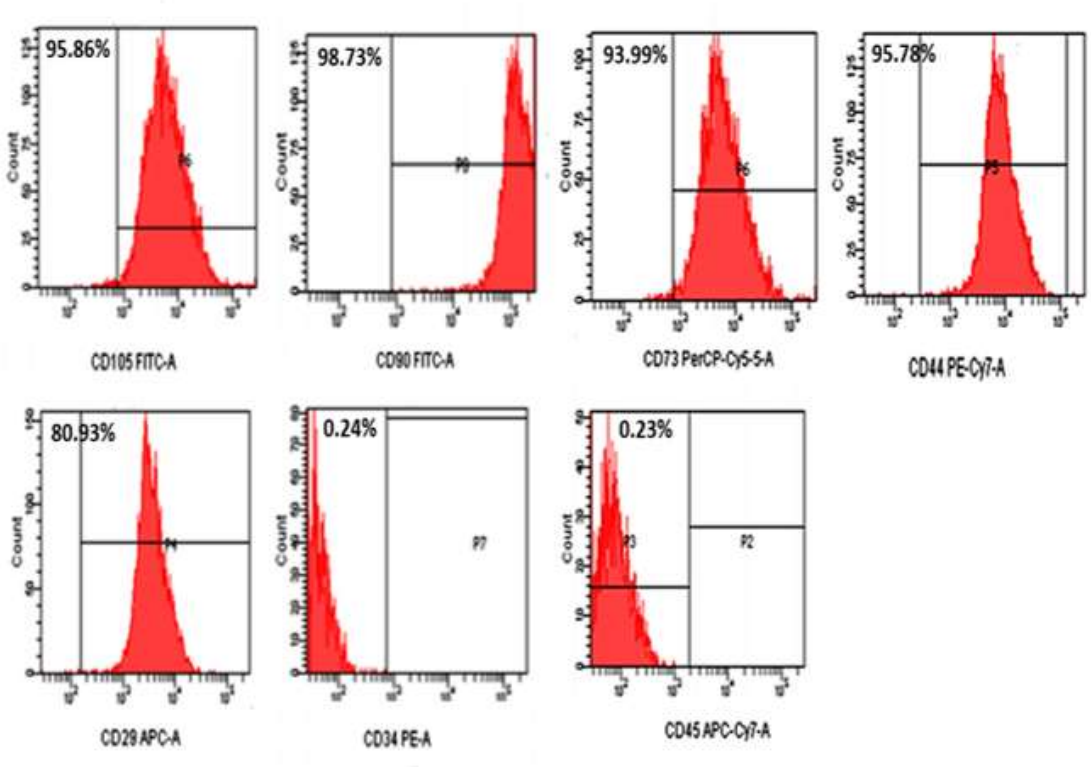

Figure 1: Representative histogram of the flow cytometry analysis (FACS) of MSC-related CD surface marker expression on PF-MSCs at early passage

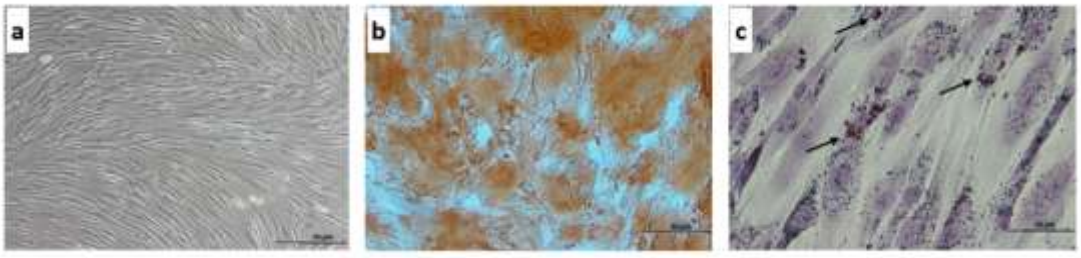

Figure 2: Differentiation capacity of the PF-MSCs into adipocytes. (a) represent the control; (b) osteocytes ; (c) adipocytes (Arrows indicate the fat cells). Scale bar is 50µm

Effect of PF-MSCs Extracts on 22Rv1 Proliferation

The MTT assay revealed a dose-dependent effect of PF-MSCs/CM (30%–100%) and PF-MSCs/CL (30

µg/ml–100 µg/ml) on 22Rv1 cells compared to that observed for the control (untreated). The most significant cytotoxic effect was observed in 22Rv1 cells treated with 100% PF-MSCs/CM and 100 µg/ml PF-MSCs/CL at 72 h. This cytotoxic effect increased gradually with the duration of incubation and PF-MSCs/CM and PF-MSCs/CL concentrations. Proliferations were significantly lower in 22Rv1 cells treated with PF-MSCs/CL at 50 µg/ml than in the control ($p = 0.001$). Moreover, proliferations were significantly lower in 22Rv1 cells treated with PF-MSCs/CL than in the control and 22Rv1 cells treated with PF-MSCs/CM at 30, 75, and 100 µg/ml ($p = 0.001$ for all) (Figure 4).

PF-MSC Extracts Altered 22Rv1 Cell Morphology

Following 24 h, 48 h, and 72 h of culturing 22Rv1 cells, noticeable alterations in morphology were observed in cells treated with 100% PF-MSCs/CM and 100 µg/ml PF-MSCs/CL. In general, 22Rv1 cell morphology displayed cell shrinkage and death when treated with PF-MSCs/CM and PF-MSCs/CL (Figure 5).

Effect of PF-MSCs/CM and PF-MSCs/CL on 22Rv1 Cell Cycle

Cell cycle analysis of sub-G1 revealed a significant reduction in 22Rv1 cells treated with PF-MSCs/CL at 100 µg/ml compared to that in the control. Cell cycle analysis of sub-G2/M revealed a significant decrease in 22Rv1 cells treated with PF-MSCs/CL at 100 µg/ml compared to that in the control and 22Rv1 cells treated with PF-MSCs/CM at 100% concentration (Figure 6).

Effect of PF-MSCs on Gene Expression

PF-MSCs/CM-treated 22Rv1 cells had substantially decreased BIRC 5, BAX, BCL2, and CASP3 gene expression than control ($p = 0.001$, $p = 0.002$, $p = 0.001$, and $p = 0.019$, respectively). PF-MSCs/CL-treated 22Rv1 cells had considerably reduced BIRC 5, BAX, and CASP3 gene expression than the control and 100% CM-treated cells ($p = 0.001$ and $p = 0.001$, respectively, for all). BCL2 gene expression was considerably lower in PF-MSCs/CL-treated 22Rv1 cells than in the control ($p = 0.001$) (Figure 7a).

There was a significant increase in the gene expression levels of IL-4, IL-10, IFN-γ, TNF-α, IL-1β, IL-6, IL-8, and IL-12 A in PF-MSCs/CM-treated 22Rv1 cells compared to the control ($p = 0.001$, $p = 0.005$, $p = 0.001$, $p = 0.001$, $p = 0.008$, $p = 0.001$, $p = 0.001$, and $p = 0.003$, respectively). IL-4, IL-10, IFN-γ, TNF-α, IL-1β, IL-6, IL-8, and IL-12 A gene expression was notably elevated in PF-MSCs/CL-treated 22Rv1 cells compared to control and 22Rv1 cells treated with PF-MSCs/CM at 100% ($p = 0.001$ and $p = 0.001$, respectively, for all) (Figure 7b).

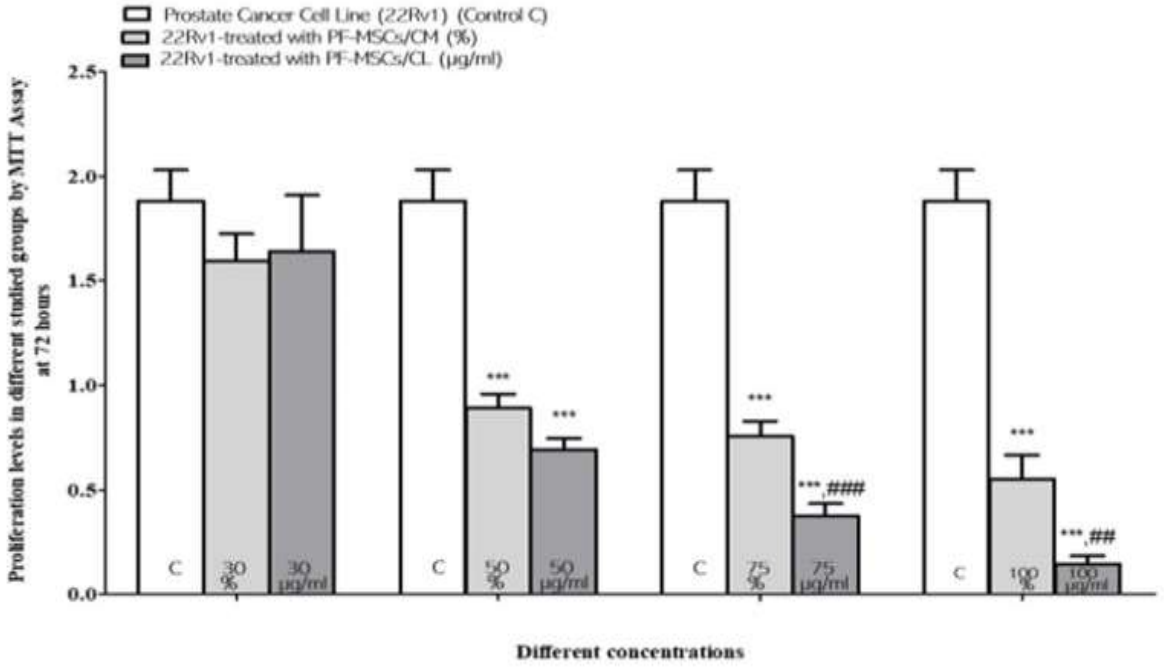

Figure 4: Comparison of 22Rv1 cells following treatment with PF-MSCs extracts (PF-MSCs/CM and PF-MSCs/CL) proliferation levels in different studied groups by MTT Assay at 72 h. *: significance versus 22Rv1-untreated (control) group; #: significance versus 22Rv1-treated with PF-MSCs/CM. ***p <0.001

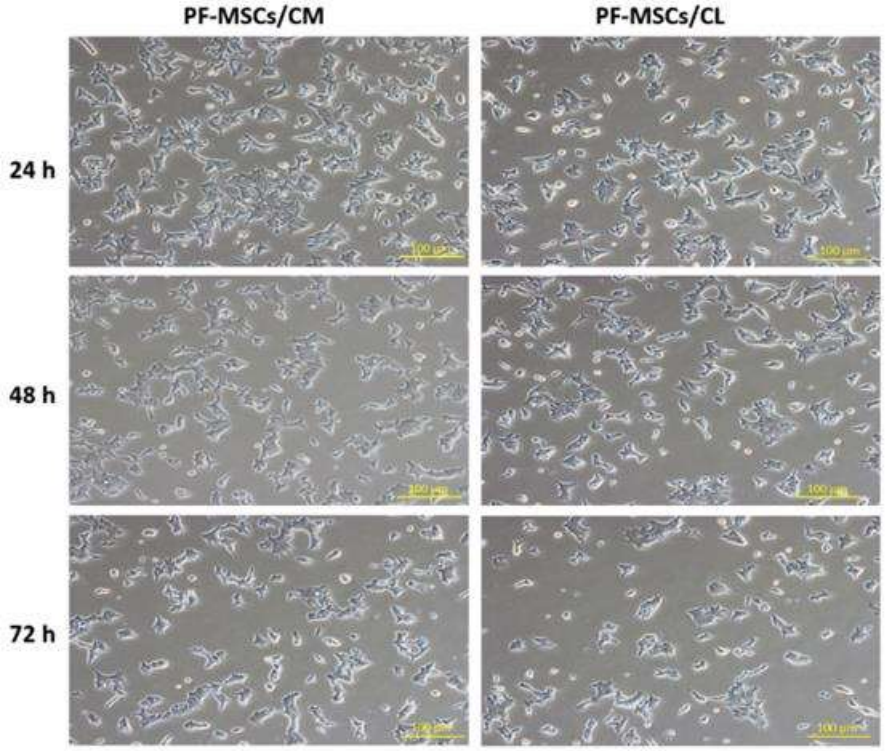

Figure 5: Phase contrast images of the 22Rv1 cells following treatment with 100% PF-MSCs/CM and 100µg/ml PF-MSCs/CL for 24 h, 48 h, and 72 h. An increase in cell death of 22Rv1 cells with time following treatment with PF-MSC extracts was noted

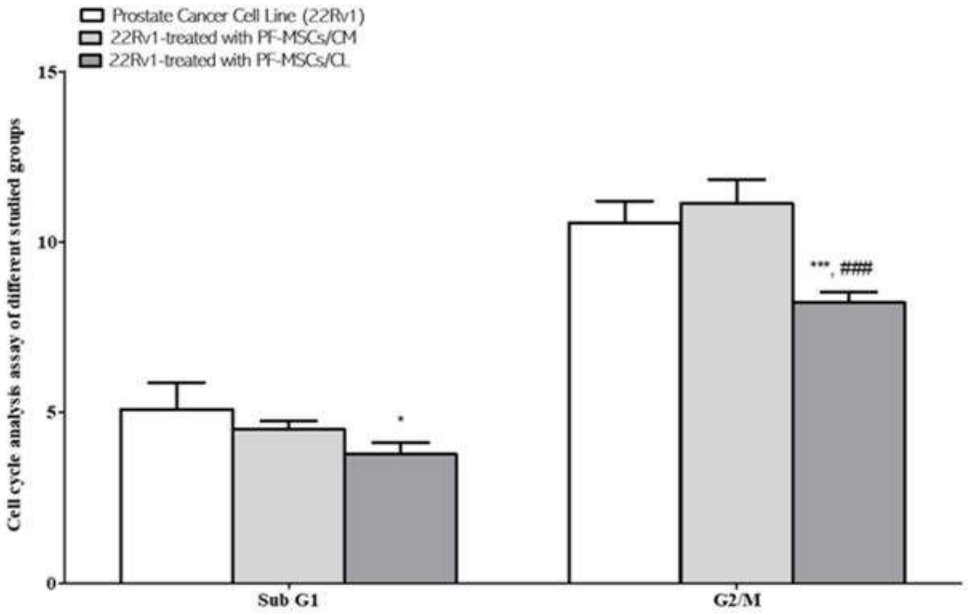

Figure 3: Cell cycle analysis assay of 22Rv1 cells following treatment with PF-MSCs/CM and PF-MSCs/CL.*: significance versus 22Rv1-untreated (control) group; #: significance versus 22Rv1-treated with PF-MSCs/CM. *p <0.050, ***p <0.001

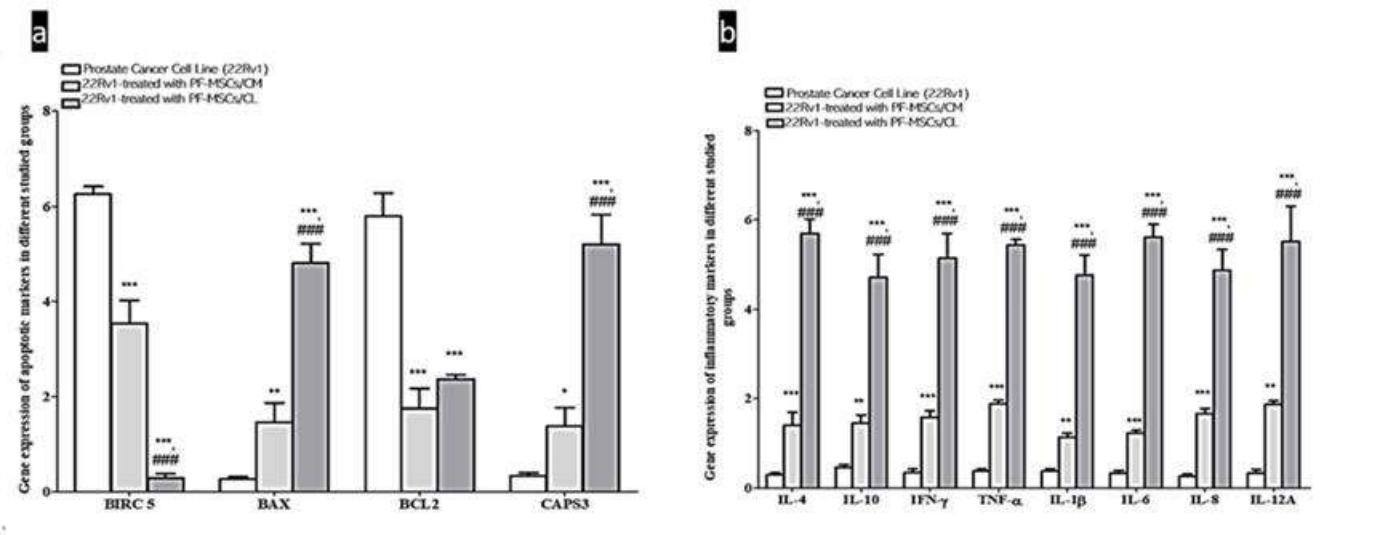

Figure 7: (a) Gene expression of apoptotic markers in 22Rv1 cell line following treatment with PF-MSC extracts (PF-MSCs/CM and PF-MSCs/CL). *: significance versus 22Rv1-untreated (control) group; #: significance versus 22Rv1-treated with PF-MSCs/CM. *: p <0.050, **: p <0.010, ***p <0.001. (b) Gene expression of inflammatory markers in 22Rv1 cell line following treatment with PF-MSC extracts (PF-MSCs/CM and PF-MSCs/CL). *: significance versus 22Rv1-untreated (control) group; #: significance versus 22Rv1-treated with PF-MSCs/CM. **: p <0.010, ***p <0.001

DISCUSSION

MSCs may be used in regenerative medicine as cell-based treatments and have pro- or anti-cancer qualities when designed to carry tiny medicines or apoptotic inducers (Hmadcha et al. 2020; Wu et al. 2020). In this study, we conducted multiple independent experiments to examine the in vitro anticancer characteristics of PF-MSC extracts using the 22Rv1 cell line. Our findings indicated that PF-MSCs successfully developed into mesodermal tissue lineages such as osteocytes and adipocytes and expressed MSC-related CD surface markers. Moreover, the cell morphology revealed spindle-shaped fibroblast-like cells.

Prior to this study, the differential effects of PF-MSCs/CM and PF-MSCs/CL on 22Rv1 cells had remained unelucidated. However, it is plausible that MSCs may indirectly induce 22Rv1 cell death through factors present in either their CM or CL. Therefore, the purpose of the current research was to evaluate the anticancer effects of PF-MSCs/CM and PF-MSCs/CL on 22Rv1 cells. PF-MSC extracts induced shrinkage and membrane damage in 22Rv1 cells, culminating in cell death (Figure 5). The main finding of this study is the observed presence of a higher anticancer potential of CL than that of CM against 22Rv1 cells. This effect exhibited dose-dependent cytotoxicity on 22Rv1 cells, with the highest observed for 100 µg/ml PF-MSCs/CL at 72 h. 22Rv1 cell death might be attributed to the indirect mechanism involving the release of chemokines, cytokines, and other inflammatory chemicals from PF-MSCs into their CM or CL (Jantalika et al. 2023; Opo et al. 2023; Sasportas et al. 2009). Additionally, MSCs-CM demonstrated antitumor capabilities by inhibiting cancer cell proliferation. Previous studies have indicated that umbilical cord MSCs and/or their extracts exhibit paracrine activities, which inhibit ovarian, breast, lung, and bone cell lines *in vitro* (Chao et al. 2012; Fang et al. 2023; Kalamegam et al. 2018). Moreover, evidence suggests that the microenvironment of human amnion MSCs secretes soluble substances that may halt the proliferation of PCa cells (Rolfo et al. 2014).

Given that cell cycle dysregulation is a characteristic of tumorous cells, interventions that arrest cell cycle progression or induce apoptosis are regarded as crucial approaches for cancer treatment (Hanahan and Weinberg 2011). Our study demonstrated the ability of PF-MSCs/CM and PF-MSCs/CL to induce 22Rv1 cell cycle arrest in the G2/M phase. Notably, a roadblock in the G2/M phase was observed, suggesting a possible target for cancer treatment. Cell cycle progression ceases when cells sustain DNA damage during the late "S" or "G2" phases, owing to their inability to enter the mitotic phase (Wang et al. 2009).

Our results revealed that PF-MSC extracts induced 22Rv1 cell death, as observed through qPCR analysis targeting BIRC 5, BAX, BCL2, and CASP3. The highest apoptotic effect was observed following treatment with the highest ratio/concentration of PF-MSCs/CM (100%) and PF-MSCs/CL (100 µg/ml), suggesting a dose-dependent response to the PF-MSC extracts. Consistent results have been observed in a previous study where

MSCs inhibited Bcl2 and Wnt signaling, thereby triggering apoptosis in cancer cells (Wang and Scadden 2015). Kalamegam et al. also described that the extraction of human Wharton's jelly MSCs induced cellular damage, upregulated CASP3 gene expression, and regulated genes involved in the cell cycle, ultimately suppressing the growth, proliferation, and apoptosis of ovarian cancer cells (Kalamegam et al. 2018). Similarly, Han et al. demonstrated that MSCs extracted from the human cord inhibited the growth and proliferation of PCa cells (Han et al. 2014).

Chronic inflammation promotes tumor growth and spread by enabling cancer cells to escape immune surveillance via soluble and cellular inflammatory mediators (Huwaikem et al. 2021). Our study found an increase in the expression of pro-inflammatory cytokines, including IFN-γ, TNF-α, IL-1β, IL-6, IL-8, and IL-12, which are known to accelerate cancer progression. A previous study found a link between pro-inflammatory cytokines and cancer risk, including IL-1β, IL-6, and TNF-α (Trompet et al. 2009).

CONCLUSIONS

PCa treatment costs can vary depending on the stage, which may involve surgery, radiation, and chemotherapy. The current study assessed the effects of PF-MSC extract on prostate cancer cell lines *in vitro*. These samples reduced the amount of 22Rv1 cells by triggering apoptosis and halting the cell cycle. PF-MSC may suppress 22Rv1 cells by increasing anti-inflammatory cytokines and decreasing pro-inflammatory genes and cytokines. Nonetheless, more in vivo and clinical trials must be conducted before utilizing these extracts for PCa treatment**.**

Supplementary materials

Not applicable.

Author contributions

AA, EH, SS, SA, GA, NA, and AB conceived this work. AA and GA conducted the experiments and analyzed the data. AA wrote the manuscript. SS and GA were involved in co-ordination of the work, review, and editing of the manuscript. All of the authors discussed the results and reviewed the manuscript.

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Institutional Review Board Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The collection of placenta fetal mesenchymal stem cells was approved by the Biomedical Research Ethics Committee, Faculty of Medicine at KAU, Jeddah, Saudi Arabia (Ref-No: 21-71, KAU).

Informed Consent Statement

Informed consent was obtained from all individual participants included in the study.

Data Availability Statement

The materials and data used and/or analyzed in the present study are available from the corresponding author on reasonable request.

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Not applicable

Conflict of interest

The authors declare no conflicts of interest.

REFERENCES

Abomaray FM, Al Jumah MA, Alsaad KO, Jawdat D, Al Khaldi A, AlAskar AS, Al Harthy S, Al Subayyil AM, Khatlani T, Alawad AO, Alkushi A, Kalionis B, Abumaree, 2016. Phenotypic and Functional Characterization of Mesenchymal Stem/Multipotent Stromal Cells from Decidua Basalis of Human Term Placenta. Stem Cells International 2016.

Adedapo KS, Arinola OG, Shittu OB, Kareem OI, Okolo CA, Nwobi LN, 2012. Diagnostic Value of Lipids,

Total Antioxidants, and Trace Metals in Benign Prostate Hyperplasia and Prostate Cancer. Nigerian Journal of Clinical Practice 15(3):293–97.

American Cancer Society, 2018. Global Cancer Facts & Figures 4th Edition.

Bergengren O, Kelly RP, Matsoukas K, Fainberg J, Mungovan SF, Bratt O, Bray F, Brawley O, Luckenbaugh AN, Mucci L, Morgan TM, Carlsson SV, 2023. 2022 Update on Prostate Cancer Epidemiology and Risk Factors—a Systematic Review. European Urology. 84(2):191-206.

Chao KC, Yang HT, Chen MW, 2012. Human Umbilical Cord Mesenchymal Stem Cells Suppress Breast Cancer Tumourigenesis through Direct Cell–Cell Contact and Internalization. Journal of Cellular and Molecular Medicine 16(8):1803–15.

Chen X, Rycaj K, Liu X, Tang DG, 2013. New Insights into Prostate Cancer Stem Cells. Cell Cycle 12(4):579–86.

Fang J, Wei H, Wang H, Wang J, Liu H, Chen Y, Chen L, Lu L, Zhang Q, Pan R, Cui E, Luo X, 2023. Human Placenta-derived Mesenchymal Stem Cell Administration Protects against Acute Lung Injury in a Mouse Model. Journal of Cellular Biochemistry 124(9):1249–58.

Gauthaman K, Yee FC, Cheyyatraivendran S, Biswas A, Choolani M, Bongso A, 2012. Human Umbilical Cord Wharton's Jelly Stem Cell (HWJSC) Extracts Inhibit Cancer Cell Growth *in Vitro*. Journal of Cellular Biochemistry 113(6):2027–39.

Giridharan M, Rupani V, Banerjee S, 2022. Signaling Pathways and Targeted Therapies for Stem Cells in Prostate Cancer. ACS Pharmacology & Translational Science 5(4):193–206.

Gondi, CS, Gogineni VR, Chetty C, Dasari VR, Gorantla B, Gujrati M, Dinh DH, Rao JS, 2010. Induction of Apoptosis in Glioma Cells Requires Cell-to-Cell Contact with Human Umbilical Cord Blood Stem Cells. International Journal of Oncology 36(5):1165–73.

Gopalarethinam J, Nair AP, Iyer M, Vellingiri B, Subramaniam MD, 2023. Advantages of Mesenchymal Stem Cell over the Other Stem Cells. Acta Histochemica 125(4):152041.

Han I, Yun M, Kim EO, Kim B, Jung MH, Kim SH, 2014. Umbilical Cord Tissue-Derived Mesenchymal Stem Cells Induce Apoptosis in PC-3 Prostate Cancer Cells through Activation of JNK and Downregulation of PI3K/AKT Signaling." Stem Cell Research & Therapy 5:1–9.

Hanahan D, Weinberg R A, 2011. Hallmarks of Cancer: The next Generation. Cell 144(5):646–74.

Hmadcha A, Martin-Montalvo A, Gauthier BR, Soria B, Capilla-Gonzalez V, 2020. Therapeutic Potential of Mesenchymal Stem Cells for Cancer Therapy. Frontiers in Bioengineering and Biotechnology 8:43.

Huwaikem MAH, Kalamegam G, Alrefaei G, Ahmed F, Kadam R, Qadah T, Sait KHW, Pushparaj PN, 2021. Human Wharton's Jelly Stem Cell Secretions Inhibit Human Leukemic Cell Line K562 in Vitro by Inducing Cell Cycle Arrest and Apoptosis. Frontiers in Cell and Developmental Biology 9:614988.

Jafari A, Rezaei-Tavirani M, Farhadihosseinabadi B, Zali H, Niknejad H, 2021. Human Amniotic Mesenchymal Stem Cells to Promote/Suppress Cancer: Two Sides of the Same Coin. Stem Cell Research & Therapy 12(1):1–11.

Jantalika T, Manochantr S, Kheolamai P, Tantikanlayaporn D, Thongsepee N, Warnnissorn N, Saijuntha W, Pinlaor S, Tantrawatpan C, 2023. The Human Placental Amniotic Membrane Mesenchymal-Stromal-Cell-Derived Conditioned Medium Inhibits Growth and Promotes Apoptosis of Human Cholangiocarcinoma Cells In Vitro and In Vivo by Suppressing IL-6/JAK2/STAT3 Signaling. Cells 12(24):2788.

Kalamegam G, Sait KHW, Ahmed F, Kadam R, Pushparaj PN, Anfinan N, Rasool R, Jamal MS, Abu-Elmagd M, Al-Qahtani M, 2018. Human Wharton's Jelly Stem Cell (HWJSC) Extracts Inhibit Ovarian Cancer Cell Lines OVCAR3 and SKOV3 in Vitro by Inducing Cell Cycle Arrest and Apoptosis. Frontiers in Oncology 8:592.

Kerr CL, Arif H, 2014. Regulators of Prostate Cancer Stem Cells. Current Opinion in Oncology 26(3):328–33.

Khakoo AY, Pati S, Anderson SA, Reid M, Elshal MF, Rovira II, Nguyen AT, Malide D, Combs CA, Hall G. 2006. "Human Mesenchymal Stem Cells Exert Potent Antitumorigenic Effects in a Model of Kaposi's Sarcoma." The Journal of Experimental Medicine 203(5):1235–47.

Kološa K, Motaln H, Herold-Mende C, Koršič M, Lah TT, 2015. Paracrine Effects of Mesenchymal Stem Cells Induce Senescence and Differentiation of Glioblastoma Stem-like Cells. Cell Transplantation 24(4):631–44.

Kumar P, Kandoi S, Misra R, S V, K R, Verma RS, 2019. The Mesenchymal Stem Cell Secretome: A New Paradigm towards Cell-Free Therapeutic Mode in Regenerative Medicine. Cytokine & Growth Factor Reviews 46:1–9.

Madrigal M, Rao KS, Riordan NH, 2014. A Review of Therapeutic Effects of Mesenchymal Stem Cell Secretions and Induction of Secretory Modification by Different Culture Methods. Journal of Translational Medicine 12(1):1–14.

Opo, FADM, Moulay M, Alrefaei GI, Alsubhi NF, Alkarim S, Rahman MM, 2023. Effect of Co-Culturing Both Placenta-Derived Mesenchymal Stem Cells and Their Condition Medium in the Cancer Cell (HepG2) Migration, Damage through Apoptosis and Cell Cycle Arrest. Saudi Journal of Biological

Sciences 30(2):103519.

Portillo-Lara R, Alvarez MM, 2015. Enrichment of the Cancer Stem Phenotype in Sphere Cultures of Prostate Cancer Cell Lines Occurs through Activation of Developmental Pathways Mediated by the Transcriptional Regulator ΔNp63α. PloS One 10(6):e0130118.

Reya T, Morrison SJ, Clarke MF, Weissman IL, 2001. Stem Cells, Cancer, and Cancer Stem Cells. Nature 414(6859):105–11.

Rolfo A, Giuffrida D, Giuffrida MC, Todros T, Calogero AE, 2014. New Perspectives for Prostate Cancer Treatment: *In Vitro* Inhibition of LNCaP and PC3 Cell Proliferation by Amnion-Derived Mesenchymal Stromal Cells Conditioned Media. The Aging Male 17(2):94–101.

Safari F, Shakery T, Sayadamin N, 2021. Evaluating the Effect of Secretome of Human Amniotic Mesenchymal Stromal Cells on Apoptosis Induction and Epithelial-mesenchymal Transition Inhibition in LNCaP Prostate Cancer Cells Based on 2D and 3D Cell Culture Models. Cell Biochemistry and Function.

Saleh AKS, Adly HM, Abdelkhaliq AA, Nassir AM, 2020. Serum Levels of Selenium, Zinc, Copper, Manganese, and Iron in Prostate Cancer Patients. Current Urology 14(1):44–49.

Sasportas LS, Kasmieh R, Wakimoto H, Hingtgen S, Water JAJMVW, Mohapatra G, Figueiredo JL, Martuza RL, Weissleder R, Shah K, 2009. Assessment of Therapeutic Efficacy and Fate of Engineered Human Mesenchymal Stem Cells for Cancer Therapy. Proceedings of the National Academy of Sciences 106(12):4822–27.

Saudi Health Council Saudi Cancer Registry, 2018. Cancer Incidence Report Saudi Arabia 2014.

Sun B, Roh KH, Park JR, Lee SR, Park SB, Jung JW, Kang SK, Lee YS, Kang KS, 2009. Therapeutic Potential of Mesenchymal Stromal Cells in a Mouse Breast Cancer Metastasis Model. Cytotherapy 11(3):289–98.

Takahara K, Ii M, Inamoto T, Komura K, Ibuki N, Minami K, Uehara H, Hirano H, Nomi H, Kiyama S, 2014. Adipose-Derived Stromal Cells Inhibit Prostate Cancer Cell Proliferation Inducing Apoptosis. Biochemical and Biophysical Research Communications 446(4):1102–7.

Tang DG, Patrawala L, Calhoun T, Bhatia B, Choy G, Schneider-Broussard R, Jeter C, 2007. Prostate Cancer Stem/Progenitor Cells: Identification, Characterization, and Implications. Molecular Carcinogenesis: 46(1):1–14.

Trompet S, Craen AJM, Mooijaart S, Stott DJ, Ford I, Sattar N, Jukema W, Westendorp RGJ, 2009. High Innate Production Capacity of Proinflammatory Cytokines Increases Risk for Death from Cancer: Results of the PROSPER Study. Clinical Cancer Research 15(24):7744–48.

Vaiasicca S, Melone G, James DW, Quintela M, Xiao J, Yao S, Finnell RH, Conlan RS, Francis LW, Corradetti B, 2024. Transcriptomic Analysis Reveals the Anti-Cancer Effect of Gestational Mesenchymal Stem Cell Secretome. Stem Cells Translational Medicine szae024.

Voinea F, Mazilu L, Micu IS, Suceveanu AP, Iliescu M, Dumitru A, Constantin VD, Paunica I, Suceveanu AI, 2021. Modern Approaches for Antiandrogen-Resistant Prostate Cancer Therapy. Journal of Mind and Medical Sciences 8(1):71–75.

Wang YH, Scadden DT, 2015. Harnessing the Apoptotic Programs in Cancer Stem-like Cells. EMBO Reports 16(9):1084–98.

Wang Y, Ji P, Liu J, Broaddus RR, Xue F, Zhang W, 2009. Centrosome-Associated Regulators of the G 2/M Checkpoint as Targets for Cancer Therapy. Molecular Cancer 8:1–13.

Wu M, Zhang R, Zou Q, Chen Y, Zhou M, Li X, Ran R, Chen Q, 2018. Comparison of the Biological Characteristics of Mesenchymal Stem Cells Derived from the Human Placenta and Umbilical Cord. Scientific Reports 8(1):1–9.

Wu Z, Liu W, Wang Z, Zeng B, Peng G, Niu H, Chen L, Liu C, Hu Q, Zhang Y, 2020. Mesenchymal Stem Cells Derived from IPSCs Expressing Interleukin-24 Inhibit the Growth of Melanoma in the Tumor-Bearing Mouse Model. Cancer Cell International 20(1):1–10.

Yang ZX, Han ZB, Ji YR, Wang YW, Liang L, Chi Y, Yang SG, Li LN, Luo WF, Li JP, 2013. CD106 Identifies a Subpopulation of Mesenchymal Stem Cells with Unique Immunomodulatory Properties. PloS One 8(3):e59354.

Yu C, Yao Z, Jiang Y, Keller ET, 2012. Prostate Cancer Stem Cell Biology. Minerva Urologica e Nefrologica 64(1):19.